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MECHANISMS OF FLAVOR RELEASE AND PERCEPTION IN SUGAR-FREE CHEWING GUM

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by

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ABSTRACT

The flavor properties of chewing gum are undoubtedly a key product attribute for consumption. However little is known about how many of the various ingredients (i.e. flavor solvents) in chewing gum alter flavor delivery. Consequently, defining mechanisms which influence flavor release in chewing gum is important for understanding its product quality and possibly may also translate to drug delivery applications for the pharmaceutical industry.

The first objective of this study investigated the influence of three flavor solvents on the aroma/taste/textural properties in a sugar-free chewing gum. Model chewing gums made with [0.67%; triacetin (TA) or propylene glycol (PG) or medium chain triglycerides (MCT)] and without flavor carrier solvent. The chewing gums were analytically characterized *in vivo* for three panelists over a period of 12 min. Volatile analysis of cinnamaldehyde, L-carvone, piperitone, jasmone was conducted using Atmospheric Pressure Chemical Ionization-mass spectroscopy (APCI-MS). Sorbitol release from saliva was tracked using High pressure Liquid Chromatography (HPLC) coupled with Refractive Index Detector (RID), while the textual properties (softness) were measured using TA-XT2. Furthermore, the perceived flavor properties of these chewing gum samples were measured using a time-intensity sensory study (TI) on the aroma (cinnamon-like), taste (sweetness) and textural (effort to chew) attributes using a trained sensory panel.

Flavor solvent addition to chewing gum did not significantly influence the aroma release profiles of all the 4 compounds. However, the sorbitol release rate was significantly lower for chewing gum made with TA compared to the other treatments.

The sensory analysis was in agreement with the analytically data; lower levels of sweetness and cinnamon-like flavor intensity were perceived for chewing gum made with TA were observed, suggesting taste-aroma interactions. Chewing gums formulated with TA or MCT were reported to be softer (based on texture analysis) then with PG or no flavor solvent addition. However, no correlations were reported between the instrumental texture analyses of the chewing gum (softness) and the flavor release. Overall, flavor solvent choice did influence the sorbitol release in chewing gum matrix due to unique plasticizing/softening mechanism of the solvent utilized.

The second objective of this study was to investigate the mechanisms for cinnamaldehyde release in a sugar-free chewing gum. Chewing gums containing (25%-Paloja gum base, 61 %-sugar alcohol, 4%-glycerine, 0.46% sweeteners, and 0.02% lecithin) were made with varying concentrations of cinnamaldehyde. Additionally chewing gums were made with p-cresol (similar log P as cinnamaldehyde). A cinnamaldehyde or cresol flavored gum base (no sugar alcohol) was also made to investigate the role of the gum base on flavor release. Three panelists were asked to chew gums or flavored gum base, while aroma release profile was tracked from the nose exhaled breath using APCI-MS over a period of 8 min. The release profile of cinnamaldehyde from chewing gum was found to correlate with the sugar alcohol release in a sugar free gum. Chewing gums made with varying amounts of cinnamaldehyde (0.29 - 2.9 mg/g of chewing gum) did not show any differences in release pattern suggesting no concentration effect. Furthermore, the cinnamaldehyde release pattern from the gum base was similar to cresol or as

predicted from the log cP value (distribution coefficient between the gum base and water). These findings suggested cinnamaldehyde was interacting with the sugar alcohol phase, possibility due to transient hemi-acetal reactions mechanisms, which resulted in a more rapid release rate than would be predicted based on the hydrophobicity of this compound.

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Chapter 1

Introduction

In order to sustain competition as well as growth, the candy and gum industry has focused on novel products with high flavor intensity, long lasting flavor and as unique applications drug delivery systems (i.e. nicotine). However most of these emerging technologies are in the form of patents, and do not provide any scientific literature to understand the mechanisms that impact compound delivery or flavor release and perception. This research project was conducted to investigating the mechanisms that impact flavor release and perception in chewing gum matrix.

Flavor solvents, such as triacetin, propylene glycol, medium chained triglycerides, are used as dispersing agents in many food systems as well as plasticizing agents in chewing gum. However little is known about how these solvents, with unique physical-chemical properties ultimately influence the aroma, taste and texture properties of chewing gum. No scientific study has been conducted to understand the influence of flavor solvents in a chewing gum matrix. The first objective of this thesis was therefore to investigate the influence of flavor solvent type on aroma, taste and texture properties in a sugar-free chewing gum was investigated as well as any cross-modalities among these flavor stimuli.

Furthermore, the gum and flavor industry use a simplified model to predict the flavor release of compounds in chewing gum; log P or log cP values (indicator of compound hydrophobicity or company affinity for the gum matrix). Compounds with

lower log cP (or log P) value are predicted to release faster any compounds with a high log cP (or log P) value. Based on the complexity of the flavor compounds and chewing gum ingredients, the use of log P or log cP model was considered to be likely an over simplified approach and therefore was the focus of the second objective of this research project.

Chapter 2

Review of Literature

2.1 Confectionary industry

The total sales of confectionery products ranked 3rd behind carbonated beverages and milk in the United States, according to the 2005 IRI report with sales surpassing $28 billion [1]. Overall, these confectionery products consisted of 5 main categories: chocolate, non-chocolate, mints, gums and others. Based on the 2005 US Department of Commerce, NCA estimated an increase in retail sales for chocolate, non-chocolate and gum categories by 2.0%, 0.9%, and 4.1% respectively [1].

2.2 Chewing gum

2.2.1 Market and trends:

According to the latest Mintel's report (2003), the consumption of breath freshening products such as gums, mints, and breath strips has increased by 41% since 1997[2] in the US marketplace. With respect to gum confectionery products, total sales reached a value of $385 million in UK and $ 2.2 billion in the US in 2002 (Conway, 2003). Sales of regular gums have declined by 5.6% from 2000 to 2002 while the demand for sugar-free gums has been increased with a jump in sales by 28% from 2000

to 2002 (Table 2-1). The primary reasons for this sales boost may be due to new varieties of gums using different sweeteners, less calories, better mouth-feel and/or strong advertising. Currently, chewing gum has been consumed to a greater extent not only as a commercial flavor gum but also as a drug delivery system in the field of pharmacy, which demonstrated a 7% growth in sales for dental and nicotine gums from 2000 [3]. These gums carry various key functional compounds such as nicotine, fluoride (Fluogum®, Flourette®), calcium carbonate (Surpass®), caffeine (Stay Alert®) and other compounds (Conway, 2003, #23). The basic factors affecting the drug release from a medicated gum includes the physiochemical properties of the drug and gum as well as the chewing efficiency [3].

Mintel's report further predicts the total retail sales will grow by 27% from 2002 to 2007 in the category of gums and mints [2]. In the case of gum confectionary alone, sales are projected to rise by 13% to $4.7 billion, between 2003 and 2007 in the global marketplace [1]. This expanding market will be mainly driven by new product introductions for consumers at various age groups. New gum products making their way into the market include antacid gum (contains calcium carbonate), caffeine-containing gum as well as anti-emetics for travel sickness [3].

2.2.2 Benefits of chewing gum [4]

- Improves concentration
- Eases tension
- Freshens breath and reduces the urge to smoke
- Provides a low-calorie snack

- Helps fight tooth decay
- Helps one stay alert and awake
- Acts as a pleasant way to take vitamins and medicine
- Reduces ear discomfort during flight travel

Making a Mint
Sales of gum and mints segmented by type, in $ millions

	2002	2000	% Change
Sugarless gum	$1,356	$1,063	27.6%
Regular gum	$836	$886	-5.6%
Breath mints	$616	$619	-0.5%
Anti-smoking gum	$312	$292	6.8%
Candy mints	$279	$276	1.1%
Dental gum	$65	$61	6.6%

Note: Excludes sales through Wal-Mart and warehouse clubs. Also, breath strips accounted for $225 million in sales in 2002.

Source: Mintel/Based on Information Resources Inc. Infoscan® Reviews Information/*Convenience Store News/Automatic Merchandiser/PF*

Table 2-1 Sale comparison of gum and mints segment between years 2000 and 2003, according to the mintel report [2]

2.2.3 Typical chewing gum composition

Chewing gums consist of two phases, 1) water-insoluble gum base phase and 2) water-soluble sugar or sugar alcohol phase. The ratio of soluble to insoluble phases has a great impact on the flavor release characteristics. Basic chewing gum composition for a sugar gum as well as a non-sugar gum is given in Table 2-2.

Ingredients	Sugar gum (%)	Sugar free gum (%)
Gum base	20	25-30
Sugar	60	-
Glucose syrup / Corn syrup	18-20%	-
Polyols	< 1%	50-60
Glycerin	< 1%	5-6
Flavor	0.5 -1	1-1.5
High intensity sweeteners	-	Aspartame (0.01 -3%) Based on the sweetener type

Table 2-2 Typical chewing gum composition for sugar and sugar-free gums [3]

2.2.3.1 Water Insoluble phase

2.2.3.1.1 Gum base:

A typical gum base composition consists of elastomer, elastomer solvent, polyvinyl acetate, emulsifier, low molecular weight polyethylene, waxes, plasticizer and fillers. An example of a non-adherable chewing gum base composition and the function of these ingredients in chewing gum are given in Table 2-3 and Table 2-4, respectively [5]. The properties of these ingredients are furthermore discussed in more detail below.

Elastomers provide the desired body, along with rubbery texture and cohesiveness. When present in low quantities, these gums may lack elasticity, while high concentrations renders the gum hard and too rubbery [5]. In addition to texture, flavor release characteristics of the gum base have also been reported to be affected by the type

of elastomer used. For example, gum bases made with poly (Isobutylene) showed higher affinity for flavor compounds (such as ethyl butyrate, cis-hexenal. 1-octanol, and limonene) compared to poly (vinyl acetate) [6]. Higher affinity results to longer lasting flavor during chewing. On similar lines, Sostmann et al. (2003) found that synthetic gum base made with Styrene Butyl Rubber (SBR) has greater affinity for ¶-electron flavor compounds such as anethole, octanal and isopropyl-pyridine [7]. Some elastomers have been reported to sequester the flavor molecules, thus preventing flavor release during chewing [8].

Elastomer solvents are often resins such as terpene resins. These solvents help in softening the elastomer rubber components. When present in low percentage, the chewing gum has unacceptable chewing characteristics [5]. On the other hand, excess solvent result in stickiness to dental surfaces. Ester resin gums are also known to have a high affinity for polar molecules such as alcohol and aldehydes [6].

Ingredients	**Weight (%)**
Elastomer	10-30%
Elastomer solvent	2-18%
Plasticizer	20–35%
Polyvinyl acetate	15–45 %
Emulsifier	2–10%
Low MW Polyethylene	0.5 – 15%
Waxes	0.5 – 10%
Filler	0 – 5%

Table 2-3 Typical gum base composition of chewing gum [5]

Polyvinyl acetate *(PVAc;* (MW- 15,000 to 30,000) at low levels can destabilize the base resulting in non-uniform flavor release[5]. However, at higher levels, the gum bases are to hard and plastic. PVA has also been found to have a higher affinity for polar alcohols [7]. Emulsifiers (HLB: 1.6 – 7.0) provide a smooth surface to the gum and reduces its adhesive nature as well as aid in mixing the immiscible components to form a stable dispersion system leading to texture acceptability and stability [5]. However, higher amounts can lead to an unstable paste-like product. Furthermore, emulsifiers can sometimes function as plasticizers.

Ingredients	**Examples**
Elastomer	Styrene- butadiene copolymer (SBR), Polyisobutylene, Isobutylene-isoprene copolymers (Butyl gum), Natural gums such as chicle, natural rubber, jelutong, gutta-percha, lechi caspi, sorva, Polyvinyl acetate, LW Polyethylene
Elastomer solvent	Methyl, glycerol or pentaerythriol esters of rosins,Modified roisins such as hydrogenated, dimerized or polymerized roisins (wood roisin, tall oil roisin, terpene resins including polyterpene and polymers of alpha, -pinene or bete pinene)
Plasticizer / softeners	Hydrogenated vegetable oil, Lanolin, Stearic acid, Sodium stearate, Potassium stearate, Glycerine
Emulsifier	Glycerol monostreate (3.8), glycerol monooleate (2.8), Lecithin fatty acid monoglycerides (4.2), Diglycerides, Triglycerides, Proplylene glycol monostearate (3.4), Sorbitan monostearate (4.7)
Wax	Rice bran wax, polyethylene wax, microcrystyalline wax, natural wax, petroleum wax, paraffin, bee wax, carnauba wax, candelilla wax, cocoa butter, degreased cocao powder

Fillers	Titanium oxide, Aluminum hydroxide, Alumina, Aluminum salicates, Dicalcium phosphate, Talc, magnesium carbonate, kaolin, silicium oxide

Table 2-4 List of various ingredients used across different categories in a typical gum base [5]

Waxes are used to soften the rubbery elastic gum base. Typical waxes are crystalline in nature with melting points (MP) above 170°C [5]. These texture modifiers provide better chewing properties compared to the low MP waxes which are known to impart tackiness to the product [5]. Waxes have a greater affinity for non-polar flavor molecules such as limonene, ethyl nonanoate and p-cymene [7].

Plasticizers are low molecular weight compounds that penetrate the structure of gum base, resulting to better chewability and mouth feel of the gum [5]. Also plastizers tend to absorb moisture and thus aid in flavor migration which lead to flavor loss [5].

Finally, fillers are added to the gum to provide color as well as reduce tackiness providing better mouth feel [5].

2.2.3.2 Water-soluble phase

2.2.3.2.1 Sweeteners in chewing gum:

Sugar sweeteners such as sucrose, dextrose, maltose, dextrin, dried invert sugar, fructose, galactose, and corn syrups can be used for regular (sugar) gums. In addition to providing sweetness, sugars also help mask the harshness of flavor compounds such as menthol and menthone [9]. In recent years, the huge growth of the sugar free gums has

led to the usage of sugar alcohols and high intensity sweeteners. The different polyols also act as bulking agents. The sugar alcohols used in sugar-free chewing gums include sorbitol, mannitol, xylitol, maltitol, lactitol, hydrogenated isomaltulose and hydrogenated starch hydrolysates. Xylitol containing gums have become popular due to its similar sweetness levels to sugar as well as cooling ability (endothermic heat of salvation) which complements the effect of the cooling agents [9]. However, because of the relatively high cost of this polyol, it is commonly used in combination of a lower cost polyol such as sorbitol, lactitol, or manitol with xylitol. Sorbitol, a commonly used polyol, has its own manufacturing challenges since this product is very hygroscopic and does not readily crystallize. Hence, various patents are in place suggesting different ways to incorporate these different polyols through different coating techniques [10-13]. With respect to flavor release, real-time breath analysis studies have reported a faster volatile release from sorbitol chewing gum compared to xylitol chewing gum for a given flavor dosage [14]; possibly because sorbitol would be predicted to undergo a relatively more rapid dissolution during mastication (more hygroscopic).

In addition, efforts to incorporate different high intensity sweeteners such as aspartame, acesulfame –K, saccharine, and thaumatin into chewing gum have increased in the recent years [5]. These sweeteners usually range from 0.005 % to 5 % of the gum composition. However, these materials are mainly affected by factors such as pH, moisture, temperature, microbial growth and chemical reactions [15]. Aspartame, the most commonly used non-caloric sweetener in chewing gum, is prone to hydrolytic degradation in the presence of moisture, leading to loss of sweetness [13]. Furthermore, other factors such as temperature and pH can speed up this degradation process [13].

Aspartame also reacts with aldehydes and ketones present in flavor oil leading to further loss of sweetness [13]. Hence, various technical use of HIS have been patented based on novel sweetener delivery systems which not only aid in protecting the sweetness over time but also helps in controlling it's release rates. These different techniques include dispersing sweetener in a hydrophobic matrix [13] and encapsulation followed by drying and grinding [16-20]. Recently, chewing gums containing Neotame, a modified N-substituted form of aspartame, have been utilized to overcome the above mentioned limitations of aspartame [21]. Similarly, efforts have been made to control the release rates of acesulfame-K through encapsulation [13, 22].

2.2.3.2.2 Physiological cooling agents (PCA):

According to Wolf et al. [9], physiological agents are perceived as cold or cool when in contact with the mucous membranes of the mouth, nose, and throat during consumption. The basic advantage of adding these agents is to provide a cooling effect with an unexpected high flavor impact while reducing the harsh notes that are most commonly encountered in sugarless gums [9]. The harsh flavor is mainly caused by menthol, which is ubiquitous in chewing gum flavors such as spearmint, peppermint, wintergreen, and fruit flavors. The presence of menthol not only provides a cooling flavor in the initial stages of chewing, but also imparts a bitter, harsh and burning taste [9]. In the case of regular sugar gums, sucrose masks these harsh qualities, whereas in sugarless gums, polyols other than xylitol have been reported not to have this flavor masking quality. Another reported advantage of using PCA is a lower required usage of xylitol, an expensive sugar alcohol. Different PCAs used for chewing gum manufacture

include menthyl succinate, menthyl lactate, 3-1-menthylpropane-1, 2-diol (TCA), menthone glycerol ketals, N-substiuted p-menthane carbamide (WS-3), acyclic carboxamide (W-23) and menthyl lactate [9]. These above cooling agents are normally used at low concentrations of about 0.001% to 2% per weight of the chewing gum. These PCAs have been categorized into three general groups based on their release rate during mastication: (1) slow release PCAs which includes menthone glycerol ketal, menthyl lactate and menthyl succinate; moderate release PCAs such ase WS-3 and WS-23 fall; and moderately fast release PCAs like TCA [9]. Efforts have been made to control the release of these cooling agents through various types of encapsulation: 1) agglomeration 2) spay drying followed by fluid bed coating, spray chilling and coacervation 3) absorption and 4) extrusion [9].

2.2.3.2.3 Warming agents:

Warming agents provide a heating sensation in the mouth in chewing gum. Some of the warming agents are: polyhydric alcohols, capsicum powder, capsaicin, vanillyl ethyl ether, vanillyl pentyl ether, gingeol etc [23]. These compounds are typically used in very low quantities (0.000001 to 0.001%) since they can cause strong skin irritation as well as excessively high warming effect [23]. Hence, these are typically preferred in combination with a cooling agent.

2.2.4 Process of making chewing gum

Conventionally, chewing gum is made by melting the gum base at a temperature ranging between 70ºC - 120ºC [5]. The molten gum base is mixed with a liquid plasticizer with or without an emulsifier for a targeted amount of time (2 to 8 minutes). About 2/3rd of the sugars along with coloring agents are then added to the mix and stirred for another 1 to 4 minutes [5]. A slow mixing process is subsequently continued with the addition of the remaining sugar ingredients followed by addition of flavoring agents for 1 to 4 minutes [5]. The final step consists of addition of fillers, humectants as well as antioxidants with further mixing for 1 to 4 minutes [5]. The resultant gum mixture is then typically rolled to form thin ribbons which are coated with finely ground sugar powder to enhance the flavor as well as to keep the gum from sticking to the rollers cut into gum sticks [24]. If gum tablets are made, after rolling, the sheets subsequently broken up and spray dried with syrup mixture containing water, sweeteners, and color [24]. The hard coated gum centers are then packed in tablet form.

2.3 Flavor release and perception

During the consumption of food, aroma compounds are initially released into the headspace of the mouth. The compounds are then further transferred to the nose, where the flavors are perceived by the brain via the nasal receptors [25]. Upon consumption of solid foods such as candies and chewing gums, the aroma compounds are released into the saliva before they are released in the headspace or air in the oral cavity. Hence, understanding the physiological process of consuming foods provides a greater insight

into the mechanisms of flavor release in the mouth and to the olfactory receptors which ultimately transmit sensory signals to the brain.

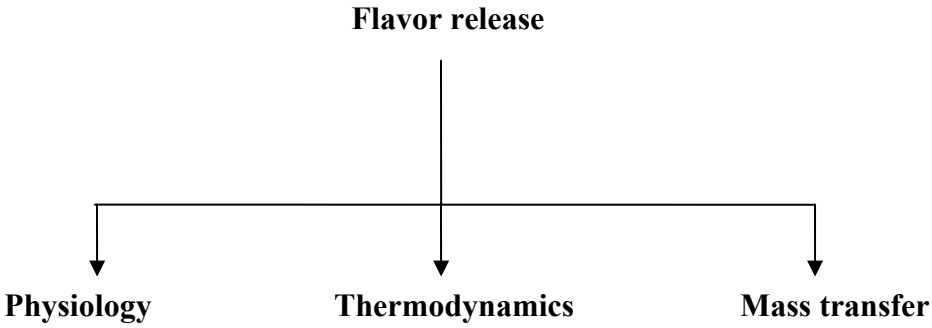


Figure 2-1 Various components that affect the flavor release

2.3.1 Physiological process:

In general, the process of eating involves the mastication of food till it breaks down while it is being mixed with saliva. After saliva hydration, the breakdown product is swallowed. However, the link between how these physiological movements affects the flavor release were not fully understood fully till recent years. Harvey et al. [26] first reported that the act of swallowing is followed by the exhalation of 4 to 15 ml of air. Buettner et al. [27] studied the transfer of ethyl butyrate during the swallowing process using a videofluoroscopic and real-time MRI. This work observed a 3 stage swallowing process during the consumption of liquids. These 3 stages include: 1) the closure between the soft palate and the pharyngeal region upon introduction of food followed by, 2) the propulsion of the liquid into the orthopharynx by the action of the soft palate against the nasal cavity, and 3) the transfer of liquid into the epiglottis which closes the entrance of the trachea till

the food reaches the stomach, followed by reopening of the epiglottis and the velum-pharynx border [27]. During this closure process (third stage), the flavor compounds from the liquid partition into the pharnx area and form a 'plug' of volatiles which is delivered to the nose during the reopening of the velum-pharynx border [28, 29]. These intermittent opening of the epiglottis and the velum-pharynx border in the mouth results to the retronasal transfer of flavor to the nasal receptors during swallowing [28, 29]. However, during mastication a slightly different process is involved where the volatiles transfered from the mouth into the pharynx and the upper waves, exhaled by the lungs into the nasal receptors [28, 29]. This exhalation retronasal route volume during chewing has been estimated to be 72 ml while it is 48 ml during swallowing.

Physiological parameters such as saliva flow rate, bolus weight, mastication, swallowing, and nasal flow may have an impact on flavor delivery to the olfactory receptors (discussed in more below with mass transfer models). Using model mouth systems, the salivary components such as alpha-amylase and mucin were found to interact with some aroma compounds or bind to these compounds, causing changes in flavor release/perception [30-32]. With increasing salivary dilution (flow rate), more flavor compounds were reported to be present in the saliva leading to a lower in-nose aroma concentration [33-35]. However a mathematical model predicted no effect of air flow rate on the initial flavor release rates while at longer times however, the gas flow rates influenced flavor release rates.

Recent studies conducted by Linforth et al. (2005), investigated the influence of bolus weight on flavor release using a wide range of foods including chewing gums, sweet mints, potatoes and chocolates [36]. They observed a linear relationship between

the bolus weight and flavor release in lower sample weight range (0.25-1g). At a higher sample weight range, the relationship was found to have a power law shape (weight: 4-12 g) [36]. These differences have been attributed to the bolus surface area as well as the bolus to in-mouth gas volume. With increasing surface area, the saliva flow rates increases which may lead to lower volatile concentration in the saliva due to saliva dilution and frequent swallowing. At higher bolus weights, bolus to in-mouth volume becomes a limiting factor compared to the bolus surface area [36]. However the presence of fat seems to adhere to the oral activity, thereby changing the effective surface area for the release of hydrophobic compounds [36]. The perceived flavor will also be influenced by the presence of other food components (e.g. carbohydrate, proteins and lipids) and on the food structure itself (liquid, semi-sold or solid).

In general, the various factors affecting flavor release can be divided into two categories: 1) flavor partitioning factors and 2) flavor transport factors.

2.3.2 Thermodynamics:

In thermodynamic terms, partitioning can be defined as the ratio of activities of the compound in two immiscible phases. Since the concentration levels of volatiles in foods are very low, the activities are proportional to concentrations (typically obey ideal law). Hence, at equilibrium (no effective mass transport), the gas-product partition coefficient can be expressed as [37]:

$$\mathbf{K_{gp} = C_g / C_p} \qquad \text{Equation 1}$$

where C_g and C_p are the concentration of the aroma compound in the headspace and product phase, respectively.

2.3.2.1 Partitioning factors

Composition of foods: Presence of salts, sugars as well as alcohols in foods influence the vapor pressure of flavor compounds by means of negative or positive solvation in the aqueous phase, leading to lower or higher levels of flavor partitioning [25].

Flavor binding/Acid-base equilbria: The interaction between flavor components and protein molecules in food affects the flavor release through 2 types of interactions: (1) reversible binding via van der waals interactions and, (2) irreversible binding where chemical reaction takes place via covalent or electrostatic linkages [38]. Irreversible binding leads to reduced flavor availability resulting. Also, factors such as pH of food and degree of protein denaturation may also influence flavor release [25, 38].

Phase partitioning: Most food systems are either oil in water (O/W) or water-in-oil (W/O) emulsions. Common examples of emulsions are milk, mayonnaise, ice cream butter, salad dressings, etc. Partitioning studies conducted by Ruth et al., (2002), showed higher levels of hydrophobic compounds in the lipid phase with increasing oil concentration in O/W emulsions [39, 40]. Similarly, Doyen et al. (2001) showed that the emulsions act as a reservoir for flavor release even at very low concentrations of oil content in water [41]. This reservoir effect is more pronounced for the hydrophobic

compounds which were correlated to the compound's solubility in the lipid phase. The volatile partitioning equation for food emulsions is given as [42]:

$$\frac{1}{K_{ge}} = \frac{\phi_o}{K_{go}} + \frac{\phi_w}{K_{gw}} \qquad \text{Equation 2}$$

whereby K_{ge} is the overall gas-emulsion partition coefficient and ϕ_o, ϕ_w are the volume fraction of lipid phase and aqueous phase in the emulsion and K_{go} and K_{gw} are the individual gas-oil and gas-water partition coefficients respectively. As ϕ_o increases, the reduction in the volatility of hydrophobic volatile molecules are much more significant compared to the volatility of hydrophilic volatile molecules.

Phase effect: Studies by Roberts et al (2003) showed the importance of solid to liquid content on flavor release depending on the type of fat as well as the temperature of the medium [43]. The release of an aroma compounds was found to dependent on the liquid fat content for milk fats below their melting points, while no release differences were found when the same fats were in a liquid state [43]. Similar results suggesting the importance of solid-to-liquid content were shown recently in complex emulsions made with animal or vegetable fat [44]. Also, the chosen flavor compounds were shown to exhibit differences in their release from the emulsions based on the hydrophobicity and chemical class [44].

Flavor adsorption: Starch and cyclodextrins present in food can form molecular inclusions of flavor which might result to flavor losses that can be seen during

partitioning [45]. The higher the amylose content of starch, the greater the capacity to form molecular inclusions through hydrophobic interactions rather than chemical interactions [45]. A recent study conducted by Ghosh et al.(2006) showed the lowering of the partition coefficient between the headspace and product was due to flavor adsorption(hydrophobic compounds) on solid lipid fat in emulsions [46].

2.3.3 Mass transfer

Flavor Transport mechanisms and factors effecting flavor release: When the equilibrium between two phases is disturbed, a concentration gradient will be built between the two phases leading to mass transport from the higher concentration region (higher chemical potential) to the lower concentration region (lower chemical potential)[25].

For steady state diffusion in one dimension, Fick's first law states that the mass flux is directly proportional to the concentration gradient [37].

$$\mathbf{J_i = -D_{ij}\,(dc_i/dx)} \qquad \text{Equation 3}$$

Where J_i is the molar diffusion flux of the migrating molecules (mol cm^{-2} s^{-1}) , D_{ij} is the mutual binary diffusion coefficient and dc_i/dx is the concentration gradient in the x direction.

The above equation can also be written in terms of mass transport coefficient k_i as [25]

$$J_i = k_i (C_i^i - C_i)$$ Equation 4

Where C_i, C_i^i are the flavor compound concentrations in the bulk and interphase (in the x-direction) respectively.

Three mathematical models that have been used extensively to predict the flavor release are [25]:

1) Stagnant film model [47]
2) Penetration theory [35, 48-50]
3) Non-equilibrium partition model [25, 51, 52]

2.3.3.1 Stagnant-film Model:

According to this model, the boundary layers at the interface are considered stagnant and the mass transport coefficient k is equal to D/t (where D is the diffusion coefficient of the flavor compound and t is the thickness of the stagnant layer). As a result, mass transfer is due to molecular diffusion [47].

$$J = (D/t) (C_i^i - C_i)$$ Equation 5

Based on this theory, Hills and Harrison described the release of flavor compounds from solid and semi-solid foods [34, 47, 53]. The transition of flavors from solid food to saliva is assumed to be the rate limiting step. Instead of flavor, dye was used to track the dissolution changes in candy over a period of time (*in vivo* study). The experiments showed a good fit with the stagnant theory [47]. Similarly, this theory has

been applied to gelatin gels along with heat transport [47]. Harder gels with melting points above body temperature showed the diffusion of sucrose from the surface into the adjacent saliva as the rate limiting step (similar to that found in solids). However, when the gel's melting point is below the mouth temperature, heat diffusion plays a vital role [47]. Assuming the stagnant layer theory for solid foods, Harrison et al.(1998) developed a computer simulation based on this model to understand the influence of saliva flow, mastication and swallowing during eating [34]. Based on the simulations, factors such as fracture mechanics (further dependent on food structure/ composition) were found to influence the initial rates while individual variations in mastication, saliva flow rates and swallowing patterns had a greater impact on the release rates at longer times [34].

2.3.3.2 Penetration theory:

This theory is based on convection where the interface boundary layers are not completely stagnant and that mass transport is by eddy diffusion. At a fixed time t, the volume element of the bulk phase comes in contact with the phase boundary, where mass transfer takes place by molecular diffusion [35, 48, 49]. According to this model, the mass transfer coefficient varies with the square root of the diffusion coefficient.

$$J = 2(\sqrt{D/\pi t})\,(C_i^i - C_i) \qquad \text{Equation 6}$$

Harrison and Hills (1997) modeled the dynamic flavor release of liquid emulsions in the mouth using the penetration theory [49]. Partitioning of flavor molecules between oil and water phases is assumed to be extremely fast compared to transport of flavor molecules between the emulsion-air interfaces [49]. Mass transfer coefficient, initial

flavor concentration and gas-emulsion partition coefficient were found to be the major physical factors affecting flavor release in emulsions [49]. Furthermore, the mass transfer coefficient decreases with increasing oil fraction, due to slow release rates in high fat systems. However, the physio-chemical properties of flavor (hydrophobicity as well as volatility) can influence the flavor rate via partitioning term [48, 49]. Using the penetration theory, the influence of saliva flow and gas flow rates during breathing were predicted to have no effect on the initial flavor release rates, however at slightly longer times however, the saliva flow rates significantly influenced the flavor release rates [48].

The penetration theory model was also used to understand flavor release for aqueous solutions at different sucrose concentrations [54]. At lower sucrose concentrations, the factor influencing release rates was the partition coefficient while at higher concentrations, viscosity of solutions significantly influenced release rates by reducing mass transfer coefficient [54]. Penetration theory has been also used to model the flavor release from liquids containing aroma-binding macromolecules such as proteins [35]. The basic assumptions include a first order reversible binding between the flavor molecule and the protein and the rate limiting step for release is the transfer of flavor across the gas-liquid interface [35]. Volatiles with longer chain length were found to strongly bind to the protein, suggesting a greater affinity constant which leads to decreased release rates and lower headspace concentrations [35].

2.3.3.3 Non-equilibrium partition model:

For this model, mass transfer is due to eddy diffusion (convection) and is analogous to solvent extraction in multiple steps. This model describes the flavor release

when phase equilibrium at each step is distributed due to continuous renewal of the headspace [25, 52]. The amount of flavor released as a function of time is given by:

$$M^{t}_{p}/ M^{o}_{p} = 1- \{ (V^{*}_{p}/V_{p})\{P_{pg} + V^{*}_{g}/V^{*}_{p}\} + (1- V^{*}_{p}/V_{p})\}^{n} \quad \text{Equation 7}$$

Whereby Vp is the total product volume, n is the number of extraction steps and superscripts t and 0 refer to time. V*g/V*p reflects the resistance to mass transfer. Similar to the penetration theory, this model also helps explain the effect of viscosity on flavor release rates based on partitioning as well as mass transfer resistance (Ppg and V*g/V*p , respectively) [25, 52]. In the case of chewing gums, the model aids in explaining the release of flavor compounds based on solubility in the first 5 minutes. This is due to the release of flavor from the water soluble phase (sugar or sugar alcohol phase) in the gum [52]. However after 5 minutes, this theory showed a weak relationship between the release of flavor compounds and their gum to water partition coefficient [52]. Based on this observation, it was predicted that the contribution of air to water partitioning coefficient (Paw) plays an important role on flavor release. Also, this theory predicts that the 2nd phase of the chewing process (after 5 minutes) is diffusion controlled with greater emphasis on mastication efficiency [52].

2.4 Flavor perception/measurement:

Flavor perception can be defined as a response where the brain processes various stimuli such as aroma, taste, texture, mouth-feel and temperature, individually or in combination as interactions. This perception process is dependent on factors such as food

matrix, physiochemical properties of aroma compounds, thermodynamic interactions, kinetic interactions as well as physiological factors. Ideally conducting real time analysis of these various stimuli (aroma, texture, taste) and linking this information with sensory and neural imaging, should provide a better understanding of flavor perception. The link between these inputs (stimuli) and perceived outputs (sensory) would be most accurate when measured close to the receptors.

However, flavor measurement of these various stimuli can be challenging. The limited sensitivity as well as selectivity of various instruments and analytical techniques (SPME, solvent extraction) used may not provide the same information that nasal receptors can detect (nose 10^{-17} g of flavor detection for some compounds) [55]. Also, as the number of aroma compounds that can be detected in real time increases, the sensitivity of the instrumentation commonly decreases. In terms of non volatiles in the mouth, developing an analytical technique to detect the concentrations of tastants at threshold levels that the tongue can detect can be challenging. Factors affecting taste stimuli not only include release rates of non-volatiles but also depend on oral factors such as saliva dilution, chewing efficiency and swallowing frequency. Real food systems, with its highly complex matrix (liquid, solid, semi-solid) render the study of flavor release as a major analytical challenge.

2.4.1 Commonly used analytical methods to measure flavor release:

2.4.1.1 Static/Dynamic headspace analysis

Static headspace analysis is a simple analytical method which analyzes the volatiles from the headspace above the sample at equilibrium. This analysis provides basic information regarding volatile partitioning between the food/air at thermodynamic equilibrium conditions [55]. Since this analytical technique represents a steady state system, this method does not represent an eating process which is dynamic in nature.

Dynamic headspace analysis includes purging of volatile compounds from the headspace onto a trap by passing an inert gas (i.e. N_2), at a constant flow rate (10-50 ml/min) and constant temperature (~25C) over the sample [56]. The different materials used as traps include charcoal, silica or Tanex materials. This absorption step is followed by a desorption process where the volatiles are desorbed from the trap with an inert gas (i.e N_2) at high temperatures (~250C) for a certain amount of time (~ 20 min) [56]. These desorbed volatiles are commonly cryofocused before injected onto a GC or GC-MS for analysis.

Dynamic headspace analysis provides better sensitivity to detect volatile compounds compared to the static headspace technique {Schober, 2004 #351. However, the sensitivity is dependent on the type of adsorbent material used. Although this method is measured at non-equilibrium, it has its own drawbacks for measure flavor release. Some of them are: 1) the adsorption capacity of volatiles is dependent on the properties of the trap being used 2) since the volatiles are absorbed on the trap at regular time intervals for flavor release studies; the quantitative data is still an average data point over

the period of time. This implies that the results do not mimic a real time eating process [56].

More realistic understanding of flavor release of foods has been conducted so far in several ways: 1) simple model systems mimicking the mouth coupled with a detector such as FID or MS {Elmore, 1996 #110; Roberts, 1995 #104; van Ruth, 1995 #449}, and 2) through breath analyses where the air exhaled during eating will be analyzed through a MS or GC/MS [57-61].

2.4.1.2 Model mouth systems:

Model mouth systems tend to imitate the basic mechanisms of the mouth during eating by controlling the mouth/nasal cavity parameters such as air flow rate, shear rate, moisture and temperature [56]. Elmore et al., (1996) developed a novel vessel to measure the dynamic flavor release from ethanol-water mixtures [62]. This vessel was water jacketed to maintain mouth temperature (37^{o}C), while a magnetic stirrer with a small amount of water mimics the movement of food along with saliva dilution. The headspace from the top of this vessel was flushed using helium into a GC-MS at a constant flow rate [62]. The authors successfully demonstrated the influence of alcohol content on flavor release properties of various flavor compounds in ethanol-water solutions. However, this model did not provide a real life model of the mouth based on the larger scale up of the vessel [62]. Also, a temperature gradient problem due to condensation near the non-water jacketed portion of the vessel was another noted limitation [62].

Roberts et al. (1995) developed a Retronasal Aroma Simulator (RAS) equipped with purge and trap, controlled gas flow rate, regulated shear mechanism and temperature control [63]. The RAS system was found to be an effective tool to understand the influence of temperature, saliva and shearing parameters on flavor release using a model grape beverage [63]. Using the RAS model system in conjunction with SPME and GC-MS, Deibler et al. (2000), focused on understanding the influence of beverage base parameters such as total acidity, pH, sweetener, aroma and solvent concentrations along with temperature, air flow on volatile flavor release properties [64, 65]. The authors then compared the effluent volatile content (release profile) from the RAS with those from *in vivo* breath analyses and showed that the time-average RAS measurements correlated well with volatile content in the breath by breath measurements obtained through in vivo studies [66]. Although none of the model mouth systems mimic the mouth conditions completely, they provided a better understanding flavor release by allowing the researcher to control the variables involved during eating at a low cost and time [56].

2.4.1.3 Breath/volatile analysis (*in vivo*):

By developing *in vivo* methods that can consistently monitor several volatile compounds from the breath, aroma inputs close to the nasal receptors can be measured. However, analytical challenges such as air as a carrier, air flow rate, and moisture from the breath hindered the development of real time *in vivo* volatile detection method until recently.

Taylor et al. (2000) developed a technique to measure flavor compounds directly from the exhaled breath using an atmospheric pressure chemical ionization-mass

spectrometer (APCI-MS) (Figure 2-2, [60]). This technique basically sampled the exhaled air from the nose into the quadrupole MS for detection [60]. In the APCI process, water from the atmosphere is believed to be ionized to hydroxonium ions $[H_3O]^+$ ions by the corona pin. This hydroxonium ion transfers its charge to the flavor molecules since they possess a higher proton affinity than water (Figure 2-2) [60]. This ionization process is called a soft ionization because the transfer of a proton from hydroxonium ions $[H_3O]^+$ to the flavor compound is relative a low energy process and hence minimizes molecular fragmentation..

Overall, this APCI-MS breath analysis technique provides approximately an 10 ppb level sensitivity as well as good linear range of detection for a wide range of flavor compounds. Consequently, this direct method of measuring volatile release while eating has become the basic tool of current flavor research for studies investigating a link between flavor release and sensory perception [14, 26, 56, 58, 67-69]. Since the development of the breath analysis technique, it has been used on foods which are mainly solid or semisolid in nature. For example, this flavor analysis technique has been applied to foods such as gels, vegetables, dairy products and chewing gums [68-73].

Volatile analysis

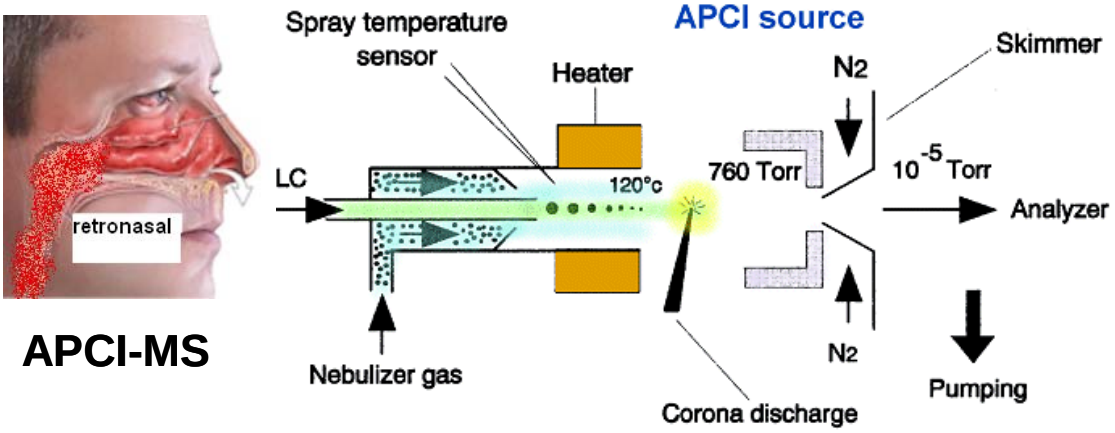


Figure 2-2 Retronasal breath/volatile (*in vivo)* analysis using APCI-MS; [60]

Grab and Geller [74] modified the sampling technique of an APCI-MS breath analysis instrument by using vacuum near the source to collect the sample into ionization chamber; previous method used a venture sampling device [60]. This vacuum sampling technique provided better sensitivity (100 X) compared to the venturi inlet system however, the baseline noise was higher and somewhat compound specific as well as quenching affects near the source were some of the major drawback of this system [75]. Other researchers have tried to optimize the APCI-MS response by adding humidified air into the APCI source chamber in combination with the venture inlet system [76]. This resulted in a better MS response or higher sensitivity by decreasing fragmentation however these studies were performed at very high aroma concentration and where not typical of aroma concentration detected in the breath of humans during eating. Furthermore, the sensitivity as well as the detection limits of APCI-MS was found to be dependent on the cleanliness of the instrument as well as on the laboratory environment. Although the ionization process is an acid/base reaction (transfer of a proton), no

relationship was reported between the sensitivity of flavor compound detection to it gas-phase basicity [75]. Jublot et al. [77] furthermore compared two different mass spectrometers for breath analysis, an atmospheric pressure chemical ionization-ion trap mass spectrometer (APCI-IT-MS) and an APCI-quadrupole mass spectrometer (APCI-MS). Although there were differences in the operating principles with respect to in-vivo studies, the quadrupole MS (in scan mode) was found to be faster with less fragmentation compared to APCI-IT-MS (Automatic gain control mode) [77]. However, for analyses where speed was not critical, APCI-IT-MS was found to be more sensitive. However, the identification of isobaric compounds using a single quadrupole APCI can be difficult [77].

A second type of MS that has been used to study flavor release by breath analysis called a Proton Transfer Reaction Mass Spectrometer (PTR-MS) This technique has been used to monitor the flavor release from various food systems including red bell peppers, coffee roasting and flavor absorption in milk fat [43, 78, 79]. PTR-MS also uses a soft chemical ionization technique in which the flavor molecules react with charged hydroxonium ions (hydroxonium ion) produced in an external ion source [43, 80]. However, in this method pure water vapor is sent into a glow discharge chamber where an abundance of the H_3O^+ ions are formed (with APCI only generated from the moisture in the air). These H_3O^+ ions enter the drift tube through a venturi type inlet where the H_3O^+ is flushed continuously with ambient air [43, 80]. In this drift tube, H_3O^+ provides a proton to the flavor molecules which have higher proton affinity than water.

$$FM + H_3O^+ \longrightarrow FMH^+ + H_2O \qquad \text{Equation 8}$$

The basic advantages of using PTR-MS include [80]: 1) is a rapid measuring technique 2) provides better linear response range 3) a general response for a wide range of organic compounds 4) a rapid detection of unknown compounds. The disadvantages of this technique include: 1) no chemical ionization for compounds with low proton affinity 2) sample losses within the instrument 3) complication in identification and quantification with the presence of too many compounds as well as with fragments with similar mass to charge ratio.

In the past 5 years, breath analysis has been used to investigate flavor release for various foods such as yogurts, chewing gum, hard candy, crackers, emulsions etc [14, 26, 56, 58, 67-69]. However, few studies have used this technique to measure flavor release from liquids/beverages due to short residence time of the beverage in the mouth as well as irregular drinking patterns of panelists which leads to high variation in the results. More recently standard protocols are being developed to overcome these limitation [81], making this analytical technique a versatile method to study the aroma release from any type of food.

2.4.1.4 Non-volatile analysis:

Flavor perception from a food is dependent not only on volatile compounds but also on non-volatile and related interactions. For example, a few studies have shown the importance of non-volatiles in enhancing volatile perception (discussed in more detail in the 'Taste-aroma interactions' section). Consequently a key limitation of many of the recent studies focused on flavor release is that they have only focused on understanding only the temporal changes in volatile release in relation to flavor perception. Few studies

have however looked into temporal changes in non-volatile release over time. Davidson et al [72] studied the influence of sucrose concentration in the salvia on the perception of mint flavor intensity in chewing gum over a period of 5 minutes. They used a swab method to sample saliva from the tongue at a given time period. These swabs are then extracted in a 3 ml methanol: water mixture (50:50) and subsequently analyzed using direct liquid mass spectrometry (APCI-MS) in negative mode. The swab method was also recently used by Pionner et al [69] to study the non volatile release in model cheese systems. They used APCI-MS in combination with high performance liquid chromatography to track the release of amino acids (leucine, phenyl alanine, glutamic acid), acids (citric acid, lactic acid, proponic acid) and minerals (sodium, potassium, calcium, magnesium, and phosphates) [69] during cheese mastication. Although this method can be effective and rapid, the swab sample at a particular location in the oral cavity may not be representative of the overall saliva concentration. An alternate method which is time-consuming but representative of the overall saliva concentration is the chew and spit method [72]. Other studies have used sensors to measure non volatiles (i.e. salt) directly from the saliva sample directly in the oral cavity [82]. Recently, a real-time analytical technique was developed to track the release of sugar alcohols, sweeteners, physiological agents and warming agents directly from the saliva while masticating chewing gum using the chew and spit method [83]. This was achieved by using HPLC in combination with electron spray ionization (ESI-MS) /refractive index detector (RID)/ultra violet detector); the instrumental configuration utilized is illustrated in Figure 2-3 [83]. Overall, non-volatile detection techniques are still being developed and likely

will be dependent on the food matrix, concentration levels of non-volatiles as well as on the rapid/sensitivity limits of the analytical tools being used.

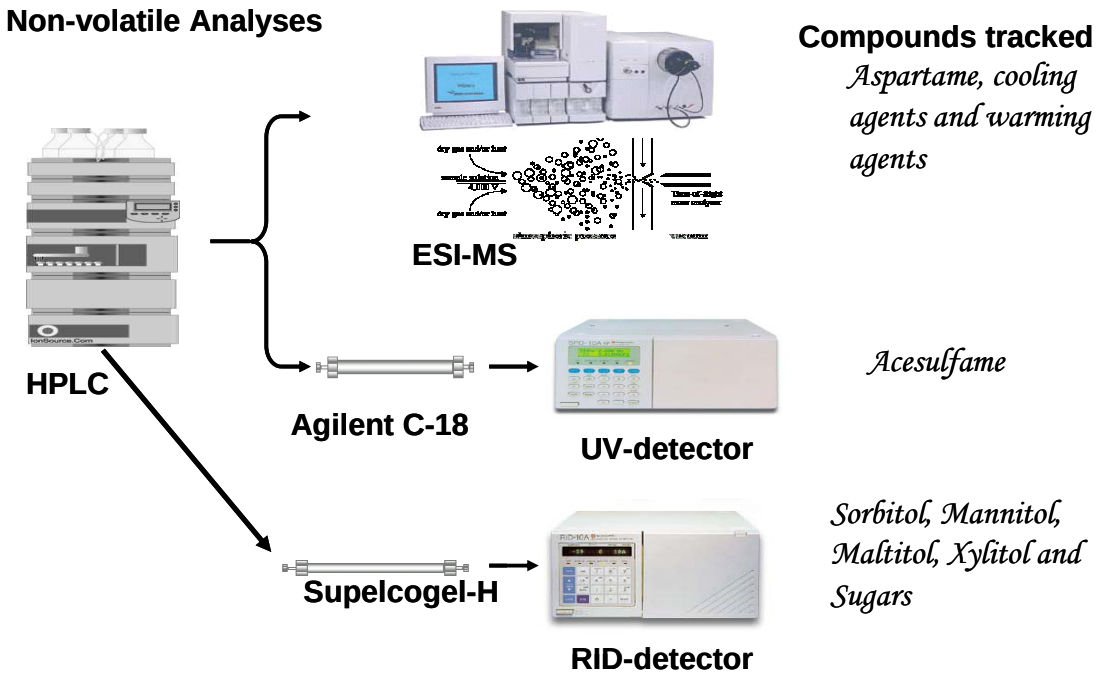


Figure 2-3 Non-volatile analysis by HPLC in combination by ESI-MS/UV/RID [83]

2.4.1.5 Time-Intensity sensory analysis:

Time-intensity sensory analysis (TI) is a temporal perception technique that measures the dynamic flavor and texture attributes in food during eating [84, 85]. From the time food is placed in the mouth, panelists are asked to record the intensity of an attribute until intensity reaches zero or a specified time had been reached. This time interval is dependent on the type of food being consumed [86]. Compared to descriptive analysis which provides an integral value to perception over time, time intensity study provides information on perception from a dynamic stand point [87]. This dynamic

process aids in better understanding flavor perception by considering is a series of steps [88]. This techniques has been used extensively in various kinds of food systems such as beverages, gels, chewing gums and more [72, 87, 89, 90]. Davidson et al. (1999) monitored the temporal release of menthone and sucrose concentration from chewing gum, while the panelists were asked to rate the mint flavor using time-intensity analysis [72]. Panelists perceived mint flavor intensity along the lines of sucrose release rather than menthone release suggesting taste-aroma interactions on overall flavor perception [72]. Guinard et al. (1995, 1997) used this analysis to study the flavor release in model gel systems and chewing gums [89, 90]. Frost (2005), using TI evolutions, showed the dynamic flavor intensity being affected by the fat level and flavor compounds in ice cream [87].

Similarly, dual attribute time –intensity analysis (DATI) is a TI analysis where two attributes are evaluated simultaneously. DATI is a more rapid technique which aids in understanding the interaction between the 2 evaluated attributes [91, 92]. Also, the halo-dumping problems can be reduced using DATI analysis [92]. Duizer et al. (1997) studied the dual attribute TI sensory study of sweetness and peppermint flavor in chewing gum [92]. The authors observed longer duration of peppermint flavor with faster release of sucrose from chewing gums.

2.5 Taste, aroma and texture interactions

2.5.1 Taste-aroma interactions

Flavor perception involves integration of smell and taste sensations as well as their interactions [93]. People tend to confuse taste and smell senses during food consumption which indicates the occurrence taste aroma interactions [93]. A recent review article by Noble [94]explains the various factors that affect taste-aroma interactions, such as the type of simulation, instruction from the judges as well as the tastants and odors used in the experiments [94]. This review focuses mainly on taste-aroma interactions on a physical, cognitive or psychological scale.

Frank et al. [95] observed an increased sweetness intensity of whipped cream in the presence of strawberry flavor. Alternately, no sweetness enhancement was observed in the presence of peanut butter flavor. In addition, the presence of strawberry flavor in a salt solution did not enhance the saltiness signifying that taste-odor interactions are both odor and tastant dependent [95]. Along similar lines, Stevenson et al. (1995), observed enhancement as well as suppression effects when tastants and odors interact in the mouth [96]. For example, a sweet smelling caramel aroma not only increased the sweetness perception of sucrose but also suppressed the sourness of citric acid [96]. With respect to the sweetness response, studies so far suggested that an increase in odor concentration showed an increasing affect on the taste and vice versa [97-99]. This kind of dependency is stronger in the case of taste-odor pairs that are most commonly encountered in foods, namely the harmonious odor-taste combination [95, 100, 101]. However, such

harmonious mixtures were found to have lower overall intensities compared to the added intensities of the unmixed components [97, 98].

Increasing the sucrose content in fruit juices not only reduced harsh notes such as sour, bitter, astringent and puckery but also increased the fruity flavor, though the volatile concentration did not change [102]. On the other hand, increasing the peach extract content while maintaining the same concentration of glucose increased the sweetness intensity. However, the reverse sequence of increasing the glucose content did not affect the fruit flavor perception [103]. This shows that the taste-odor interactions can be a result of a complex perceptual phenomenon or a halo-dumping artifact.

The halo dumping has been defined as "a problem that arises in data collection when there is a carry over from one judgment to the other" [104, 105]. This effect is most commonly found during the evaluation of similar stimuli. A halo dumping effect leads to dumping the sensory score into an available attribute when one of the more relevant attributes were not included in the sensory ballot. Previous studies have shown that halo-dumping effect can result in an increase in sweetness intensity in the presence of odor when the available sensory response was limited to only sweetness attribute [105, 106]. In addition, Clark et al. (1994) showed how the data obtained from time intensity scaling can be biased based on the halo dumping effects [107]. Experiments that included flavor as well as sweetness response options were found to have lower levels of odor-enhanced sweetness compared to the studies with only sweetness scaling [107]. However, providing too many responses showed negative odor effects on sweetness due to inappropriate partitioning of responses. Some of the ways to minimize halo dumping effects are to reduce fuzzy concepts while incorporating specific scales depending on the

subject's requirements. Overall, this provides a well defined conceptual boundary that provides less overlap as well as gradient shifts between responses [108]. In other words, providing a very specific standard definition for odorant attributes may reduce the dumping effects [94]. A second option involves training the panelists as extensively as possible since training lowers occurrence of halo dumping effects [108].

Another major area that affects the outcome of sensory experiments depends on the type of experimental protocols used. As a result, the modes of simulations can be varied. For example, [109] asked panelists to rate the overall intensity of a pair of mixtures consisting of a blank, an odorant and a tastant in various combinations Gillian (1983). Their experiment protocol includes sipping sucrose solutions while sniffing odorants. Meanwhile there are studies that used only the sipping protocol [99, 106] or a sip and spit protocols [103, 110]. While these methods may help to understand the interaction differences in stimuli, these cases may not be compared to normal eating processes. Recently Hort et al. [111] used a controlled flavor delivery system which mimics the normal eating condition to study the taste – aroma interactions with trained and untrained panelists. The authors concluded the presence of perceptual cross model interactions between taste and smell, in both untrained as well as trained sub group of panelists [111]. Based on this information, the authors were not convinced of the need to use trained panelists in cross-model investigations.

To understand if the cross model interaction is a profusion of odors being perceived as a taste attribute, Murphy et al. [97] asked panelists to consume a flavor sweetened sample with plugged and unplugged noses. When the flavor sweetened samples were consumed when wearing a nose plug, the taste intensity values were found

to be lower compared to the non-plugged samples suggesting that olfactory simulation induces taste simulation [97].

Similarly, in the temporal study conducted by Kuo et al. (1993), citral intensity was evaluated with and without nose plugs, in the presence of sugar, citric acid or a combination of both [100]. The unplugged nose data were rated to have a higher odor intensity as well as higher persistence in the presence of sugar, acid and as a combo. However, when the nose was plugged, 10 out of 19 panelists showed higher citral values in the presence of citric acid showing the association of sourness to the lemon odor [100]. Also the same experiment showed that a different odorant such as vanillin only increased sweetness intensity perception when the nose is unplugged [100]. This information illustrates that even after training, more than half the panelists expect a certain mixture combination, in this case sourness to lemon odor which leads to a cognitive association at some level [100]. This association in certain panelists was explained by the individual's prior experience to the type of taste-odor combination.

This taste-aroma interaction phenomena has also been explained by associative learning regarding taste properties of odor [96, 112]. The taste enhancement effect by an odor was been shown to depend on the learnt association between sweetness with some flavors [112]. The authors propose that the panelist's experience of an odor-taste mixture occurs at a minimal conscious level which will be encoded in the memory unanalyzed. This encoded data might activate the memory when the same odor is sniffed at a different time, leading to an overall odor-taste profile instead of just an odor profile [112].

For a long time, the cognitive effects of taste-aroma interactions were thought to occur at the central processing level [94]. Only recently, Dalton et al. [113] used a cross

model summation method with subthreshold concentrations of tastant (saccharin) and odorant (benzaldehyde) to understand taste-aroma interactions. This cross-modal summation, which requires central point intermodal convergence of neurons, suggested a central neural integration of taste and odors resulting in an increased sensitivity to benzaldehyde at subthreshold saccharin concentrations [113]. Conservely, the same study showed no such effects with benzaldehyde and monosodium glutamate, suggesting the need for congruency [113].

Moreover, there is some neurological evidence using Positron Emission Tomography (PET) as well as functional Magnetic Resonance Imaging (fMRI) to show activity differences between taste and odor when presented separately or in combination [114-118]. Neuroimaging should be a promising tool to understand perceptual differences when coupled with in vivo methodologies such as breath analysis and non-volatile HPLC analysis.

2.5.2 Irritant effect on taste-aroma interactions:

Irritant perception is mediated by chemo-sensitive fibers which are different from taste and smell fibers [119]. Irritants were found to have a slower onset than the tastants and aroma compounds [120, 121]. Earlier studies looked at the interaction between irritants and taste perception. Tastants such as citric acid and sucrose were found to suppress the burn intensity caused by capsaicin and piperine [122, 123]. Conversely, sweetness suppression by capsaicin was also observed in foods [124]. In some studies, the mutual pungency effect of salt and capsaicin showed an increase in the burning sensation [124].

Though the total flavor impact of a variety of foods is dependent upon combinations of taste, flavors, and pungency— less attention has been given to understanding the relative role of each component on the overall flavor impact in different food matrices [124]. Oral irritation produced by capsaicin at low concentrations has been shown to enhance the flavor intensity of tomato and apple purees [125]. On the other hand, the highest level of capsaicin suppressed the flavor intensity. Furthermore, mixtures of oral irritants have been shown to have both additive and synergistic interactions, depending on the concentration [126]. Studies using electrophysiological and psychological evidence indicated that odors at lower concentrations than those generally considered to be non-irritating can simulate both olfactory and trigeminal chemoreceptors, which can contribute to the perceived odor intensity [127]. Consequently, irritants can inhibit the perception of odors, while odor compounds can shift the flavor profile by making make food more pungent [128, 129].

2.5.3 Texture effects on flavor release:

In addition to aroma and tastant simuli, the textural properties of foods are also known to influence flavor perception. Beak et al. [70] through time-intensity sensory studies on gelatin gels showed that softer gels reached higher maximum flavor intensity in lower times compared to harder gels suggesting faster release of volatiles. The influence of texture on aroma release and flavor perception in viscous/gel systems (e.g. whey gels, yogurt; model desserts) was obtained by varying the amount/type of texture agents, tastants and aroma compounds. With increased viscosity or gel hardness, the sensory intensity (aroma/taste) was found to be lower [70, 130]. Similar results were

observed with liquid food systems containing higher amounts of hydrocolloids (guar gum, xanthan, sodium alginate) or solid gel systems with proteins or carbohydrates (whey protein, carragean) [130, 131]. This difference for harder gels or more viscous foods was reported to be due to low flavor transfer rates via molecular diffusion from the interior of the food system into the nasal cavity during mastication [45, 132]. However, two recent in vivo studies showed no changes in in-nose aroma profiles with variation in texture agents in food systems such as yogurts and whey gels [58, 67]. In the case of different whey gels with different viscosities, Weel et al. [58] showed no differences in the volatile breath concentration which was monitored by PTR-MS, however it report the textural differences did affect the flavor perception.

Based on the hypothesis of 'first impression', Mestres et al., [133, 134] attempted to explain that the perceived aroma intensity was dictated by the initial release rates of the aroma compounds rather than textural perception. These authors explained the temporal resolution of retronasal aroma perception based on the opening and closing of velum-tongue border, based on the texture of the gels (softer vs. harder gels). The border was found to open intermittently only for softer gels, suggesting no transfer of volatiles from the oral to the nasal cavity for harder gels until swallowed [133, 134].

However, the influence of these three stimuli in combination (aroma, taste, texture) on the overall flavor perception of foods has not been investigated until recently. Leurent et al. [135] investigated the aroma/taste/texture interactions in model desserts by varying the texture agent, sucrose and aroma concentrations. Although sweetness intensity was not affected by aroma concentration, the texture and sucrose release differences were found to have a major impact on the perceived aroma intensity, at higher

aroma concentrations. However, at lower aroma concentrations, texture effects did not make a major impact [136].

2.6 Food Matrix interactions on flavor release:

2.6.1 Proteins

A few studies have focused on understanding the influence of proteins on flavor perception [137-139]. Due to the complexity of real food systems (meats, tofu, milk etc), simplified models systems are frequently used to investigate the various flavor interactions with respect to the protein source.

Generally, the influence of proteins on flavor release and perception are attributed to the complex conformational structure of proteins which leads to chemical and/or physical interactions with flavor compounds. These interactions have been divided into two types: 1) reversible van der waals interactions (physical adsorption), and (2) irreversible chemical covalent or electrostatic interactions (chemical interaction) [25]. Factors which are known to influence protein interactions with flavor compounds include the volatility of the aroma compounds, ratio of protein and volatile compounds, ionic strength of the food, pH, temperature, and processing time length [38] and can be related to changes in shape, surface structure, and other physical characteristics of the protein. Upon denaturation, the surface hydrophobicity increases typically leading to more flavor binding.

Most of the protein-flavor interactions have been focused on the study of milk proteins such as casein and whey protein (β-lactoglobulin) [140-142] as well as soy

protein [143-146]. β-lactoglobulin has been found to interact with many flavor compounds such as hydrocarbons [147], aldehdyes and ketones [146] and ionones [140]. These interactions were reported to be due to hydrophobic interactions between the volatile compounds and the central cavity of the globular protein, leading to lower levels of odor perception in 'headspace' [141]. In the case of soy proteins, based on the temperature history profile, off-flavor components were found to be more tightly bound to heat-denatured soy proteins than to native soy proteins [143]. A few studies have also investigated the interaction of animal proteins with volatile compounds. A recent study by Gianelli et al. (2006) studied the retention of flavor peptides on skeletal and sarcoplasmic peptides such as carnosine, anserine and myoglobin using Solid Phase Micro Extraction (SPME) analysis [148]. Carnosine and myoglobin contained more binding sites which may affect the sensory properties of muscle foods [148]. Also, certain specific peptides may provide taste attributes described by Yamasaki and Maekawa (1978) as the "delicious" peptide or "beefy-meaty" peptide (BMP), or savory taste enhancing peptide (STEP) [149]. On similar lines, Maehasi et al. (1999) isolated various types of peptides which imparted umami taste from soy protein, casein and chicken protein through enzymatic hydrolysis [150].

2.6.2 Carbohydrates:

The influence of carbohydrates on flavor perception can be divided into two main categories: 1) thermodynamic effect (salting out, ligand binding or molecular inclusions) and 2) mass transfer effect (viscosity effects). The type of interactions is dependent on

factors such as type and concentration of ingredients, as well as the physiochemical properties of the flavor compound [38].

In the case of mono- and disaccharides, carbohydrates contributed to increased levels of aroma in the headspace due to salting out effects [45, 151]. However in some cases, presence of carbohydrates may increase the solubility of esters, which in turn leads to lower headspace aroma concentration [152].

Recently, there is much interest in carbohydrate-flavor interactions such as molecular inclusions with respect to starches and cyclodextrins [45]. These inclusions are due to hydrophobic interactions between the flavor molecules and the interior core of the starch molecular structure. Most studies conducted so far suggest amylose-flavor complexes to be more favorable than amylopectin-flavor complexes [153, 154] in sequestering flavor compounds. This is attributed to the greater interaction between amylose with the flavor due to its flexibility or ability to form a helical structure [153]. However, the exact mechanism of this inclusion type is very complex and still little understood.

Carbohydrates along with other food components complicate the release of flavor volatiles. Philippe et al. (2003) showed the influence of carbohydrates on the retention of ethyl butyrate and 2-pentanone in a complex carbohydrate-lipid matrix [152]. For more hydrophobic compounds such as ethyl hexanoate, the partitioning effect into lipid was more pronounced than the interactions with carbohydrate. Similarly, in the case of iron-carbohydrate complexes, the perceived flavor was influenced by the type and amount of carbohydrate as well as the pH of the system [155].

At a critical concentration (c*), hydrocolloids such as starch, gelatin and pectin were found to impart changes in viscosity of a food system, which reduced the overall perceived flavor intensity [45, 156]. Increasing viscosity causes lower flavor transfer due to impeded molecular diffusion from the interior of the food system to the surface. The reduced flavor diffusion rates were reported to influence the amount of flavor which reached the retronasal cavity leading to differences in the flavor profile and perception [45, 132]. However, some studies have shown no significant viscosity effects upon applying eddy diffusion or mastication to the experimental protocol [157, 158]. Other studies reported that softer gels were found to release volatiles faster leading to higher perceived flavor intensity compared to harder gels [70].

2.6.3 Lipids:

The influence of lipids on flavor release in food products has been well documented. Because most flavor compounds are generally more hydrophobic than hydrophilic, the lipid phase typically has a pronounced role in flavor volatility/release. Since most of the food systems containing lipids include emulsions, this section will focus on flavor emulsion literature. Food emulsion consist of oil in water (O/W) or water-in-oil (W/O) systems where the former phase is dispersed in the later. Some of the more common examples of emulsions are milk, mayonnaise, ice cream butter, salad dressings etc.

The various factors which have been reported to influence flavor release from emulsions include the compositional and structural components such as emulsion type, lipid concentration/type, particle size distribution and the emulsifier type/fraction [159]

showed greater flavor release rates from O/W emulsions compared to W/O emulsions for a given emulsifier-type and oil level. This scenario was previously predicted by the flavor release model formulated by McNulty et al. [160] who suggested this was due to lower dilution effects of the continuous oil phase (in W/O emusions) leading to slow flavor release [160], or in other words, mass transfer differences at the interfaces [161].

Partitioning studies conducted by van Ruth et al. [39], showed higher levels of hydrophobic compounds in the lipid phase with increasing oil concentration in O/W emulsions. Similarly, Doyen et al. [41] showed that emulsions can function as a reservoir for flavor release even at very low concentrations of oil in water. This reservoir effect was more pronounced for hydrophobic compounds which would be anticipated.

The lipid composition has also been reported to influence flavor release in emulsions.. For example, the chain length as well as the degree of saturation of the fatty acid composition of the oil can affect the air-oil partition coefficients [162]. Flavor release from a saturated fat (stearin) was found to be slower than in an unsaturated fat (olein) due to differences in melting points of these fats [162]. Roberts et al. [163] and Roudnitzky et al. [164] indicated that flavor release in emulsions was also dependent on solid to liquid content as well as the type of fat and the temperature of the medium. The release of various aroma compounds were found to depend on the liquid fat content of milk fats below their melting points, while no release differences were reported for the various fat-type (milk, palm fat, coconut oil) when in liquid state. Similar results were also reported by Relkin [44] who investigated the importance of solid-to-liquid content in complex emulsions made with animal or vegetable fat on flavor release.. Additionally, the physical-chemical properties of the flavor compound have been shown to exhibit

differences in release in these emulsions based on the hydrophobicity and chemical class [44]. A more detailed study on the role of solid fat versus liquid fat on flavor release was conducted by Ghosh et al. [46], who reported that flavor adsorption (hydrophobic compounds) onto solid lipid fat in emulsions lower the partitioning coefficients between the headspace and product.

Flavor release has been reported to be inversely related to the emulsion particle size. van Ruth et al. [40] indicated that a larger particle size of the dispersant phase has been found to increase the aroma retention regardless of the lipid fraction and the polarity of the flavor compounds. This research group and others have also reported that the air/liquid partitioning values for flavor compounds increased when the tween-20 concentrations increased in O/W emulsions, and generally that hydrophilic compounds were better retained compared to the hydrophobic compounds, indicating the concentration of the emulsifying agent can also influence the flavor properties of an food emulsion [39, 40].

To further investigate the temporal effects of fat on aroma intensity on flavor perception, numerous studies measured the aroma release using an instrument (APCI) in connection with sensory analyses (time intensity studies) in simple food systems and complex flavor mixtures [71, 165-169]. Although all of these studies demonstrated a reduction in flavor intensity in the presence of fat, no conclusions were made with respect to temporal data such as the duration of perceived intensity as well as rate of aroma release. Some studies, however, have showed that perceived flavor duration times ranging from short [167-169] to long [38] in low fat samples compared to high fat samples. Similarly, several studies have observed longer release rates of flavor

compounds in high fat sample [165]while conversely others have shown no differences [89, 168] in release rates. Likely, these discrepancies in the literature may be due to variation of food systems (biscuits, ice creams, milk, yogurts, garlic, pepper etc.), choice of instrumental or sensory methods as well as differences in experimental designs.

A recent study conducted by Miettinen et al. [168] compared the temporal flavor release profile obtained from in vivo study (APCI-MS) with time intensity study with variation in milk fat (0-5%). However, exact parallels could not be drawn between the results obtained from instrumental analysis with the time-intensity study due to challenges such as panelist variation, instrumental sensitivity, as well as taste-aroma interactions which cannot be captured by instruments [168].

2.6.4 Flavor compound-compound interactions

Due to low concentration of flavor compounds in many food systems, the role of flavor compound to compound molecular interactions has traditionally not been considered to influence flavor perception compared to the flavor interactions with macromolecules such as proteins, carbohydrates and lipids. Recently, Schober et al (2004) studied the influence of flavor-flavor interactions on flavor perception using menthol and 1,8 – cineole in hard candy [170]. Using breath analysis (APCI-MS), the release of menthol and 1,8-cineole was found to be rapid and higher when added separately into the candy compared to their addition as a mixture [170]. In addition, the time intensity showed a higher level of cooling sensation for candy containing compounds added singularly [170].

2.7 Flavor aspects of a chewing gum

One of the main criteria during the production of commercial chewing gum is to obtain high levels of flavor burst in the initial chewing stage and also controlled flavor release/perception for a longer period of time [171]. Controlled release is defined as, "a method by which one or more active ingredients are made to release at a desired site and time at a specific rate" [172]. Several patents have been filed for different encapsulation techniques to produce a long lasting flavored chewing gum. These commercial encapsulation techniques range from extrusion, coacervation, cocrystallizaton, spray cooling/ chilling, and cross linking with various chewing gum ingredients [173].

Although most of these encapsulation techniques are used to micro-compartmentalize volatile compounds in chewing gum, no sufficient scientific literature is available to explain how these microenvironments can influence the flavor release profiles for various volatile compounds. De Roos [25, 51] showed the importance of compartmentalization on the release of flavor compounds (varying gum base to water partitioning) by comparing the spray dried flavor addition against the non-encapsulated liquid flavor addition in chewing gum. In the case of gums made with spray dried flavor, the volatile release was higher in the first 5 minutes compared to gums containing liquid flavor addition. These flavor release differences were suggested to be due to reduced interaction between the flavor compounds with the gum base when the gums contained encapsulated flavors.

Chewing gum can be viewed as a two phase system which consists of approximately 75% water soluble phase and 25% water insoluble phase. The release of flavor compounds during chewing is also viewed as a two stage process: first during the

initial dissolution of the water soluble phase (first 10 minutes), followed by extraction of the flavor compounds from the gum base over the next 20 minutes [51]. During the dissolution process (10 minutes), a linear relationship is observed between the amount released and the water-gum base partition coefficient (water solubility, Log P) for the flavor compounds (Figure 2-4). However, after 10 minutes, a different trend is observed (Figure 2-4). The second phase of the chewing process (after 10 minutes) was reported to be diffusion controlled with greater emphasis on mastication efficiency. Based on mathematical models the primary mechanism of flavor release from gum was based on hydrophobicity of the flavor compounds [52, 174]. However, little is known about flavor-matrix interactions (importance of each ingredient) on flavor release.

Chewing gum is also an excellent model system to understand flavor perception as the system allows for volatile and non-volatile components to be delivered from a semisolid food matrix for long time periods of time [57, 72]. Ovejero-Lopez et al. [57] studied the influence of sugar alcohol type (xylitol or sorbitol) and peppermint oil concentration on flavor release using APCI-MS and TI study in a mint-flavored chewing gum system. An increase in the menthol concentration was found to impact the level of flavor being perceived; conversely, the type of sweetener did not seem to have an impact on the perceived mint flavor [57].

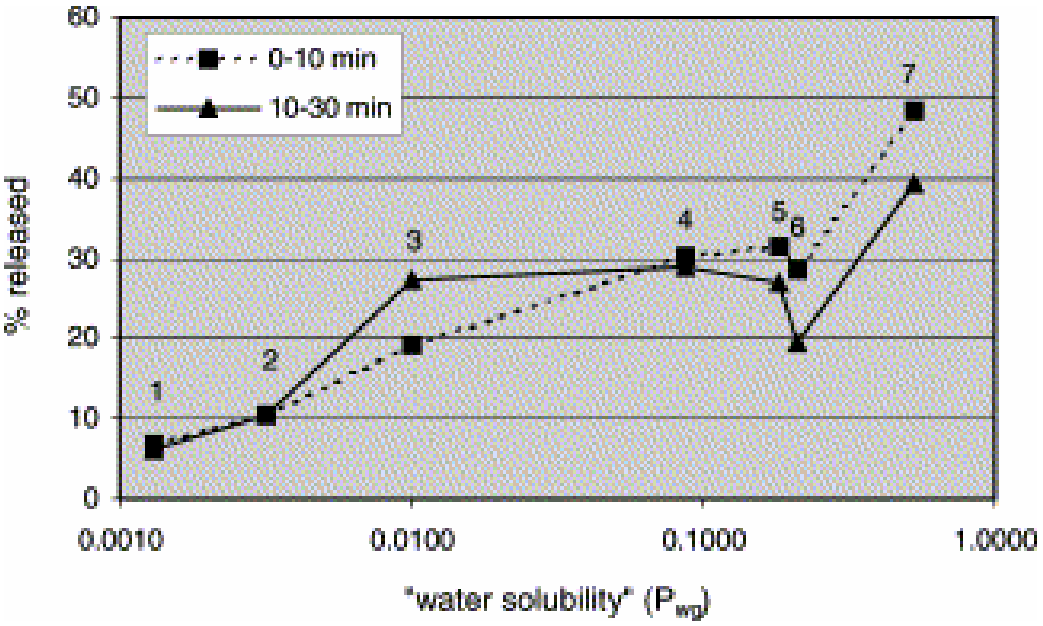


Figure 2-4 Flavor release profile from a sugar stick gum during the fist 10 min compared with that during the next 20 min (average of 10 people). 1) ethyl cinnamate (LogP: 2.99), 2)methyl cinnamate (Log P: 2.62), 3)methyl benzoate (Log P: 2.12), 4) cinnamyl alcohol (Log P: 1.95), 5) ethyl vanillin (Log P: 1.58), 6) 4-(p-hydro-xyphenyl)-butane-2-one (Log P: 1.48) and 7) Vanillin (Log P: 1.58) [51]

A study conducted by Duizer et al. [91] showed that the release rate of sucrose during chewing affected the overall duration of peppermint flavor intensity. Recently, Davidson et al. [72] monitored the temporal stimuli close to the receptors by measuring the in-mouth sucrose and in-nose menthone concentrations over a period release from chewing gum over 5 minute during mastication. Simultaneously, panelists rated overall mint intensity using time intensity analysis (TI). Figure 2-5 shows the temporal release inputs (sucrose and menthone release) along with output signals from the brain (TI data). The perceived mint intensity pattern followed sucrose release instead of menthone release suggesting perceptual interactions between taste (sucrose) and aroma (menthone) [72].

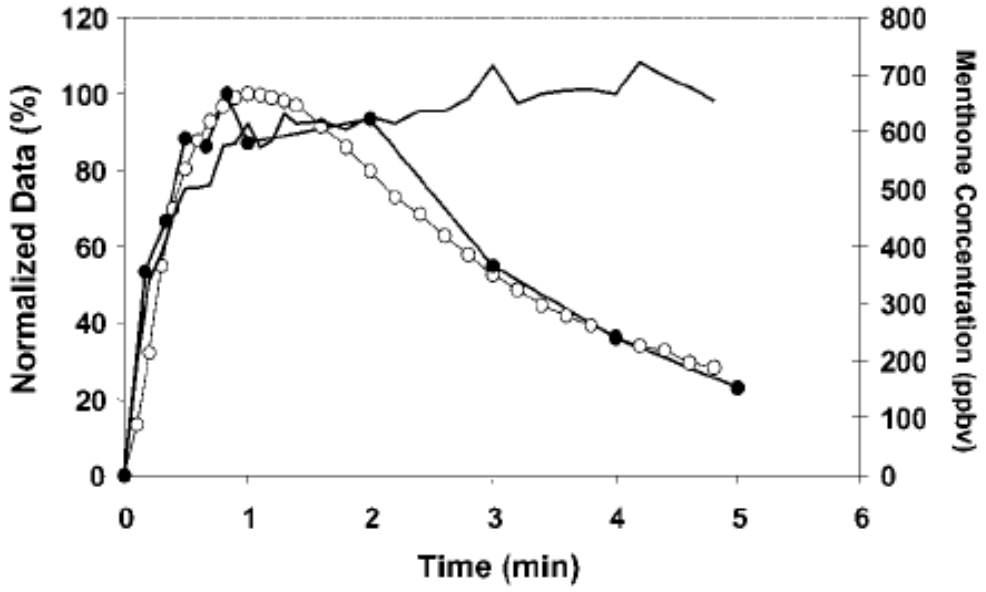


Figure 2-5 Release of Sucrose release (·), menthone (-), and perceived intensity of overall mint flavor (TI curve) (○), from a stick type commercial chewing gum [72].

In addition to the reported influence taste-aroma interactions on gum flavor, the influence of texture (hardness or softness of food) as mentioned previously can also influence flavor release and perception. The influence of texture on flavor release/perception have been typically studied in food systems such as yogurts and gels. However, de Roos [51] did investigate the influence of gum texture on flavor release from flavored gum bases [51]. Textural differences in gum bases were obtained by varying gum base composition or by adding a plasticizer (glycerine monostreate) [51]. In both cases, softer gum bases were found to release the flavor compounds at a faster rate than the harder gum bases. However, the authors could not conclude if the textural effects on flavor released were based on a non-equilibrium partition model [51]. For chewing gum, factors that may affect texture properties include composition, gum base type, type of softeners, manufacturing conditions as well as flavor carrying solvent type.

Flavor solvents in chewing gum in addition to functioning as plasticizing agents (modify the texture), as the name implies are also used as dispersing agents to facilitate flavor incorporation into food systems. Reineccius et al. (2005) investigated the influence of the flavor carrier solvent type (propylene glycol (PG), triacetin and triethyl citrate) on flavor release in cyclodextrin/flavor inclusions [175]. Based on the structural properties and polarity, these different solvents were found to compete with the aroma compounds, leading to partitioning differences [175].

Based these observations, it was hypothesized that due to the different physical-chemical properties of common flavor solvents (propylene glycol, medium chained triglycerides, or triacetin) used to manufacture chewing gum, each of these ingredients may uniquely impact either the aroma, taste or textural properties and therefore the flavor of chewing gum. Furthermore, these finding would also provide incites into the importance of aroma-taste-texture interactions on chewing gum flavor which is currently poorly understood. Overall, this research project is focused on investigating mechanisms of flavor release and perception in a sugar-free chewing gum system.

Chapter 3

Hypothesis I

The physical-chemical properties of common flavor solvents (propylene glycol, medium chained triglycerides, or triacetin) uniquely modify the flavor properties of chewing gum.

Objectives:

1) Analytically characterized the aroma, sweetener and textural profiles during the consumption of chewing gum made with different flavor solvents
2) Correlate the analytically data from objective 1 with time-intensity (TI) sensory analyses of the perceived aroma, sweetness and textural properties of chewing gums made with different flavor solvents

Chapter 4

Influence of Flavor Solvent on the Mechanisms of Flavor Release in Sugar-Free Chewing Gum

4.1 Abstract

The objective of this study was to investigate the influence of three flavor solvents triacetin (TA), propylene glycol (PG), medium chained triglycerides (MCT) on the mechanisms of flavor release in a sugar-free chewing gum system. Model chewing gums were made with either 0.67%-TA, PG, MCT or without these ingredients. The aroma release (cinnamaldehyde, carvone), sorbitol release, and textual properties of the chewing gum model systems were analytically characterized over a 12-min chewing period. Additionally, time-intensity sensory studies (TI) on the aroma (cinnamon-like), sorbitol (sweetness) and textural properties (effort to chew) were also conducted using a trained sensory panel. The analytical characterization studies indicated that the flavor solvent-type did not influence the aroma release rates however; the chewing gum samples formulated with TA reported a statistically lower sorbitol release rate (maximum concentration at 50 seconds). The textural analyses of TA or MCT were also softer (based on total work) than the PG or no flavor solvent chewing gum samples. Sensory analysis furthermore indicated that chewing gums formulated with TA reported a statistically higher perceived intensity of both the cinnamon aroma and sweetness levels. In summary, the choice of the flavor solvent-type influenced the sugar alcohol release

rate which was correlated to the perceived cinnamon aroma intensity and was not related to the textural properties of the chewing gum samples.

4.2 Introduction

The flavor properties of chewing gum are undoubtedly a key product attribute for consumption. Consequently, defining mechanisms which influence the flavor properties of chewing gum is for important for understanding its product quality.

The perception of flavor in confections or food products is a complex function consisting of multiple stimuli that can be better understood by combining analytical methods that can monitor the release profiles of key flavor stimuli in vivo in connection with sensory panel analysis. Previous studies on the flavor perception of chewing gum, for example, have correlated mint flavor intensity to the sucrose concentration. Davidson et al. (1999) monitored the temporal release of menthone and sucrose concentration from chewing gum, while the panelists recorded the mint flavor intensity over time [72]. Overall, the panelists perceived a decrease in mint flavor intensity over time which was correlated to the decrease in sucrose concentration rather than menthone release suggesting taste-aroma interactions where important for the overall mint flavor perception. On similar lines, Duizer et al. (1996), conducted a dual attribute time intensity study on chewing gums and found longer duration of peppermint flavor with faster release of sucrose from chewing gums [91] .

A few studies have also reported a correlation between flavor perception and textural properties of solid food systems (e.g. gels, yogurt; model dessert). Beak et al.,

1999 conducted a time-intensity sensory study on gelatin gels and reported a higher maximum flavor intensity and a lower time to maximum flavor intensity for softer gels compared to harder gels which they suggested was due to a faster release of volatiles from a softer gel [70]. In contrast, a study conducted by Weel et al. (2002) on whey protein gels, using breath analysis reported no differences in the volatile concentration, while the textural differences affected the flavor perception [58]. Mestres et al., (2005, 2006) attempted to explain these contradictory results based on the hypothesis of 'first impression', where the perceived aroma intensity is dictated by the initial release rates rather than textural interactions [133, 134]. Mestres et al (2005) further suggested that the temporal resolution of retronasal aroma perception was based on the opening and closing of velum-tongue border (passage way to the olfactory membrane in the oral cavity), which was influenced by the texture of the gels (softer vs. harder gels) [133]. They reported that the velum-tongue border was found to open intermittently for softer gels but not for hard gels. This suggested that there would be no transfer of volatiles from the oral to the nasal cavity for harder gels until they were swallowed.

More recently Leurent et al., (2005) investigated the influence of these three stimuli in combination (aroma, taste, texture) on the overall flavor perception of a model dessert by varying the texture agent, sucrose and aroma concentrations [135]. Although the sweetness intensity was reported not to be affected by aroma concentration, both the textural properties and sucrose release rates were found to impact the perceived aroma intensity, at higher aroma concentrations. However at lower aroma concentrations, there were no texture affects found [136].

In industry it has been reported that the flavor release/perception of chewing gum is dependent on the type of flavor solvent utilized due to difference in the texture. For example chewing gum formulated with triacetin (TA) or medium chained triglycerides (MCT) are softer than if formulated with propylene glycol (PG), and therefore would have a higher flavor intense [51, 176]. The texture of chewing gum is dependent on various factors such as chewing gum composition (gum base-type, type of softeners, flavor solvent-type) as well as the processing conditions.

Reineccius et al. (2005) investigated the influence of flavor carrier solvent (propylene glycol (PG), triacetin and triethyl citrate) interactions on cyclodextrin/flavor inclusions [175]. While PG had no effect, triacetin and triethyl citrate were found to compete with the aroma compounds within the inclusion of cyclodextrin, based on headspace analysis [175]. The authors tried to explain these differences based on polarity and molecular structural parameters of the solvent-type. A recent study conducted by Schober et al. (2004) showed the influence of flavor solvent on release kinetics of L-menthol in a hard candy system monitored by breath analysis [177]. Though perceptual differences were not observed, L-menthol was found to release faster in PG or Miglyol compared to 1,8-cineole [177].

No studies currently have investigated the influence of flavor solvents on the release of both volatile (aroma) and non-volatile (sugar-alcohol) in correlation to textural effects and how these three flavor stimuli relate to the mechanisms of flavor perception in chewing gum. Consequently, the overall objective of the study was to investigate the influence of three flavor solvents on the aroma-taste-textural properties (and related cross modalities) in a sugar-free chewing gum model. The release kinetics of both the aroma

and sugar alcohol flavor compounds and the textural properties were analytically monitored and compared to the perceived aroma, taste and textural properties by a trained sensory panel.

4.3 Materials and Methods

4.3.1 Materials.

Cinnamaldehyde, L-carvone, and jasmone were purchased from Aldrich (Sigma Aldrich, Milwaukee WI). Piperitone was from the Penta Manufacturing (Livingston, NJ). Methanol was from Fisher Scientific (Fairlawn, NJ). Hexane and formic acid were from EMD Chemicals (Gibbstown, NJ). N-Caproic acid methyl ester was purchased from TCI America (Portland, OR). VH1 gum base was obtained from Hershey Foods (Hersheys, PA). Sorbitol was from SPI polyols (Wilmington, DE). Glycerine was from Univar (Bedford park, IL). Concentrated hydrogenated glucose syrup was from Roquette Americas (Keokuk, IA). Lecithin was from Solae (St. Louis, MO). Titanium dioxide was from Sensient (St. Louis, MO). Flavor solvents such as propylene glycol and triacetin were from Givaudan flavors (Cincinnati, OH), while medium chain triglycerides (Neobee-80) was from Stepan company (Northfield, IL)

4.3.2 Chewing gum models.

The chewing gum model composition consists of a variety of ingredients was shown in Table 4-1 (gum base composition shown in Table 4-2). Table 4-3 shows the model

cinnamon mixture consisting of various flavor compounds. Chewing gums were made by melting the gum base in a twin screw rotating blade mixer via conduction using steam (98°C -104°C). The molten gum base was mixed with lecithin and titanium oxide using the blades mounted on the mixer till the mixture becomes homogenous. At this stage, the steam is shut off and hydrogenated glucose syrup of known weight was added to the mixer, while cooling the mixture via circulation of cold water (24°C). Approximately, 1/2 of the sorbitol were added to the mix and mixed well for another 2 min. The slow mixing process was further continued by adding the cinnamon mixture (with or without flavor solvent) and the remaining sorbitol for 2 min. Finally, the glycerin then the sorbitol syrup were added and further mixed for 1 min per each ingredient. The resultant chewing gum dough was rolled using a dough roller while spraying the dough surface with mannitol to keep the gum from sticking to the rollers. The average sheet thickness after sheeting was 0.66" (± 0.02). This step is followed a conditioning step where the sheets stored at room temperature at 45 % humidity for at least 12 hours. These sheets were further cut into small commercial size sticks and packed in cartoon boxes. The cartoon boxes are further wrapped in aluminum foil and stored at 21°C at 35 % (± 10) relative humidity for 4 months before any analyses was conducted.

4.3.3 Quantification of aroma compounds.

Twelve randomly selected gum pieces per the chewing gum treatment were further sub-sampled to a 0.5 ± 0.02 grams sample and dissolved in 1ml of hexane on a vortex shaker (Vortex Genie 2, Model CG-560, NY). The hexane mixtures were then centrifuged at 11,750 rcf for 4 min (Brinkman Instruments Inc., Model no: 5415C, NY)

and 0.3ml of the supernatant was collected and added to 1 ml of methanol. The hexane-methanol extracts were then centrifuged at 11,750 rcf for 4 min and 1 ml of supernatant was collected. This step aided in the precipitation of the gum base polymers. 100µl of methanol containing methyl hexanoate (as internal standard; 2500 mg/L) was then added to the hexane-methanol supernatant, and subsequently analyzed by gas chromatography/flame ionization detector (GC-FID).

4.3.4 Gas Chromatography (GC).

Analyses was performed using a Hewlett-Packard 5890 Series II GC equipped with a split/splitless injector, flame ionization detector (FID), autosampler (HP 7673) and a fused-silica capillary column (DB-wax, 30 m, 0.32 mm i.d., 0.32 µm film thickness, Agilent Technologies, CA). The GC operating conditions were as follows: 1 µL 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 230°C and held for 3 min; constant flow rate of 1.0 mL/min (He).

4.3.5 Analysis of sorbitol/maltitol release.

The concentration of sorbitol and maltitol were determined from expectorated salvia of three panelists while chewing a 2.5g piece of chewing gum sample over a 12 min time period. The panelist were trained to follow a defined chewing and swallowing/expectorate saliva (C/S) protocol; chew at 60 chews/min (used a metronome) and to swallow or expectorate saliva into 20ml cups with lids at 0, 10, 30,

50, 70, 120, 240, 420, 660 seconds.. 0.5 g of saliva was immediately transferred into a 1.5ml centrifuge tube and mixed with 1 ml solution of nanopure water containing 0.1 % formic acid. The samples were then centrifuged at 12000 rpm for 3 min and the supernatant was transferred into 2 ml amber bottles prior to HPLC analyses. Each chewing gum sample was analyzed in triplicate. The sugar alcohol concentration was determined using an external standard curve at 0.006, 0.013, 0.025, 0.038, 0.05 g/L for sorbitol and 0.016, 0.031, 0.063, 0.125, 0.250, 0.5, 1 mg/L for maltitol plotted versus peak area ($r^2 > 0.99$).

4.3.6 High Performance Liquid Chromatography (HPLC).

Analyses was performed using a Shimadzu HPLC system consisting of pump (LC-10ATvp), degasser (DGU-14A), an auto sampler (SIL-10Ai) and Shimadzu column heater (CTO-10ACvp) was connected to a Refractive index detector (RID; RID-10A). Separations were performed on a LC column Supelcogel-H (5 µm, 250 x 2 mm i.d.,) using an isocratic run with 0.1% formic acid in water as the mobile phase maintained at 40°C. The flow rate was 0.17 mL min-1 and the injection volume 20 µL.

4.3.7 Aroma compound release analysis (*In Vivo)*.

Breath-by-breath analysis was performed with a atmospheric pressure chemical ionization-mass spectrometer (APCI-MS) as previously described by Schober and Peterson (2004). Three panelists (1 male and 2 female) were asked to chew the chewing gum samples as described in the 'Analysis of Sorbitol Release In Vivo' section. The

experimental design includes: 3 panelists * 4 treatments * 3 replicates. The whole experiment was conducted over a 3 day period, with 4 gums per panelist per day, in random order. To minimize carry over effects and fatigue, each panelist rinsed with water waited at least 20 min between sample analyses.

The breath from the nose was directly and continuously sampled via an interface set at 65ºC into the Quattro II/Micromass mass spectrometer (Waters, Milford MA) modified for breath analysis at 0-4 min, 6-8 min, 10-12 min time intervals. The APcI operating conditions are as follows: SIM mode; sampling rate was 200 ml/min; block temperature is 110°C; transfer line 65°C; corona discharge was 3.5 kV, cone voltage was 15V. Ions monitored were 133 [M + H]+ for cinnamaldehdye, 151 [M + H]+ for carvone, 153 for piperitone and 165 [M + H]+ for jasmone. Day to day variation in instrumental signal/noise ratio was corrected for base on the peak heights obtained by injecting known amount of L-carvone (1µl of 1000 mg/L solution in pentane) before the start of the breath analyses each day) into a airtight water-jacketed 1.1-L deactivated glass vessel. This vessel was maintained at 40 °C and held for 5 min with constant stirring (200 rpm) prior to interfacing directly to the breath analysis instrument using the same operating conditions at described above. Quantification of aroma compounds from the breath was determined via standard calibration curve methodology. Different concentrations of each compound (0.009, 0.018, 0.089, 0.179, 0.536, 0.837, 1.768 µg) were injected into the deactivated glass vessel as described above. The peak height (ion intensity) versus µg weight of each compound per liter air was plotted (all compounds reported an $r2 > 0.99$).

4.3.8 Instrumental texture analysis.

Three panelists (1 male and 2 female) were asked to chew the chewing gum samples as described in the 'Analysis of Sorbitol Release In Vivo' section. At the time intervals of 30, 60, 120, 240, 420, 720 sec the panelists are asked to expectorate the chewing gum sample for texture measurements. A new piece of chewing gum was used for each analysis. After each sample, panelists rinsed their mouth with water to clear the palate, and at least 20 min breaks were taken between samples to minimize the effects of fatigue. Five samples were evaluated for each treatment.

The texture of the chewing gum samples was measured in a 3.5 ml cap (1.5 cm diameter and 1.5 cm length) using a TA-XT2 Texture Analyzer (Godalming, Surrey, UK) equipped with a cylindrical stainless steel probe (1 cm diameter and 5 cm length). The probe penetrated the first 2 mm of the product at 2 mm s-1 and the total work done was recorded from the area of the force-distance curve for chewing gum chewed at a give interval.

4.3.9 Gum volume analysis.

Three panelists (1 male and 2 female) were asked to chew the chewing gum samples as previously described in the 'Analysis of Sorbitol Release In Vivo' section. The panelists were asked at the given time intervals of 30, 60, 120, 240, 420, 720 seconds to expectorate the chewing gum sample for volume measurements. The samples were initially placed onto a Kimwipe® absorbent napkin (Kimtech, Ontario, Canada), to remove any additional saliva and then placed into a 10ml volumetric cylinder containing

10 ml of water. The volume displaced by the addition of chewing gum was then measured using a magnifying glass. Each sample was measured in triplicate.

4.3.10 Plasticity index analysis.

This analysis was conducted on gum bases made with either TA, PG, MCT or without these ingredients. Molten Gum bases containing 2.7 % of solvent was poured into a aluminum weight pan (7.5 cm diameter and 1.5 cm depth) and cooled for 24 hours, before the plasticity analysis was conducted. An indentation experiment was conducted with an Instron 4444 universal testing machine (Instron Corp., MA) using a spherical probe (dia: 12.60 mm). Probe was punched into the gum bases (1.5 cm thickness) made with different flavor solvents to a distance of 1.016 mm at a speed of 2.54 mm/min. The plasticity index was calculated based on the equation listed below. Theoretical chord value (TCV) for a given depth and diameter of probe was obtained from trigonometric calculations on a sphere. After 24 hrs of indentation, an experimental chord value (ECV) was measured using Vernier calipers. Plasticity index was calculated based on the ratio of ECV to TCV values for each sample.

$$\text{Plasticity Index (PI)} = \frac{\text{Experimental chord value after 24 hours (mm)}}{\text{Theoretical chord value (mm)}}$$

Equation 9

4.3.11 Sensory analyses.

Time-intensity sensory analysis was conducted with a trained panel of 9 people (3 males and 6 females; age range: 21-35 years) recruited from the Department of Food

Science, The Pennsylvania State University. Training and practice sessions were conducted to familiarize the panelists with the various sensory attributes of the chewing gum samples such as aroma (cinnamon-like intensity), sweetness and texture (effort to chew); defined descriptors, procedures, standards references and scales. The time-intensity practice and evaluation sessions were conducted using the Compusense program (Compusense five, Guelph, Canada). During these practice and evaluation sessions, panelists were asked to rate one sensory attribute at a time while chewing the gum (2.5 g) for 4 min. For the aroma and textural attributes, the panelists were instructed to breathe normally through their nose with their mouths closed. For the sweetness attribute, the panelists evaluated the samples wearing a nose-clip and breathing regularly through the mouth. For each attribute, a 15 point scale was used for intensity measurement, with 0 representing zero intensity while 15 being the maximum intensity imaginable. Panelists used references for sweet (sucrose solutions 2, 4, 6, 9, 12% w/w corresponding to 2, 4, 6, 9 and 12 reference value), aroma (solutions of cinnamon mixture with 4 compounds as in table 3; 25, 50, 100 µg/L of water corresponding to 3, 6, and 9 reference value) and texture (Jet-Puffed® marshmallows, Gaint® orange slices, Swedish fish® candy, Tootsie Roll® chocolate corresponding to 1, 4, 7 and 9 reference values respectively).

For the final evaluations, a 2.5 g of chewing gum was cut, wrapped in a wax paper and placed small Ziploc bags coded by a three-digit number 1 hour before each session. The evaluations were conducted over 12 sessions, for 36 samples (4 treatments x 3 attributes x 3 replicates), by the panelists in random order. Each day consisted of 2 sessions (with at least 3 hours between same-day sessions). During each session,

panelists evaluated the 3 attributes for a given treatment with 2 min interval between each attributes.

4.3.12 Statistical analysis.

4.3.12.1 Instrumental data

The ANOVA general linear model (GLM) was used to analyze the instrumental data measured mastication of chewing gum made with different flavor solvents (TA, MCT, PG, none) using SAS statistical software Version 8.0 (SAS Institute, Cary, NC). A random block design with panelists and treatments as blocks was used for each instrumental attribute. For the breath analysis, GLM analysis was conducted on a 6s average concentration at 30, 70, and 150 sec per each replicate in a given treatment. For sorbitol/glycerine release, texture analysis, gum volume analysis and PI values, GLM analysis was conducted on data set at times 30, 70 and 120 sec.

4.3.12.2 Sensory data

The ANOVA general linear model (GLM) was used to analyze the sensory analysis data with panelist, treatment, replicate and the interactions, panelist*treatment, panelist*replicate included into the model. If significant differences were found among the treatments, Tukey's pairwise comparison was used statistically compare the means at the 0.05 significance level. All statistical analyses were performed with SAS statistical software Version 8.0 (SAS Institute, Cary, NC). For attributes such as sweetness and

cinnamon like intensity, GLM analysis was conducted on maximum intensity (I_{max}), time-at-maximum intensity (T_{max}), Area under the curve, incline angle and decline angle values obtained from Compusense software for all the panelists. In case of effort to chew attribute, statistical analysis was conducted on minimum softness score (I_{min}) and time to reach minimum softness (T_{min}) values were measured from each individual time-intensity curve of the panelists.

4.4 Results and discussion

Losses of the aroma compounds may be expected during manufacturing and storage of chewing gum. Consequently, the concentration of the four aroma compounds in each chewing gum treatment (3 flavor solvents or none) were determined and are reported in Table 4-4. During the course of analytical and sensory studies, no significant differences in the aroma compounds concentration were observed between any of the treatments of the chewing gum samples.

The volatile release profiles of cinnamaldehyde and carvone monitored from the exhaled breath directly from the nose over a period of 12 min during consumption from the chewing gum samples made with the three different flavor solvents (PG, MCT and TA) and without a flavor solvent are reported in Figure 4-1 and Figure 4-2 for the individual 3 panelists. Although the 3 panelists differed in their individual breath volatile concentrations for these two compounds, similar release patterns were observed for these chewing gum samples made with different chewing gum samples. The same trends were also reported for piperitone and jasmone (breath analysis data not shown) for all 3 panelists. Statistical analyses were also conducted at three time points (30, 70 and 150

seconds) on the aroma concentration in the breath using a panelist block design and indicated no statistical differences ($\alpha = 0.05$) were reported for all four aroma compounds for each panelists. This indicated that these flavor solvents had no apparent affect on the aroma release of these four aroma compounds (log P ranged from 1.90 to 3.55, Table 4-3) in these chewing gum samples.

The release profile for sorbitol from the chewing gum samples over a 12 minute consumption time period as influenced by the three flavor solvents are furthermore reported in Figure 4-3. Overall the triacetin-containing chewing gum was reported to have lower initial sorbitol release rate in the first 50 seconds (on average) compared to the other solvent-type gums for all 3 panelists. Analogous release patterns were observed with hydrogenated glucose syrup (HGS) for all 3 panelists (data not shown). Statistical analysis conducted at 30 sec, 70 sec and 120 sec, showed significant differences in sorbitol concentration for triacetin gums at 30 sec and 70 sec (Table 4-6) but not at 120 sec for all the 3 panelists (data not shown). Similar statistical results were obtained for HGS. Considering that the sugar alcohol phase of these samples made up approximately 65% of the total sample mass, the TA containing samples should also have a higher volume during this time period which was consistent with volume measurements reported in Figure 4-4.

The influence of the flavor solvent-type on the textural profile the chewing gum samples consumed over 12 minutes is illustrated in Figure 4-5. Overall, chewing gums made with TA or MCT were found to be statistically different (were softer for most of time points for all 3 panelists; softness estimated based on 'total work done') than the chewing gum samples made without a solvent or with PG (Figure 4-5).

These analytical findings indicated the type of flavor solvent used for the manufacture of these chewing gum samples did influence the textural properties, however did not influence the aroma release, but did alter the release rate of the sugar alcohol. Furthermore, a softer textured chewing gum did not however necessarily result in faster release of the sugar alcohol rates (e.g. MCT formulated chewing gum). This suggested that the textural properties were not a good indicator of the flavor release potential of chewing gum. Based on the similar textural (softness) properties of TA and MCT chewing gum samples (Figure 4-5), yet the unique influence on the sugar alcohol release rate, it was hypothesized that TA was primarily plasticizing (softening) the gum base polymeric continuous phase (polyvinyl acetate, etc.) while MCT was mainly softening the lipid or discontinuous phase; based on the solubility properties of these two solvents. We suspected that a more plasticized continuous phase (softer) would be anticipated to entrap the discontinuous phase (sugar alcohol) more efficiently during consumption and therefore resulted in the delayed release the sorbitol phase as reported in Figure 4-3.

To support the hypothesis that TA primarily softened the continuous polymeric gum base phase, while MCT primarily softened the discontinuous lipid phase, gum base (as reported in Table 4-2) was mixed with an equivalent load of flavor solvent (in comparison to the chewing gum composition - Table 4-1) and the plasticity index was analytical measured and was reported in Table 4-5. The lower the plasticity index (min = 0) means the more elastic the gum base was while a higher the value (max = 1) means the gum base was more plastic. A flavor solvent which plasticized the continuous phase would be expected to have a more elastic behavior (flow after deformation) then a flavor solvent which plasticized the discontinuous phase. Based on the reported plasticity index

values for the three flavor solvents, our hypothesis was supported as the TA containing gum base was the most elastic while MCT was the least elastic in texture or was statistically equivalent to a gum base with no solvent and indicated different plasticization mechanisms for different solvent types in these chewing gum systems.

To investigate the role of the instrumental findings in this study on flavor perception, a time-intensity sensory study was conducted on sweetness, cinnamon-like flavor and 'effort to chew' attributes on different solvent-type formulated chewing gum models (shown in Figure 4-6). Notably both the maximum cinnamon-like flavor and sweetness intensity (I_{max} and T_{max}) were found to be significantly lower for the triacetin containing chewing gum (Table 4-7) in comparison to the other chewing gum samples which was agreement with the slower sorbitol release rates reported from the instrumental data (Figure 4-3). Previous studies on the flavor perception of chewing gum have correlated mint flavor intensity to the sucrose concentration, suggesting taste-aroma interactions [72] and were further supported by our analytical and sensory analysis.

The influence of flavor solvent-type on the perceived chewing gum textural properties (effort to chew, Figure 4-6) was in agreement with the analytical textural measurements (Figure 4-5). Overall, triacetin and MCT gums were found to be softer or required less effort to chew versus PG or no-solvent containing chewing gums (I_{min} and T_{min} from Table 4-7). Although the analytical textural measurements reported that the chewing gum samples made with triacetin and MCT were comparable in softness (Figure 4-5), the sensory data showed the rate at which the softness was perceived in the first 2 min were statistically different ("effort to chew" in Figure 4-6). Gums with MCT were perceived the softest around 40 sec while for the gums with triacetin around 70 sec again

suggesting different plasticizing affects of these flavor solvents on the gum base (Table 4-7).

The data in this study indicated that flavor solvent can influence the flavor properties of chewing gum and was dependent on the plasticizing/softening mechanism of the solvent utilized. Flavor solvents which softened the continuous polymeric phase of chewing gum (i.e. TA) reported to influence the sugar alcohol release rate which influenced the perceived sweetness and aroma intensity. Furthermore, the use of analytical macroscopic chewing gum textural measurements did not adequately predict flavor release; softer chewing gums did not imply a more extractable flavored product.

Table 4-1 Chewing gum model formulation

Ingredient name	**Composition (%)**
Gum base	25.57
Lecithin	0.10
Titanium Dioxide	0.49
Concentrated hydrogenated glucose syrup	11.80
Sorbitol Crystals	54.48
Sorbitol Solution	1.97
Glycerine (95%)	2.95
Flavor Solvent	0.66
Flavor mixture	0.98
Total	100.00

Table 4-2 Compositional range of gum base

Ingredient name	**Composition range (%)**
Polyisobutylene	7-10
Styrene butadiene rubber	3-5
Polyvinyl acetate	15-20
Wood rosin	11-15
Polyethylene	0-2
Filler-$CaCO_3$	25-35
BHT	0-0.1
Waxes	4-8
Emulsifiers	0-2
Softeners (confectionary fat, hydrogenated)	10-17

Table 4-3 Composition of model cinnamon-like aroma and estimated Log P values

Compound name	**Percentage proportion of the flavor mixture (%)**	**Log P value**
Cinnamaldehyde	88.26	1.90[a]
L-Carvone	10.16	2.87[b]
Piperitone	0.79	2.85[b]
Jasmone	0.79	3.55[c]

a = experimental value from Hansch et al. (1995); b = experimental value from Griffin et al. (1999); c = estimated based on the K_{ow} calculation program (Syracuse Research Corporation; http://www.syrres.com/aboutsrc/default.htm)

Table 4-4 Quantification data of aroma compounds in chewing gum samples made with PG, TA, MCT or no flavor solvent.

	Compound Concentration (mg/g of chewing gum)			
Treatments	Cinnamaldehyde	L-Carvone	Piperitone	Jasmone
No Solvent	5.59 (± 0.78)	0.50 (± 0.09)	0.10 (± 0.01)	0.10 (± 0.01)
PG	5.40 (± 0.67)	0.51 (± 0.10)	0.10 (± 0.01)	0.09 (± 0.01)
Triacetin	5.10 (± 0.20)	0.45 (± 0.07)	0.10 (± 0.01)	0.11 (± 0.01)
MCT	5.28 (± 0.36)	0.52 (± 0.03)	0.09 (± 0.01)	0.10 (± 0.02)

a = average of 12 samples (± 95% CI)

Table 4-5 Plasticity index values of gum bases made with different flavor solvents

Treatments	**Plasticity Index (PI)[1]**
No Solvent	0.97[a] (± 0.06)
PG	0.83[b] (± 0.01)
Triacetin	0.76[c] (± 0.02)
MCT	0.98[a] (± 0.02)

1 = average of six replicates ± 95% C.I.; * Numbers in columns followed by the same lower-case letter are not significantly different at the 5% level

Table 4-6 Tukeys' mean comparison of sorbitol release from chewing gums with different flavor solvents at 30 and 70 sec

	Sorbitol release	
Treatments	30 sec	70 sec
No solvent	103.5[a]	96.8[a]
PG	99.8[a]	96.2[a]
Triacetin	81.0[b]	120.7[b]
MCT	109.6[a]	101.1[a]

* Numbers in columns followed by the same lower-case letter are not significantly different at the 5% level

Table 4-7 Tukeys' mean comparison of sensory parameters of chewing gums with different flavor solvents

	Sensory Parameters*					
	Sweetness		**Cinnamon flavor intensity**		**Effort to chew**	
Treatments	I_{max}	T_{max}	I_{max}	T_{max}	I_{min}	T_{min}
No solvent	9.06^{a}	39.56^{a}	8.83^{a}	49.44^{ab}	4.43^{a}	62.11^{a}
PG	9.06^{a}	37.41^{a}	8.67^{a}	50.04^{ab}	3.76^{b}	68.89^{a}
Triacetin	8.34^{b}	47.74^{b}	8.15^{b}	59.44^{a}	2.69^{c}	69.11^{a}
MCT	9.07^{a}	39.11^{a}	8.69^{a}	43.85^{b}	3.15^{c}	48.85^{b}

* Numbers in columns followed by the same lower-case letter are not significantly different at the 5% level; I_{max} and T_{max} are the maximum intensity and time at maximum intensity values for the sensory attributes; I_{min} and T_{min} values are the softness and time at reach softness for different gums

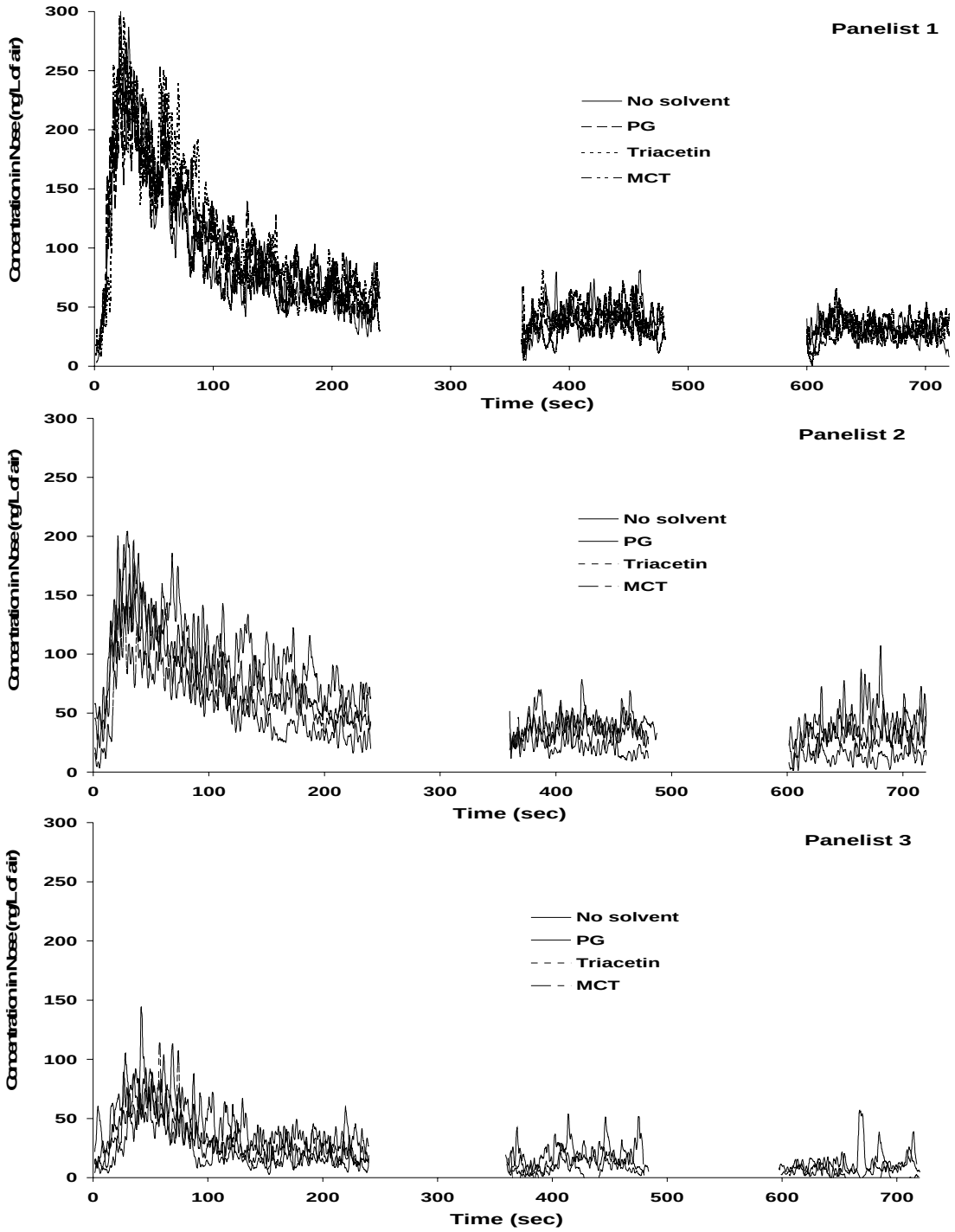


Figure 4-1 Cinnamaldehyde release (*in vivo*) analyzed by APCI-Breath Analysis for 3 panelists for chewing gums made with different flavor carriers; each curve represents the mean of three replicates subsequently smoothed by a 1.5-s moving average trendline.

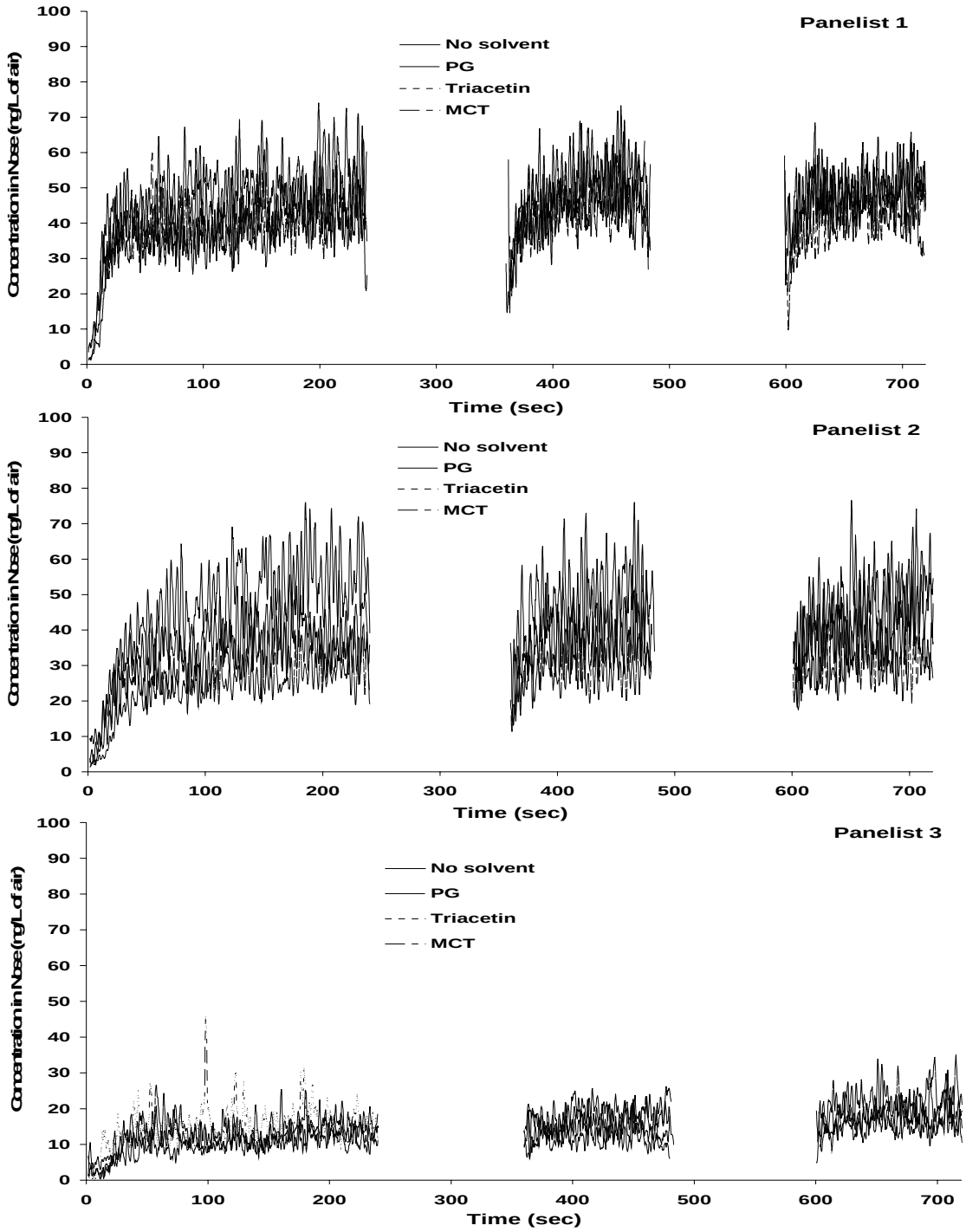


Figure 4-2 L-Carvone release *(in vivo)* analyzed by APCI-Breath Analysis for 3 panelists for chewing gums made with different flavor carriers; each curve represents the mean of three replicates subsequently smoothed by a 1.5-s moving average trendline

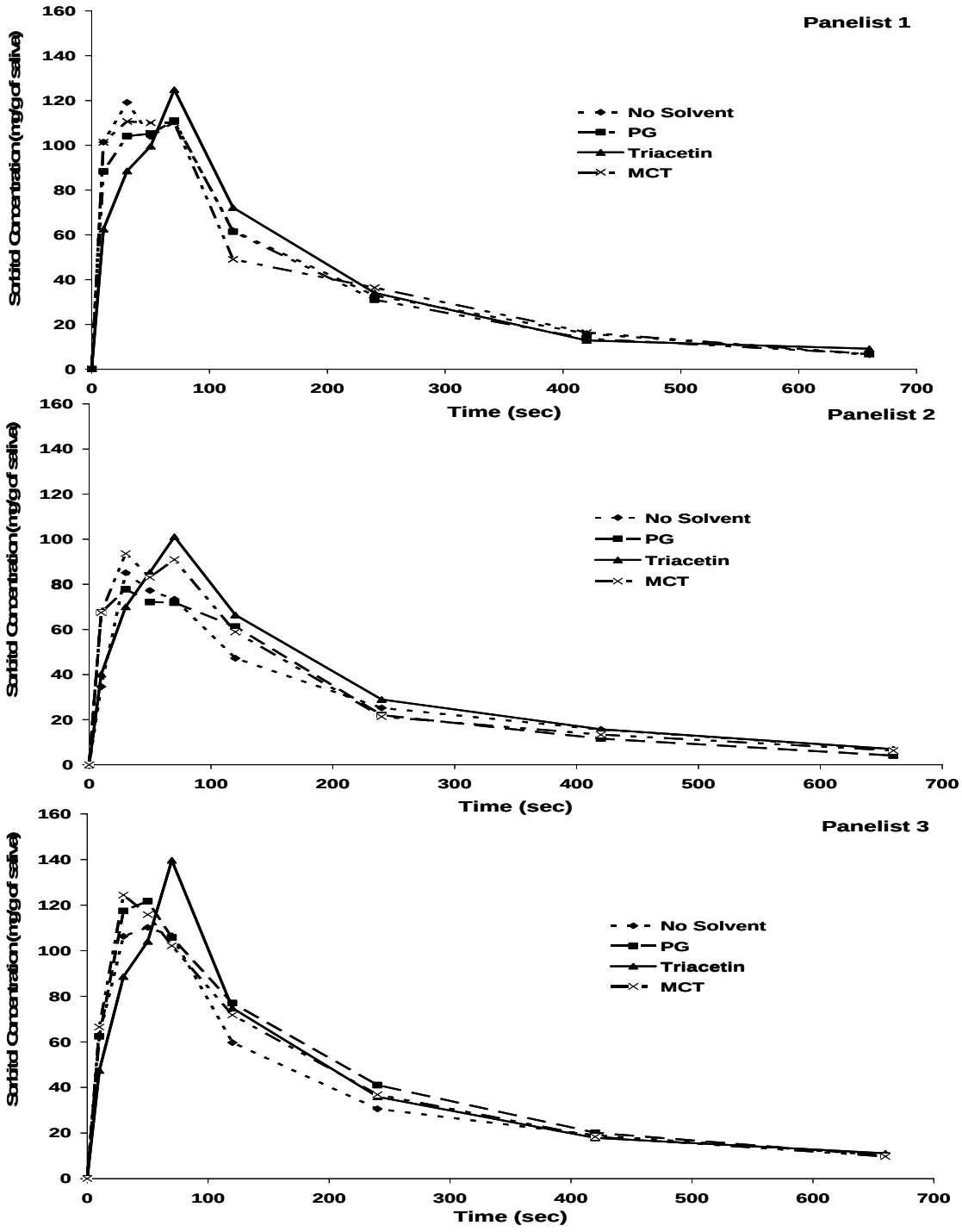


Figure 4-3 Sorbitol release for 3 panelists from chewing gums consumed for 12 mintues made with different flavor solvent carriers; average of triplicate

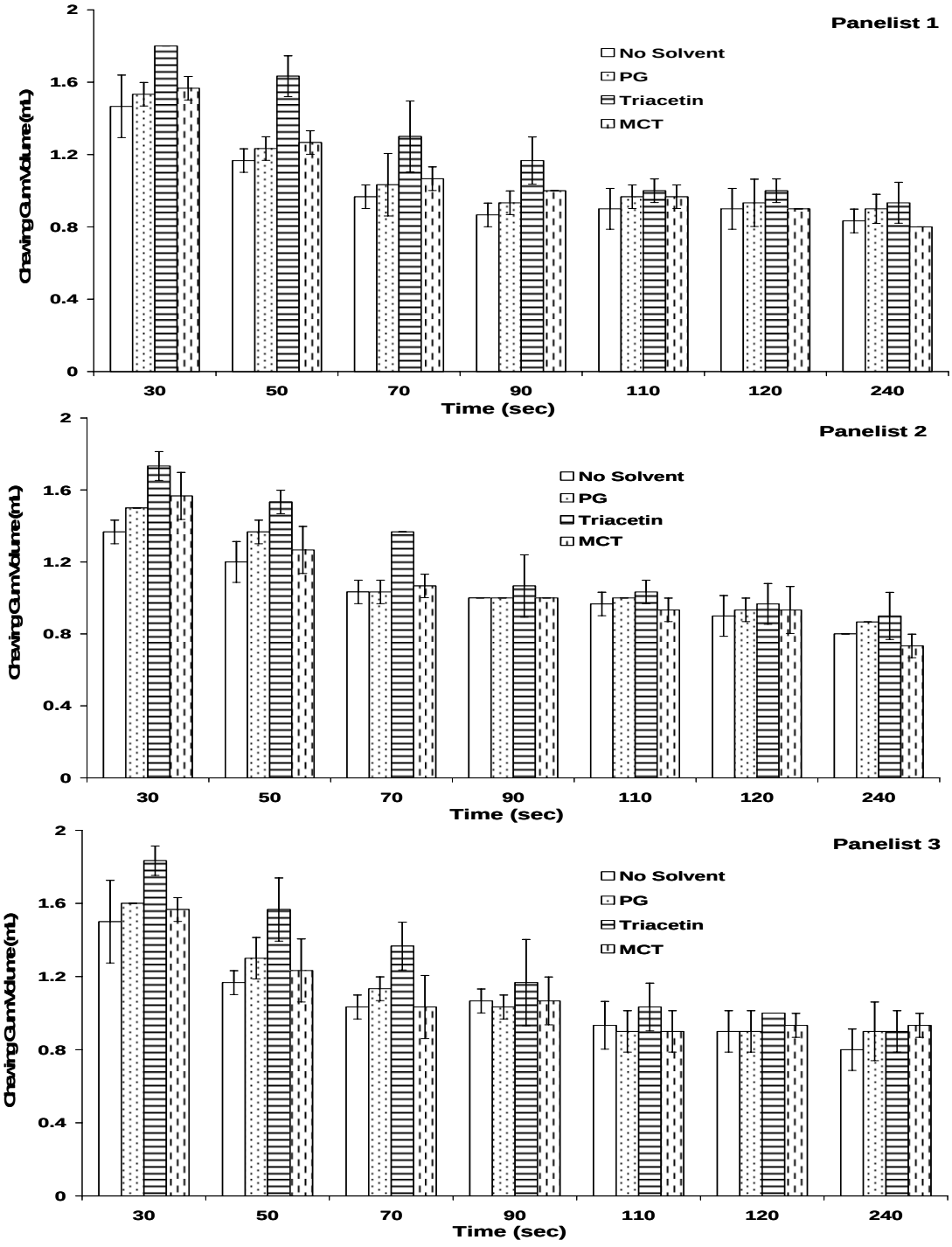


Figure 4-4 Time course gum volume measurements from chewing gums made with PG, Triacetin, MCT or No Solvent for 3 panelists consumed over 4 minutes

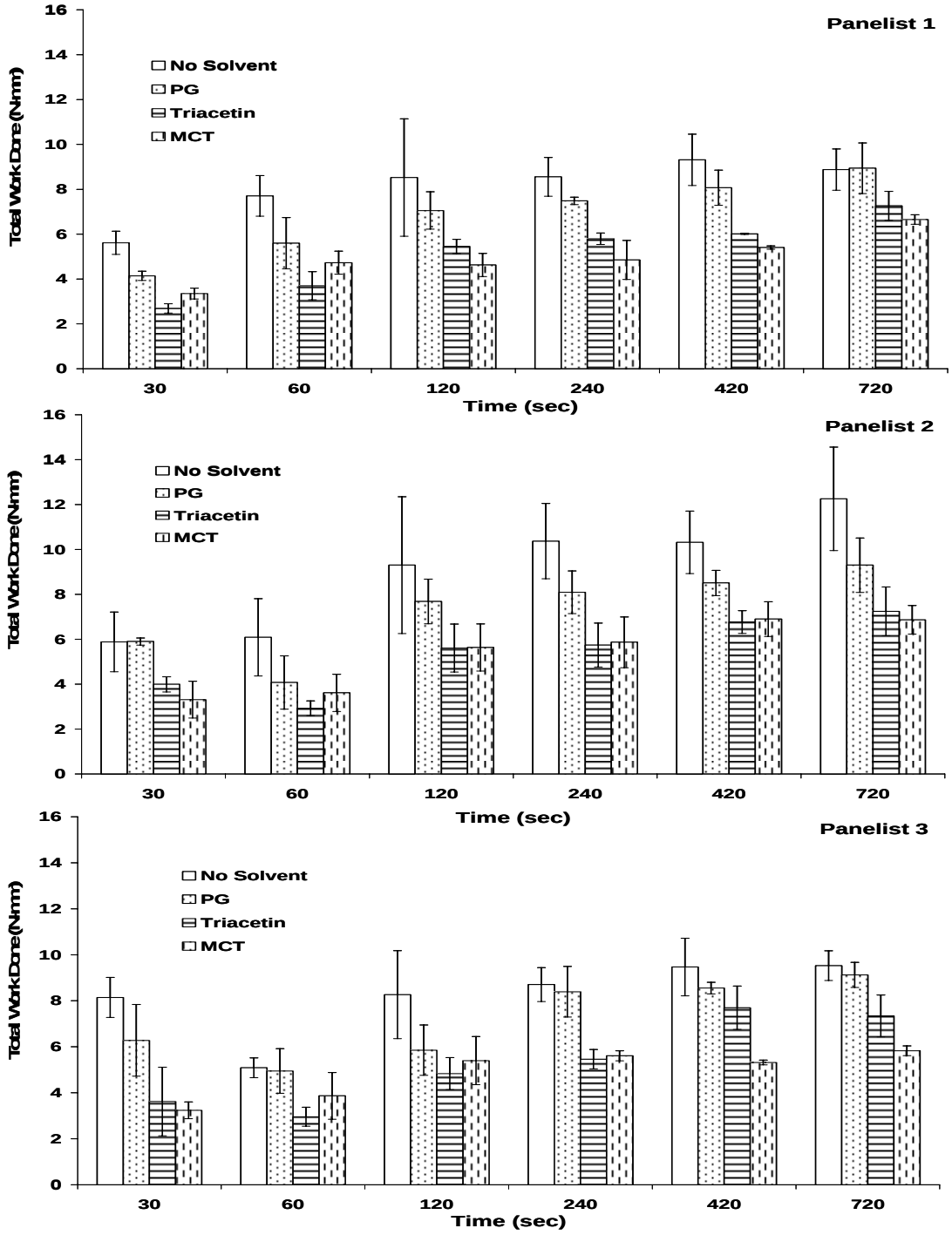


Figure 4-5 Total work done analyzed by TA-XT2 for 3 panelists from chewing gums made with different flavor solvent carriers; average of five replicates ± 95% C.I.

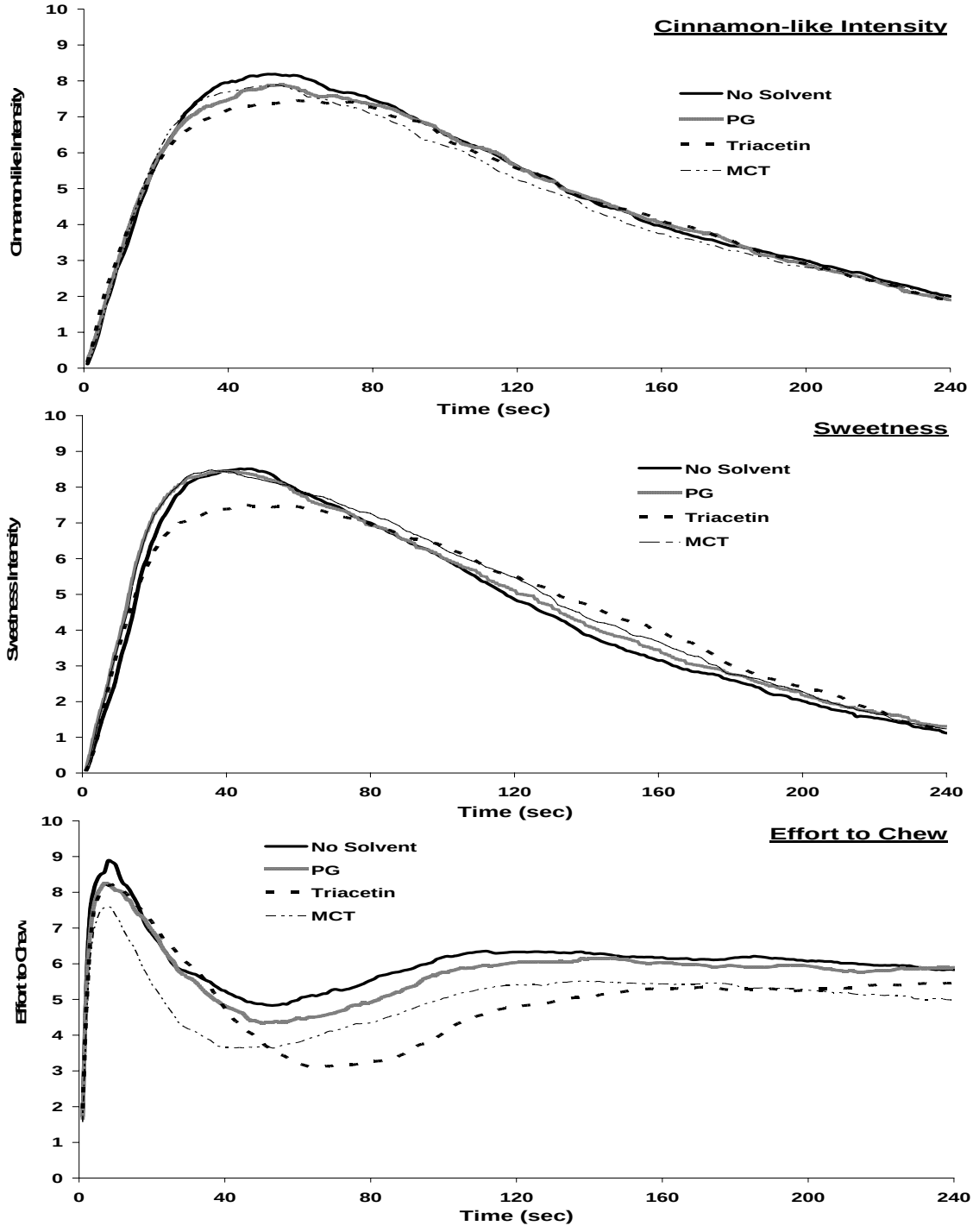


Figure 4-6 Sensory time-intensity analysis of (i) cinnamon-like flavor, (ii) sweetness and (iii) effort to chew; average of 9 panelists

Chapter 5.

Hypothesis II

The mechanism of cinnamaldehyde release from chewing gum is not adequately predicted by the log cP value (thermodynamic model); is related to interactions with the sugar alcohol phase.

Objectives

1) Analytical determine the log P and Log cP value (thermodynamic model) for cinnamaldehyde release in a chewing gum model system.
2) Define a similar log P/log cP aroma compound for direct comparison with cinnamaldehyde
3) Characterized the release properties of cinnamaldehyde, cresol and the sugar alcohol from chewing gum and a gum base model system.
4) Define the influence of flavor load on the flavor release profile

Chapter 6

Mechanisms of Flavor Release in Chewing Gum: Cinnamaldehyde

6.1 Abstract

Recently we reported the release profile of cinnamaldehyde from a sugar-free chewing gum was correlated with the sugar alcohol release rate or was not as predicted by log P determination (octanol/water partition coefficient – hydrophobicity index). The objective of this study was therefore to investigate mechanisms of flavor release for cinnamaldehyde, in a sugar-free chewing gum model system. P-cresol was also analyzed for comparison (similar log P value). The release profile of cinnamaldehyde (or cresol), sorbitol of chewing gum or gum base were analytically characterized for three panelists over an 8-min chewing period. The release of cinnamaldehyde from chewing gum during mastication was more rapid than cresol and was correlated to release profile of sorbitol. Chewing gums made with varying amounts of cinnamaldehyde (0.29 - 2.9 mg/g of chewing gum) did not show any differences in release pattern suggesting no concentration effect. Furthermore, the cinnamaldehyde release pattern from the gum base was similar to cresol. These findings suggested cinnamaldehyde was interacting with the sugar alcohol phase, possibility due to transient hemi-acetal reactions mechanisms, which resulted in a more rapid release rate than would be predicted based on the hydrophobicity of this compound.

6.2 Introduction

Chewing gum can be defined as a two phase product consisting of a water-insoluble gum base (approx. 25%) and a water-soluble sugar or sugar alcohol phase (approx. 74%) with approximately a 1% flavor load. The distribution of the flavor compounds between the two phases would be dependent on the compound affinity for each phase and historically has been related to the compound hydrophobicity (suggested main mechanism related to flavor release). For example, compounds which are more hydrophobic would be predicted to interact more with the gum base, resulting in a relatively low release rate during mastication (water extraction).

De Roos et al (1994) investigated mechanisms of flavor release from chewing gum for a wide range of hydrophobic compounds, using a non-equilibrium partition model [52]. According to the model, the release of flavor compounds was linearly dependent on gum base to water partition coefficient (Log cP) during the first 5 min (thermodynamic control). However after 5 min, the use of Log cP was less valid due to a noted weak relationship with the flavor release measured [52]. Based on this observation, they suggested the air to water (saliva) partitioning coefficient (Paw) was controlling flavor release during this time period (after 5 min) and was diffusion controlled (greater emphasis on mastication efficiency) [52].

Harrison et al. (2000) also investigated mechanisms of flavor release for various flavor compounds based on gum to saliva partitioning coefficient (Log cP) in chewing gum model system [174]. They used the stagnant layer theory with the interfacial mass transfer from chewing gum to saliva as a rate limiting step. The authors also considered the interaction of flavor compounds with the olfactory epithelium as a controlling factor

in the model system [174]. Overall, they concluded that flavor compounds with a low chewing gum-to-saliva partitioning coefficients were found to release faster, while the release rate was constant for flavor compounds with high chewing gum-to-saliva partitioning coefficients [52, 174]. Furthermore, the rate of flavor depletion to its initial concentration was faster for compounds with high chewing gum-to-saliva partition values. Both the Harrison and De Roos et al. models emphasized the gum base as a major factor dictating the flavor release kinetics of various flavor compounds based on hydrophobicity [52, 174].

Inverse phase chromatography (IGC) has also been applied to understand the interactions between different gum bases and flavor compounds [6, 7]. Niederer et al (2003) studied the various thermodynamic parameters such as partitioning coefficients, activity coefficients, Henry constants, molar heat of solution between the flavor compounds (ethyl butyrate, limonene, 1-octanol and cis-2-hexenal) and gum bases (containing higher amounts of polyvinyl acetate or polyisobutylene), using an IGC method [6]. Based on the thermodynamic data, authors could predict flavor release was based on the affinity between the flavor compound and gum base. They indicated that a higher affinity between the gum base and flavor molecule leads to slower release or long lastingness during mastication and vice versa [6]. Similarly Sostmann et al. (2002), categorized the binding behavior of flavor compounds with gum base ingredients into 3 groups, 1) higher binding polar compounds to polyvinyl acetate (PVAc) and ester gum/lower binding to paraffin waxes (eg., pentanol, linalool, benzaldehdye, ethyl benzoate and eugenol) and 2) higher binding to paraffin waxes/lower binding to PVAc (eg., limonene, ethyl nonanoate, p-cymene) 3) medium polar compounds with high

affinity towards Styrene butylene rubber (SBR) (eg., octanone, tran-2-hexyl acetate, isopropyl-pyridine, octanal, anethole) [7].

The release of flavor compounds from chewing gum has been traditionally predicted in the flavor/gum industry based on Log P or Log cP values. Recently Potineni and Peterson (2007) reported, however, that the release profile of cinnamaldehyde from chewing gum during mastication were correlated to the sorbitol release rate or was not as would be anticipated based on the log P value 1.90,of this compound [178]. Our previously observed release profile for cinnamaldehyde and sorbitol from chewing gum is illustrated in Figure 6-1.

The objective of this study was therefore to investigate the mechanisms of cinnamaldehyde release in a chewing gum model system. P-cresol was analyzed in parallel for comparison of a similar log P aroma compound with a different functional group (alcohol).

6.3 Materials and methods

6.3.1 Materials.

Cinnamaldehyde was purchased from Aldrich (Sigma Aldrich, Milwaukee WI). p-Cresol was from Penta (Livingston, NJ). Methanol was from Fisher Scientific (Fairlawn, NJ). Hexane and formic acid were from EMD Chemicals (Gibbstown, NJ). Methylhexanoate was purchased from TCI America (Portland, OR). Chewing gum ingredients such as Paloja® gum base was from L.A.Dreyfus Company (South Plainfield,

NJ), polyols (sorbitol, xylitol and mannitol) were from SPI polyols (Wilmington, DE), glycerin was from Givaudan Flavors Corp.(Cincinnati, OH), medium chain triglycerides (MCT) was from Stepan company (Northfield, IL), aspartame was from Ajinomoto (Chicago, IL), acesulfame-K was from Wintersun Chemical (Ontario, CA), cooling and warming agents from Takasago Corporation (Rockleigh, NJ).

6.3.2 Chewing gum model.

Chewing gum was manufactured according to the general procedure previously reported by Potineni and Peterson (2007). Table 6-1 shows the various ingredients and the percent composition in the chewing gum model systems. After manufacture, the chewing gum was stored at room temperature at 45% humidity for 6 months before further analyses were conducted. Different chewing gum treatments were made by varying the concentration of cinnamaldehyde or p-cresol (shown in Table 6-2).

6.3.3 Flavored gum base model.

50g of Paloja gum base was softened by heating on a gas stow (Empire comfort systems, Belleville, IL), till the gum base reaches 75°C. Ingredients such as Medium chain triglycerides (MCT), lecithin and glycerin were added according to the gum base treatment shown in Table 4. Volatile concentrations for cinnamaldehyde and cresol were added to a concentration equivalent to the total amount added to the chewing gums CIN (control) and CRE respectively (shown in Table 2). Molten gum base along with other ingredients were stirred (5 minutes) and then poured on a slab covered with a parchment

paper (Alcoa Inc., Richmond, VA) to an approximate thickness of 0.25 cm by gently shaking the slab. After cooling, flavored gum bases were stored in a parchment paper and in glass bottles with Teflon™ lined lids.

6.3.4 Quantification of cinnamaldehyde/cresol in chewing gum or flavored gum base.

For the chewing gum samples, 5 gum pieces per treatment were analyzed from a box of 50 while for the gum base samples, 3 gum base pieces were selected for analysis. The quantification procedure was previously reported by Chapter 4. In brevity, samples were dissolved in hexane, centrifuged, the supernatant was mixture with methanol, centrifuged and the supernatant of this methanol:hexane mixture was analyzed by gas chromatography.

6.3.5 Gas Chromatography (GC).

Analysis was performed on a Hewlett-Packard 5890 Series II GC equipped with a split/splitless injector, flame ionization detector (FID), autosampler (HP 7673) and a fused-silica capillary column (DB-wax, 30 m, 0.32 mm i.d., 0.32 μm film thickness, Agilent Technologies, CA). The GC operating conditions were as follows: inlet temperature was 200°C, oven program was 35°C for 2 min, then increased at 10°C/min to 230°C and held for 3 min; constant pressure of 15psi (He); 1 μL of sample was injected in split mode (1:20).

6.3.6 Log P analysis.

Values for cinnamaldehyde and p-cresol were determined by a shake flask method. Equivalent amounts of octanol containing each flavor compound (100 mg/L) and distillated water were added together in a volumetric flask. The resulting two-phase system was shaken gently for 1 hr on an orbital shaker (Lab-Line Instruments Ltd., Melrose park, IL). After mixing, 500 µl of octanol fraction was removed and diluted in methanol (500 µl; containing 1000 mg/L of benzyl alcohol as internal standard) and this mixture was directly analyzed by GC. The GC operating conditions were as follows: inlet temperature was 200°C, oven program was 100°C for 2 min, followed by 10°C / min increase to 230°C; constant pressure of 15psi (He); 1 µL of sample was injected in split mode (1:20).

6.3.7 Log cP analysis.

Gum base sample preparation: *100*g of gum base was ground in a blender (Waring blender, Torrington, CT) and subsequently sieved using a sieve shaker (W.S. Taylor Ltd., Gostonia, NC) with sieve number 40 to 70 obtain a 212 – 425 µm particles size sub-sample. All cP analyses were conducted with this sample fraction.

Gum base to water partitioning coefficient (Log cP): Nine grams of gum base was suspended in 100 ml of water containing 0.1% of flavor compound (cinnamaldehyde or p-cresol) in a 125 ml flat bottomed flask with glass stopper. Flasks are shaken gently though out the experiment in a water bath set at 38°C. At regular intervals (0, 1, 4, 8, 12, 24, 48 and 60hrs), samples of 350 µl were taken and subsequently mixed with 500 µl of

30% acetonitrile mixture (containing 500 mg/L of benzyl alcohol as internal standard) and analyzed by an HPLC. HPLC analysis was performed on a Pinnacle II C-18 column (Restek corp., Bellefonte, PA) using a linear gradient binary mobile phase (A = water and B = acetonitrile). The initial mobile phase conditions were 5 % B in A and then increasing B to 100 % over 25 minutes. The flow rate was 1 ml/min and the injection volume was 20μL. Partition coefficients were determined from the data after 60 hr of equilibrium (analysis of time points from 0-60 were to validate the system was relatively 'steady-state').

Gum base to sugar alcohol solution partitioning coefficient: The general procedure to obtain partitioning coefficient was the same as described for the '*Gum base to water partitioning coefficient'* method described above with the following exception, 100 ml of sugar alcohol mixture in water was used. Sugar alcohol mixture contained 3.5% of sorbitol, 1.8 % of xylitol and 1.3 % of mannitol on weight basis. This ratio of sugar alcohols was obtained from the maximum release values of sugar alcohols from non-volatile analysis (at retention time 70 minutes, Figure 6-2).

Gum base to sugar alcohol/glycerine solution partitioning coefficient: The general procedure to obtain partitioning coefficient was the same as described for the '*Gum base to sugar alcohol solution partitioning coefficient'* method described above with the following exception, 0.4% of glycerine was added to the 100ml of sugar alcohol solution. Rest of the procedure was similar as described above.

6.3.8 Breath analysis.

Breath-by-breath analysis was performed with an atmospheric pressure chemical ionization-mass spectrometer (APCI-MS) as previously described by Schober and Peterson (2004) [170]. The release of cinnamaldehyde and p-cresol from chewing gums and flavored gum bases were monitored using the chewing protocol previously defined by Potineni and Peterson (2007). Chewing gums (2.5 g) or gum bases (1g) were masticated at the rate of 60 chews/min by 3 panelists (1 male and 2 female), while breathing normally by keeping their mouth closed. The breath from the nose was directly and continuously sampled via an interface set at 65ºC into the Quattro II/Micromass mass spectrometer (Waters, Milford MA) modified for breath analysis at these given time intervals (0-4 min, 6-8 min). The APcI operating conditions are as follows: SIM mode; sampling rate was 200 ml/min; block temperature is 100°C; transfer line 60°C; corona discharge was 3.5 kV. Ions monitored were 133 [M + H]+ for cinnamaldehyde and 109[M + H]+ for p-cresol at cone voltages 15 KV and 30 KV respectively . Day-to-day variation in the instrumental signal response was adjusted by the injection of a known amount of L-carvone in pentane as described in Chapter 4. Quantification of cinnamaldehyde and cresol were determined via standard calibration curve. 0.5, 1, 5, 10, 30, 48 ul of a 0.02 g cinnamaldehyde/ml pentane and 0.1, 0.5, 1, 5, 10 ul of 0.005 g cresol/ml pentane was injected into a airtight water-jacketed 1.1-L deactivated glass vessel [170] maintained at 40 °C and held for 5 min with constant stirring (200 rpm) prior to interfacing directly to the breath analysis instrument using the same operating conditions at described above. The peak height (ion intensity) versus μg weight of each compound per liter air was plotted (all compounds reported an $r2 > 0.99$).

6.3.9 Sugar alcohol/glycerine release analyses.

The concentration of sorbitol, xylitol mannitol, and glycerine was determined in expectorated salvia of three panelists while chewing a 2.5g piece of chewing gum sample over a 8 min time period. A previously defined chew/swallow protocol (Chapter 4; [178]), was used for saliva collection. In brevity, three panelists expectorated salvia at regular intervals at 0, 10, 30, 50, 70, 110, 180, 240, 360 and 480 secs which were collected into spit cups with lids. 0.5 g of saliva was immediately transferred into a centrifuge tube containing 1 ml of 0.1 % formic acid, centrifuged at 11,750 rcf for 3 min before the supernatant was transferred into 2 ml amber bottles. The whole analyses are conducted in triplicate per treatment. The sugar alcohol concentration was determined using an external standard curve at 0.006, 0.013, 0.025, 0.038, 0.05 g/L for sorbitol or mannitol or xylitol, and 0.0002, 0.0004, 0.0009, 0.002, 0.004, 0.007 g/L for glycerine plotted versus peak area ($r2 > 0.99$).

6.3.10 High Performance Liquid Chromatography (HPLC) analysis.

Analyses were performed on Shimadzu HPLC system consisting of two pumps (LC-10ATvp), degasser (DGU-14A), an auto sampler (SIL-10Ai) and Shimadzu column heater (CTO-10ACvp) was connected to a refractive index detector (RID; RID-10A). Separations were performed on a LC column Supelcogel-H (5 µm, 250 x 2 mm i.d.,) using an isocratic run with 0.1% formic acid in water as the mobile phase maintained at 40°C. The flow rate was 0.17 mL min-1 and the injection volume 20 µL.

6.4 Results and discussion

The release properties of flavor compounds from chewing gum are commonly estimated based on log P values which can be derived from computation chemistry techniques such as by quantitative structure-activity relationship method (QSAR) based on the correlation between the structures of compounds to their chemical activity [179] or by experimental determination [180]. Both the predicted and calculated Log P values for cinnamaldehyde and p-cresol are listed in Table 6-3. Although the experimental values were found to be lower than the predicted values, overall based on either method both compounds would be predicted to be of similar hydrophobicity. However, considering that the gum base is not octanol, perhaps a better prediction method would to measure the binding affinity of the flavor compounds to the gum base (known as log cP; distribution between the gum base and aqueous phase). The log cP values for both compounds are were therefore also determined and are reported in Table 6-3. Based on Log cP values using water as the aqueous phase, cinnamaldehyde was found to have a similar binding affinity as cresol for the gum base (Table 6-3); implying that the release of these compounds from chewing gum would be comparable. However the use of water and gum base as model to predict flavor release may also be too simplistic as the salvia phase would contain other water soluble compounds (i.e. sugar alcohols, glycerine) from the chewing gum which may alter the affinity of a select aroma compound for the gum base. To study the influence of the aqueous phase composition, the Log cP values were also determined with a model where the water aqueous phase contained sugar alcohol or sugar alcohol plus glycerine at levels reported in the saliva phase during mastication (see Table 6-3). The Log cP value of cinnamaldehyde was not found to be influenced by the

addition of sugar alcohol or sugar alcohol and glycerine; whereas the affinity of p-cresol for the gum base was lowest for the aqueous sugar alcohol and glycerine solution model system.

Based on log P or log cP values determined it would be anticipated that the release of cinnamaldehyde during the mastication of chewing gum would be comparable or even relatively slower than for cresol. The analytically determined release profile of cinnamaldehyde, cresol and the total sugar alcohols (sorbitol, xylitol, mannitol) from chewing gum model 1 and 2 (Table 6-2) during consumption over an 8 minute time period are shown in Figure 6-2 for one panelist. The average maximum cinnamaldehyde and cresol concentration as measured form the exhaled breath form the nose from 0-4 minutes (max 1) and for 6-8 minutes (max 2) as well as a ratio of max 1/max are presented in Table 6-5 for all three panelists. The max1/max2 ratio is an indication of a compounds release profile; a higher number indicates the compound has decreased in concentration for the second time period suggesting it was released more rapidly initially; whereas a lower number indicates a less rapid release rate initially (more stable/consistent over time). The max1/max2 ratio values for cinnamaldehyde were approximately 2-3 times higher than for cresol for all 3 panelists indicating the release of cinnamaldehyde was more rapid than cresol. The release of cinnamaldehyde did appear, however, to be correlated to the release profile of sorbitol; both reported a rapid increase in the first 30 seconds and subsequently decreased to approximately 20-30% of the initial concentration maximum over the 8 minute consumption time period (Figure 6-2; calculation not shown). This indicated that Log cP value (thermodynamic model) was not accurate in predicting the release of these aroma compounds or more specifically cinnamaldehyde.

Overall, the release of cinnamaldehyde from this chewing gum model system (formulated with Paloja gum base, Table 6-1) was in agreement with our previous findings for chewing gum formulated with VH1 gum base (Figure 6-1). VH1 consists of higher levels of polyvinyl acetate (PVAc) compared to styrene butadiene rubber (SBR) and polyisobutylene (PIB) (approx PVAc: SBR: PIB = 4:1:2). Conversely, Paloja gum base contains higher amounts of SBR compared to PVAc and PIB (PVAc: SBR: PVAc = 1:2:1). The similar cinnamaldehyde release profile for both of these chewing gum samples (made with two different commercial gum bases) suggested the release of this compound was not dependant on the gum base composition or related interactions.

The concentration of cinnamaldehyde was approximately 16-fold higher than cresol in the chewing gum model analyzed in Figure 6-2 (see Table 6-2 – model 1 and model 2, simulated a commercial flavored product) therefore the influence of flavor concentration on flavor release was also investigated. The release profile of cinnamaldehyde at 0.2880μg and 2.860μg/g chewing gum (Table 6-2 – model 3 and 4) are illustrated in Figure 6-3a,b for panelist number 1. Overall, no differences were observed in the release properties of cinnamaldehyde over this concentrations range (10-fold). Similar results were observed for all 3 panelists (data not shown). Furthermore, the lowest concentration of cinnamaldehyde in chewing gum (Table 6-2 – model 3) was at a similar concentration as cresol in chewing gum model 2 (Table 6-2; between 0.1-0.3μg/g chewing gum) and further supported that different release properties reported for these two compounds in Figure 6-2 were not due to any concentration effects.

To further validate that cinnamaldehyde release from chewing gum was not controlled by its affinity for the gum base, the release of cinnamaldehyde or cresol from

gum base (with MCT, model 1, 2 – Table 6-4) was determined and reported in Figure 6-4a and b (the release of these compounds from chewing gum is also shown for direct comparison). Additionally the maximum breath concentrations for both of these compounds at 0-4 minutes and 6-8 minutes and the ratio of these values are reported Table 6-5. The release of cinnamaldehyde from the gum base was slower and more constant compared to that in chewing gum; whereas for cresol both the gum base and chewing gum reported very similar release profiles (Figure 6-4a; Table 6-5). Furthermore, the release of cinnamaldehyde from the gum base was very similar to cresol, as would be predicted based on the log P or cP values.

Based on these observations, it was proposed that cinnamaldehyde was forming transient hemiacetals within the sugar alcohol phase during chewing gum manufacture (catalyzed heat and moisture loss) and during mastication these hemiacetal reaction products were degraded back to cinnamaldehyde and the corresponding alcohol (catalyzed by hydration); the mechanism illustrated in Figure 6-5. This proposed reaction mechanism would explain why cinnamaldehyde release was correlated to the dissolution of the sugar alcohol phase (Figure 6-2) during mastication. In theory any alcoholic compound such as glycerine (4.0% of the chewing gum composition) may also be involved in this proposed hemiacetal reaction mechanism. Glycerine was reported to be primarily associated with the sugar alcohol phase as its release from chewing gum and not from the gum base (Figure 6-6) was correlated to the sugar alcohol release profile (Figure 6-2). However, based on the Log P values estimated using Chemdraw® Ultra 10 (Cambridgesoft, Cambridge, MA), the hemiacetals formed with hexols such as with sorbitol (Log P value = - 0.4) would be predicted to be more hydrophilic compared to the

hemiacetals formed with glycerin (Log P = 1.21). Considering the release of cinnamaldehyde in this study was correlated with the sugar alcohol phase, hemiacetal relations which were also relatively hydrophilic (those with hexols) would likely explain the observed release profile of cinnamaldehyde in this study. Furthermore, this would also suggest that similar flavor compounds could be added in a hemi-acetal state with a specific hydrophobicity (alter alcoholic hemi-acetal moiety) to chewing gum to tailor their release properties.

To further test this hypothesis that flavor compounds with an aldehyde functional group (or carbonyl) can chemical react with the sugar/sugar alcohol phase of chewing gum and influence their release properties during mastication, the release properties an aldehyde (anisaldehyde – model 5, Table 5-2) and a ketone (L-carvone; model 6, Table 5-2) were determined and are reported in Figure 5-7. As predicted, anisaldehyde had a similar release profile as cinnamaldehyde. However, L-carvone did not follow the release pattern of cinnamaldehyde, and suggested either a lower reactivity of ketones to form acetals in the sorbitol phase or possibly the higher predicted hydrophobicity (log P = 2.87) of this compound (in comparison to cinnamaldehyde or anisaldehyde) influenced this reaction mechanism.

The data presented in this study supported that cinnamaldehyde release in chewing gum was a two phase process (1) the release of hemiacetal bonded cinnamaldehyde compounds during the dissolution of sugar alcohol phase (dominate mechanism during the initial stage of mastication; 0-4 minutes) and (2) the release form the gum base as predicted by the log cP value (dominate after 6 minutes)

Table 6-1 Chewing gum composition made with PALOJA gum base

Ingredient	Composition (%) (w/w)
Gum Base (PALOJA)	25
Sorbitol	32.6
Xylitol	16.6
Mannitol	12.5
Glycerine	4.0
Medium chain triglycerides (MCT)	1.0
Aspartame	1.36
Acesulfame-K	0.26
Cooling agents	0.2
Heat/hot agents	0.08
Lecithin	0.02
Others (fillers, emulsifiers, flavor)	5.0

Table 6-2 Volatile quantification of chewing gums made with PALOJA gum base

Volatile compound	Compound concentration (µg/g of chewing gum ± 95% CI)					
	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
Cinnamaldehyde	1963 (± 148)		288 (± 14)	2860 (±170)		
p-Cresol		122 (± 5)				
Anisaldehyde					325 (±18)	
L-Carvone						479 (± 28)

Table 6-3 Log P and Log Cp values for cinnamaldehyde and p-cresol

	Log P value		**Log cP**		
	Estimated	**Calculated [b,c]**	**Water [b]**	**Sugar alcohol [b] (6.6 %)**	**Sugar alcohol + glycerine [b] (6.6 + 0.4 %)**
Cinnamaldehyde	1.90[a]	1.00 (± 0.08)	1.22 (± 0.03)	1.22 (± 0.02)	1.24 (± 0.03)
p-Cresol	1.94[a]	1.20 (± 0.11)	0.90 (± 0.05)	1.02 (± 0.07)	0.73 (± 0.10)

[a] = estimated values from [179]

[b] = average of triplicate ± 95% confidence interval

[c] shake-flask method (octanol/water) [180]

Table 6-4 Cinnamaldehyde, cresol and glycerine concentrations in flavored gum base models made with PALOJA gum base

Ingredients	**Gum base models[a]** µg/g gum base ± 95% CI		
	Model 1	**Model 2**	**Model 3**
Cinnamaldehyde	7524 (± 173)		7192 (±376)
p-Cresol		784 (± 108)	
Glycerine (%)			16[b]

a = 4 % MCT added to gum bases (equivalent to 1 % in chewing gum)

b = units in % (equivalent to 4% in chewing gum)

Table 6-5 Maximum average aroma concentration in the breath from 0-4 min and 6-8 min from chewing gum and gum base[a]

a = average of triplicate ± 95% confidence intervals

	Compound Concentration (ng/L air)								
	Panelist 1			**Panelist 2**			**Panelist 3**		
Chewing gum	Max1 (0-4 min)	Max2 (6-8 min)	Ratio (Max1/Max2)	Max1 (0-4 min)	Max2 (6-8 min)	Ratio (Max1/Max2)	Max1 (0-4 min)	Max2 (6-8 min)	Ratio (Max1/Max2)
Cinnamaldehyde	51.3 (± 26)	16.2 (± 5.8)	3.2 (± 1.0)	35.3 (± 12)	16.8 (± 1.8)	2.1 (± 0.6)	18.5 (± 2.8)	6.8 (± 3.2)	3.1 (± 1.9)
Cresol	0.05 (± 0.0)	0.04 (± 0.0)	1.27 (± 0.1)	0.04 (± 0.0)	0.06 (± 0.0)	0.77 (± 0.24)	0.02 (± 0.0)	0.02 (± 0.0)	1.06 (± 0.17)
Gum base									
Cinnamaldehyde	16.2 (± 3.6)	15.5 (± 2.8)	1.1 (± 0.5)	36.8 (± 11)	43.2 (± 15)	0.9 (± 0.1)	22.5 (± 6.4)	17.8 (± 2.1)	1.3 (± 0.2)
Cresol	0.2 (± 0.0)	0.3 (± 0.3)	0.8 (± 0.51)	0.2 (± 0.1)	0.2 (± 0.1)	1.1 (± 0.3)	0.06 (± 0.0)	0.08 (± 0.0)	0.8 (± 0.16)

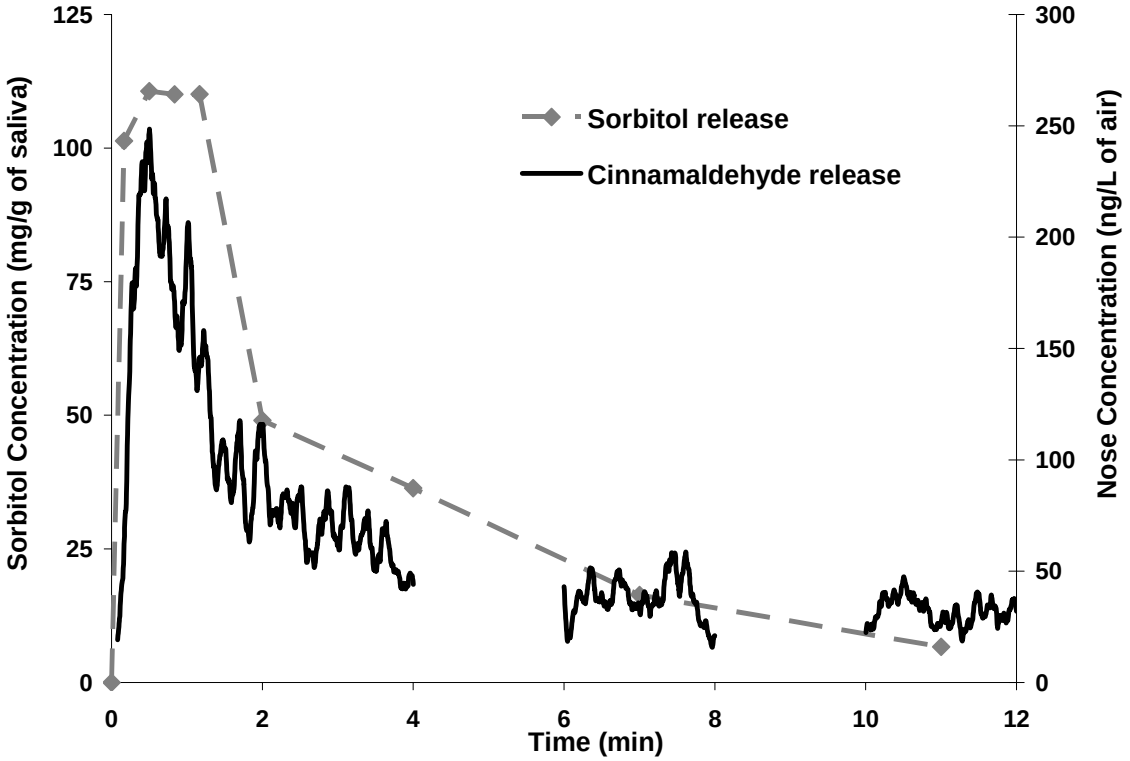


Figure 6-1 Release kinetics of cinnamaldehyde and sorbitol from a chewing gum made with VH1 gum base; adapted from Chapter 3 [178]

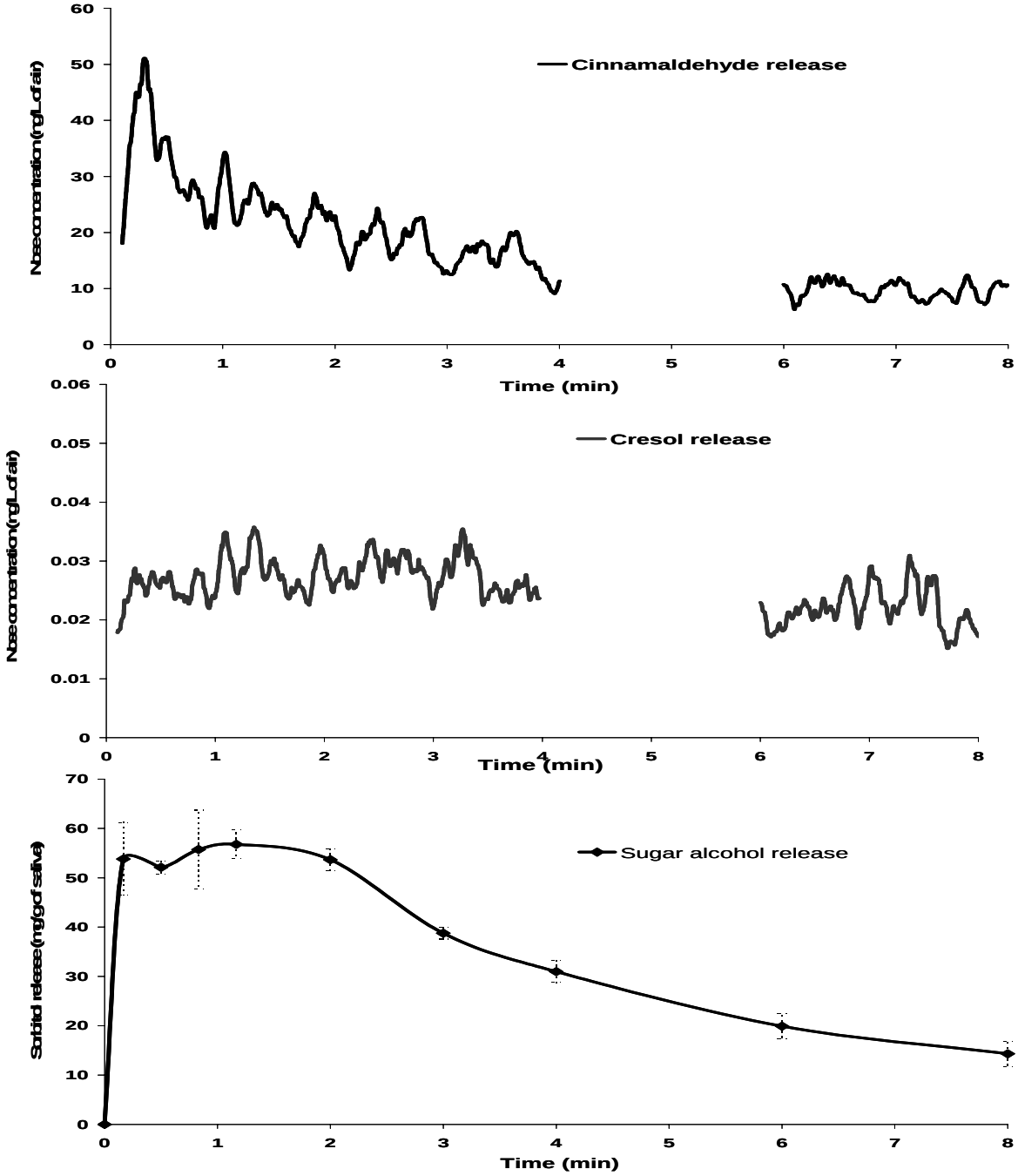


Figure 6-2 Cinnamaldehyde, cresol and total sugar alcohol release in chewing gums made with PALOJA gum base for one panelist; (a) and (b) each curve represents the mean of three replicates subsequently smoothed by a 3-s moving average trendline, (c) curve represents the mean of three replicates $\pm$ 95% confidence intervals

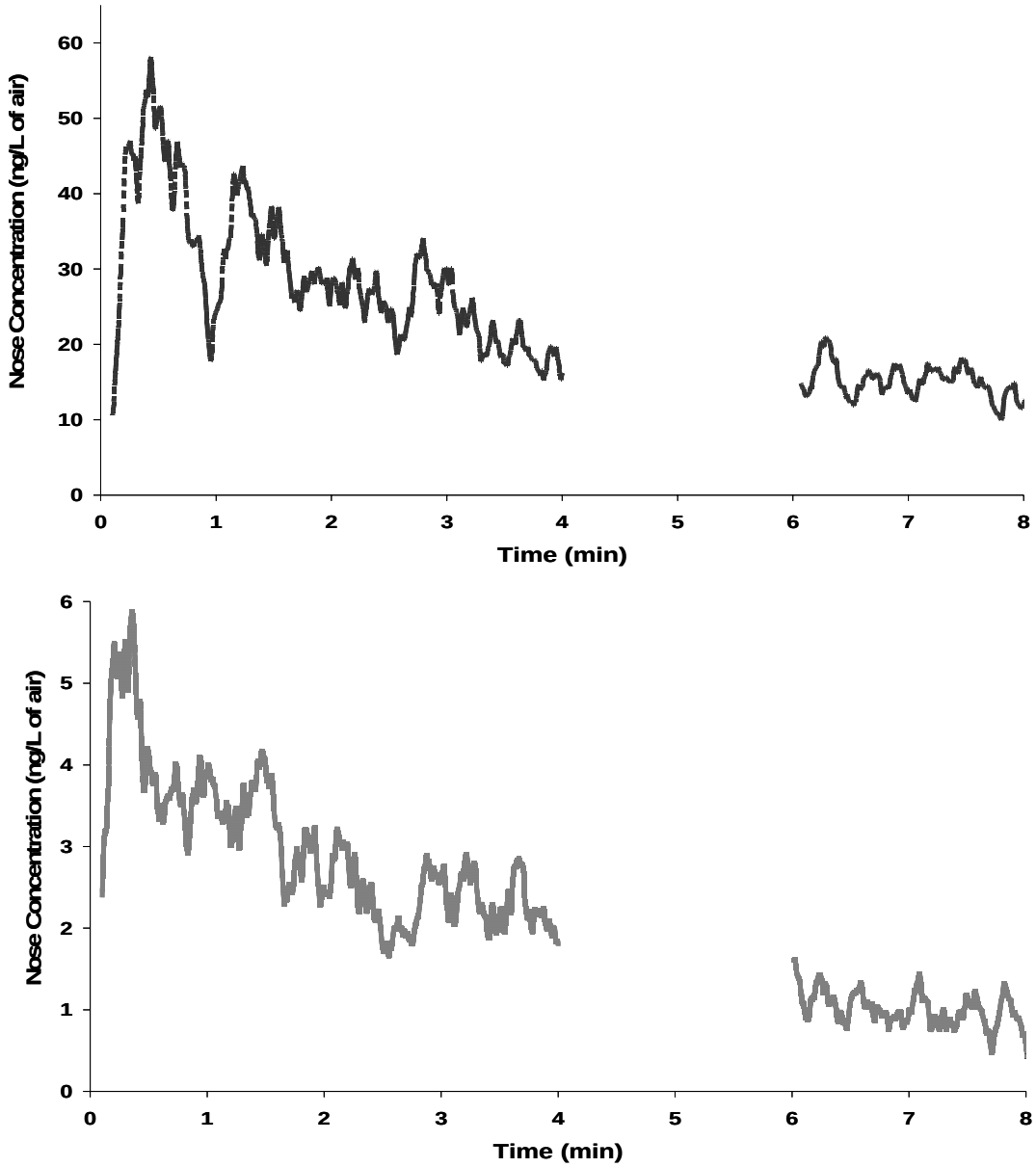


Figure 6-3 Release patterns of cinnamaldehyde in chewing gum at two different cinnamaldehyde concentrations a) 2860 μg/g chewing gum, and b) 288 μg/g chewing gum for panelist 1; each curve represents the mean of three replicates subsequently smoothed by a 6-s moving average trendline.

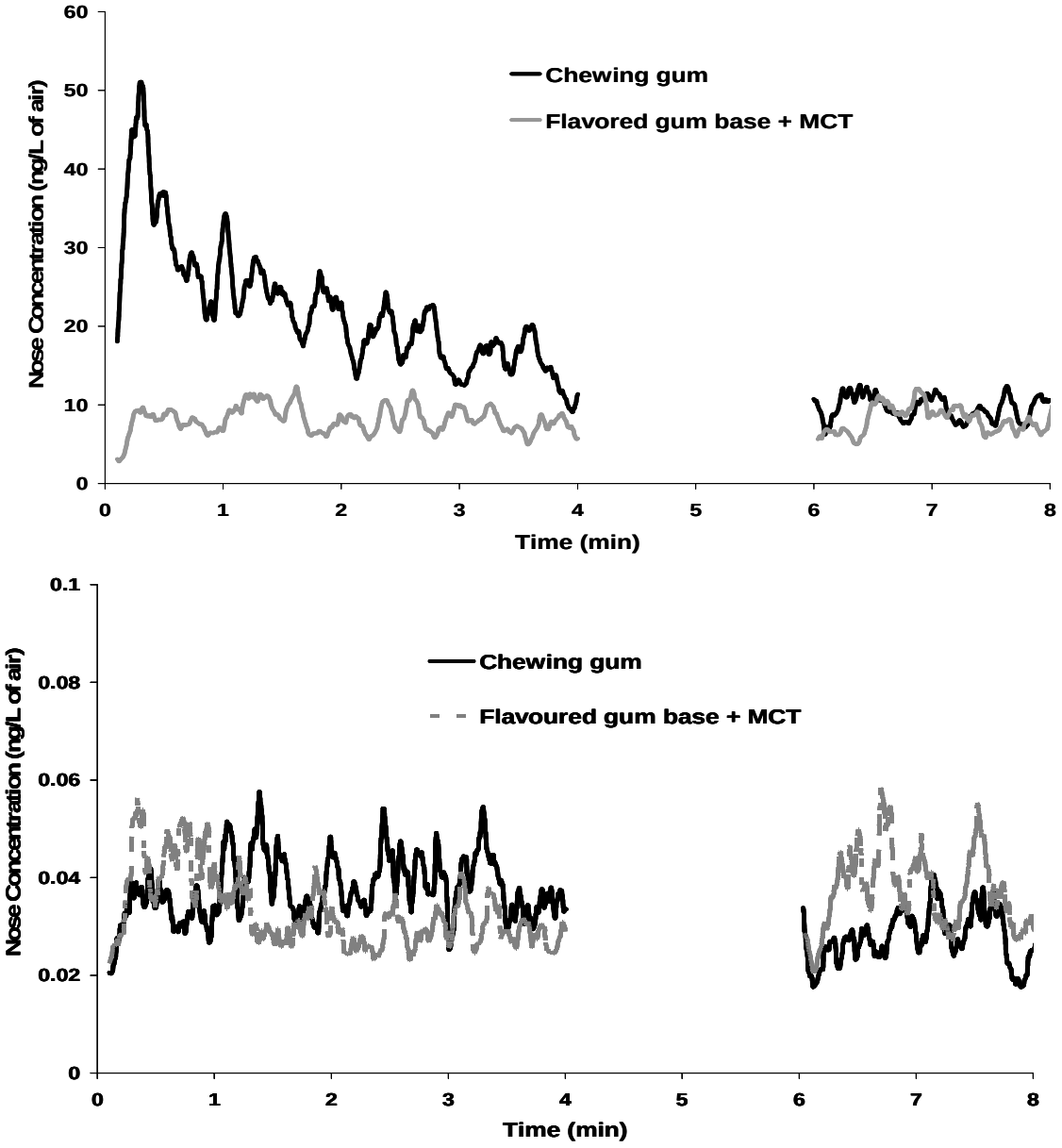


Figure 6-4 Release of (a) cinnamaldehyde and (b) cresol release from gum base with MCT [a]cinnamaldehyde or cresol release profile from chewing gum (figure 2) was also illustrated for comparison; each curve represents the mean of three replicates subsequently smoothed by a 6-s moving average trendline

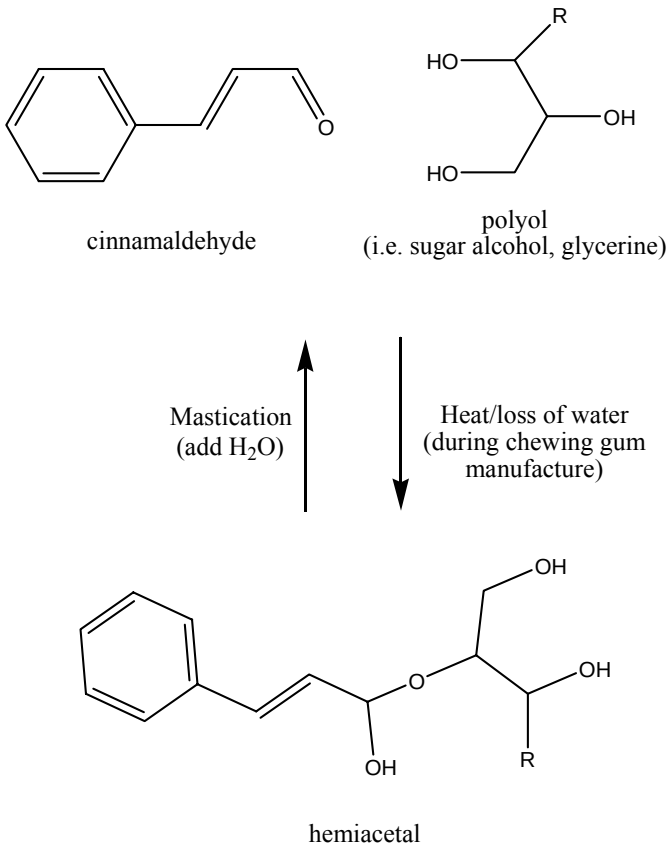


Figure 6-5 Proposed release mechanism for cinnamaldehyde in chewing gum during mastication

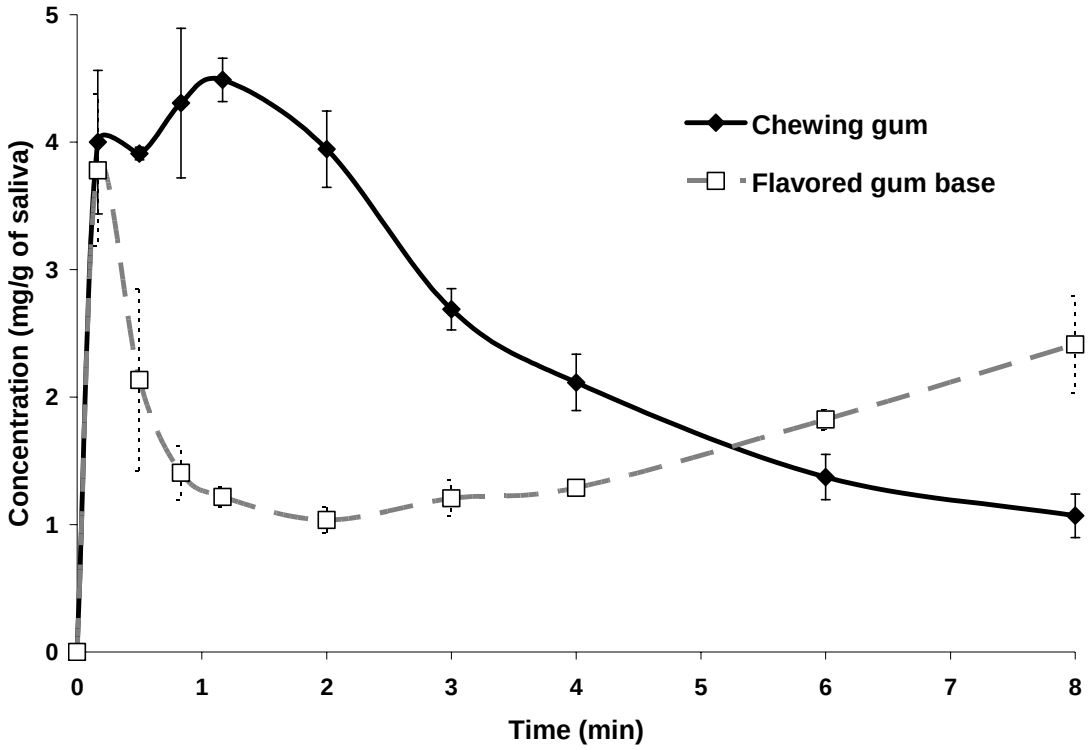


Figure 6-6 Glycerine release in chewing gum and gum base made with MCT; average of triplicates ± 95% confidence interval

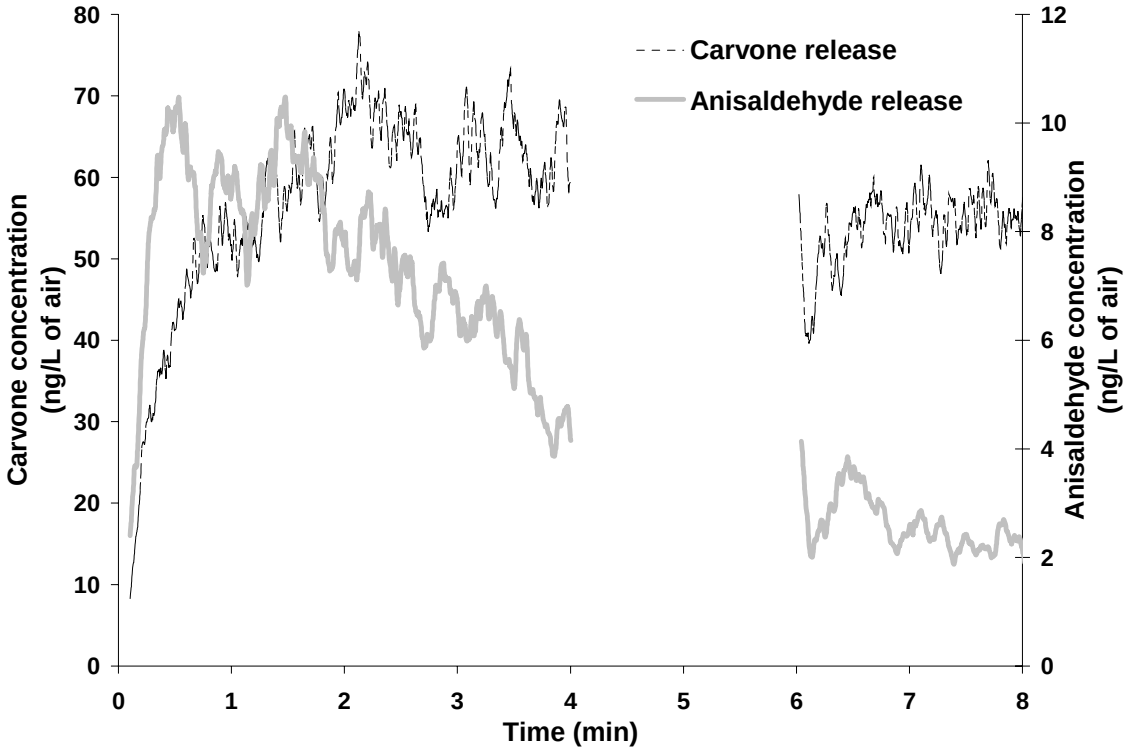


Figure 6-7 Release of anisaldehyde and carvone in chewing gum made with PALOJA gum base for panelist one; each curve represents the mean of three replicates subsequently smoothed by a 3-s moving average trendline

Chapter 7

Future Research

Understanding of taste, aroma and texture aspects on flavor perception from a neural imaging point

By integrating real time instrumental analysis with sensory analysis in Chapter 3, an attempt has been made to understand the influences of taste, aroma and texture properties on flavor perception with chewing gum as a model system. However, a better way to understand this concept will be to conduct experiments by integrating the flavor perception (via sensory and analytical data) with brain imaging using a better model food system. Some of the research questions can be solved by such integration studies are

Research question 1: Does different type of sweeteners (such as sugar, sugar alcohol, and acesulfame/ aspartame) stimulate different active regions the brain? If there do, can they be translated to perceptional differences?

Research question 2: Does presence of sweetener and aroma stimulate different active regions in brain, when presented separately or in combination? If there are differences can these differences be used to explain taste-aroma interactions?

Research question 3: How does the brain respond to the differences in aroma release rates? For example, in chapter 5, the release of cinnamaldehyde release from chewing gum and gum base was very different. One important aspect to understand

would be how these release differences for a aroma compound impact the brain's activity as well as perception.

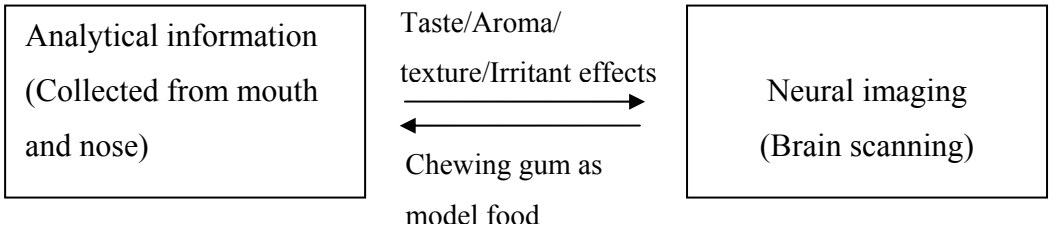
Research question 4: How does the influence of texture-aroma interaction impact flavor perception?

Research question 5: Does presence of irritant along with sweetener and aroma individually or in combination make show any variation compared to research question 2,?

Advantages of using chewing gum as a model system in these studies:

1) Variation in matrix composition with respect to sweetener type, aroma, irritant and texture differences can be easily achieved
2) Provides longer time zone for fMRI scans and analysis
3) Real time temporal influences of taste, aroma or texture variation on flavor perception can be studied by linking analytical techniques with sensory and nueral imaging.

Basic Outline



The formation of transient hemiacetals for aldehydes in chewing gum can be further studied via experiments, where the aldehydes/ketones can be crosslinked with a fatty acid chain. This cross linking process may change the hydrophobicity of the given

aldehdye/ ketone, leading to partitioning differences between gum base and sorbitol phase. These partitioning differences may be reflected in the breath analysis profiles of the given compound, thus providing better insights regarding the formation of hemiacetals.

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Appendix A: Influence of thermal processing conditions on flavor stability in fluid milk: benzaldehyde

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Influence of thermal processing conditions on flavor stability in fluid milk: benzaldehyde

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Abstract:

Flavor loss in dairy products has been associated with enzymatic degradation by xanthine oxidase. This study was conducted to investigate the influence of milk thermal processing conditions (or xanthine oxidase inactivation) on benzaldehyde stability. Benzaldehyde was added to whole milk which had been thermally processed at four different levels (1) none or raw, (2) HTST pasteurized, (3) HTST pasteurized which was additionally heated to 100ºC (PAH) and (4) UHT sterilized. Additionally, PAH and UHT milk samples containing benzaldehyde (with and without ferrous sulfate) were spiked with xanthine oxidase. Azide was also added as an antimicrobial agent (one additional pasteurized sample without) and the microbial load (total plate count) was determined on days 0, 2 and 6. The concentration of benzaldehyde and benzoic acid in all milk samples were determined at day 0, 1, 2, 4, 6 (stored at 5oC) by gas chromatography/mass spectrometry in selective ion monitory mode. Over the six-day storage period more than 80% of the benzaldehyde content was converted (oxidized) to benzoic acid in raw and pasteurized milk, while no change in the benzaldehyde concentration were found in PAH or UHT milk samples. Furthermore, the addition of xanthine oxidase or xanthine oxidase plus ferrous sulfate to PAH or UHT milk samples did not result in benzaldehyde degradation over the storage period.

(Keywords: thermal processing, benzaldehyde, benzoic acid, dairy, flavor degradation)

Abbreviation key: PAH = pastuerized additionally heated to 100ºC, UHT = ultra heat treatment, TPC = total plate count, FE = iron.

1. Introduction

Effective use of flavoring materials is an important parameter for the manufacture of flavor-enriched food products. Ideally, the flavor component of a food product would remain stable from the time of manufacture throughout the anticipated product shelf-life. However, numerous product traits (i.e. matrix composition or processing conditions) can influence product flavor stability. Consequently, defining key modes of flavor loss or degradation would provide useful information for the food industry, such as dairy industry which commonly produces flavor enriched food products (ice cream, flavored milks, yogurt, etc).

Flavor degradation in dairy products has been previously linked to enzymatic reactions (Allen and Wrieden, 1982a, Anklam et al., 1997, Baumgartner and Neukom, 1972, Chevalier, et al., 1972, Gassenmeier, 2003, Jeon, 1993, Kempe and Kohnen, 1999, Ostdal, et al., 2000). Most recently, Anklam et al. (1997) as well as Gassenmeier (2003, 2004) studied the degradation of vanillin to vanillic acid in select dairy products during storage and associated this degradation reaction to oxidative activity of the intrinsic milk enzyme xanthine oxidase. Gassenmeier (2003, 2004) furthermore suggested lipid oxidation derived off-flavor compounds could be generated via a coupled chemical reaction during the enzymatic oxidation of vanillin.

Flavor-protein interactions has been also widely investigated with regards to flavor loss in dairy products (Damodaran and Kinsella, 1980, Fabre, et al., 2002, Guichard and

Langourieux, 2000, Hansen and Heinis, 1992, O'Neill and Kinsella, 1987). Hansen et al. (1996, 1992) reported that the perceived aroma intensity of specific flavor compounds (i.e. vanillin, benzaldehyde, citral and limonene) were reduced in the presence of milk proteins in aqueous solutions. For example, they indicated that the perceived flavor intensity of benzaldehyde was inversely related to the concentration of whey protein while no significant difference in flavor intensity was observed with respect to casein. The flavor binding properties of whey protein have been primarily characterized as reversible hydrophobic binding affects of β-lactoglobulin (Andriot, et al., 1999, Guichard and Langourieux, 2000, Jouenne and Crouzet, 1996, Jung, et al., 2002, Langourieux and Crouzet, 1995).

The current study was based on empirical findings reported by a Penn State Alumni (Dr. Stuart Patton) who observed that Stuzipan Ice Cream [Penn State Creamery; a Marzipan flavored product, (Baumracker, 2000)] had a low overall flavor impact. This suggested that key character impact aroma compounds (i.e. benzaldehyde) may be unstable in dairy products. Because ice cream has a relatively high hydrophobic matrix, flavor losses due to hydrophobic protein interactions between benzaldehyde and β-lactoglobulin would not be anticipated to have a significant impact on the flavor intensity of a full fat ice cream. However, based on previous studies suggesting the oxidation of vanillin to vanillic acid in dairy-based products via intrinsic oxidative enzymes (i.e. xanthine oxidase), it was considered that benzaldehyde may likewise be degraded to benzoic acid by similar pathways. The objectives of this study were therefore (1) to analytically monitor the change in concentration of a benzaldehyde spiked milk sample

and the concomitant formation of benzoic acid over time; and (2) determine if the milk heat treatment influenced benzaldehyde stability (associate with enzymatic inactivation).

2. Materials and Methods

2.1. Materials

Benzaldehyde, benzoic acid and hydrochloric acid (12 N) were purchased from Aldrich (Sigma Aldrich, Milwaukee, WI). Methanol was from Fisher Scientific (Fairlawn, NJ). Hexane and acetone were from EMD Chemicals (Gibbstown, NJ). Methyl hexanoate was purchased from TCI America (Portland, OR). Fresh milk and pasteurized milk were purchased from The Pennsylvania State University creamery (University Park, PA), while UHT milk was from Parmalat USA (Wallington, NJ). 3M Petrifilm for Aerobic Count Plates was from 3M Company (St. Paul, MN). Bovine xanthine oxidase (10 U) was from EMD Biosciences (San Diego, CA). Ferrous sulfate was from J.T.Baker (Phillipsburg, NJ).

2.2. Sample preparation

Benzaldehyde (110 mg/L) was added to four different thermally processed whole milk samples. These different treatments include 1) raw or none, 2) High temperature short time pasteurization (HTST), 3) HTST pasteurization followed by further heating to 100ºC (PAH) and 4) Ultra heat treatment (UHT) and a UHT milk with 40 mU/ml activity of xanthine oxidase. Benzaldehyde was added to all milk samples at or below 5oC.

Sodium azide (200 mg/L.) was added as an antimicrobial agent in each of these samples. An additional pasteurized milk sample without sodium azide was also concurrently analyzed. All the samples were stored at 5ºC in the dark over a 6 day period in 200ml glass jars with Teflon closures and analyzed for the concentration of benzaldehyde and benzoic acids at day 0, 1, 2, 4, and 6.

2.3. Microbial analyses

The 3M™ Petrifilm aerobic count plate were used to enumerate total aerobic microbial plate count (TPC). For counts in 3M™ AC plates, 1 mL of dilutions 1:10, 1:100 and 1:1000 of milk samples were plated using phosphate buffer, KH2PO4 (0.0425 g/L, adjusted to pH 7.2). All plates were incubated at 32ºC ± 2ºC for 48 h. Red colonies on countable plates were enumerated regardless of colony size. Microbial analysis was conducted on days 0, 2 and 6.

2.4. Solvent extraction

Milk samples (20 ± 0.1 g) were acidified to pH 1.5 using hydrochloric acid (1N), in 50 ml centrifuge tubes (Nalgene International, Rochester, NY). This step was followed by a 2 stage extraction process. During the first stage, 8 g of hexane: acetone (10:1) solvent mixture containing N-Caproic acid methyl ester (as internal standard; 100 mg/L) was added to the acidified milk samples. Samples were then centrifuged (7025 g; 12 min) in a Beckman GPR Centrifuge (Palo Alto, CA) and the supernatant solvent mixture was collected. Moisture was removed from the solvent mixture (5g) using 1g of anhydrous Na2SO4.

Second stage solvent extraction includes extraction of flavor compounds into 1 g of methanol from 4 g of hexane/ acetone mixture obtained from the previous step. The methanol extract was then directly analyzed by gas chromatography/mass spectrometry (GC-MS).

2.5. Gas Chromatograpy-Mass Spectrometry

An Agilent 6890 GC (Agilent Technologies, Palo Alto, CA) equipped with a split/splitless injector coupled with a mass selective detector HP 5972A (Hewlett-Packard, Palo Alto, CA), CTC A2000S liquid auto sampler (Leap Tec., Carrboro, NC) and a fused-silica capillary column (DB-FFAP, 30 m, 0.32 mm i.d., 0.32 μm film thickness, Agilent Technologies, California, USA) was used for analysis. The gas chromatography operating conditions were as follows: 1□l of sample was injected in splitless mode; sample tray maintained at 5oC; inlet temperature was 200oC, oven program was 40oC for 2 minutes, then increased at 10ºC/min to 230oC and held for 3 minutes; constant flow rate of 0.7 ml/min (H2). The MS operating conditions were as follows: capillary direct interface temperature was 260°C; for SCAN mode the mass range was 35-200 amu; for SIM mode specific ions monitored were 106 for benzaldehyde 99 for methyl hexanoate (internal standard), and 122 for benzoic acid.

2.6. Quantitative/Qualitative Analysis of Target Compounds

Qualitative analyses as well as quantitative analyses were done using GC-MS, in scan (GC-MS-SCAN) and selective ion monitory (GC-MS-SIM) modes, respectively. Peak areas obtained from MS data were used to determine the concentrations through the use of standard curve based on benzaldehyde (r2 =0.99) as well as benzoic acid (r2 = 0.98)

added to UHT milk and extracted as described in this section. The standards were run in duplicates: 0, 35, 60, 85, 110 mg of benzaldehyde and benzoic acid per liter of milk.

3. Results and Discussion

The stability of benzaldehyde (or its degradation to benzoic acid) in fluid milk samples which were processed at four different levels of thermal treatment over a 6-day period of storage at 5oC are shown in Figures 1-4. The thermal treatment level of the milk sample had a definite affect on the stability of benzaldehyde. For both the raw milk and pasteurized milk samples (containing azide) more than 90% of the benzaldehyde was converted (oxidized) to benzoic acid over the 6-day period (Fig. 1 and 2, respectively). On the contrary, for both the pasteurized milk sample which was additionally heated to 100ºC and the UHT milk sample no degradation of benzaldehyde to benzoic acid over the 6-day period was apparent (Fig. 3 and Fig. 4, respectively). Furthermore, autoxidation lipid degradation products, such as hexanal, were not identified in all the above samples (data not shown) suggesting an alternative degradation pathway.

The microbial analysis of the milk samples (see Table 1) indicated that addition of azide inhibited microbial growth, while for the pasteurized milk sample which contained no azide, microbial growth was evident during storage (as anticipated). Comparison of benzaldehyde stability for the pasteurized milk sample with and without azide (see Fig. 2), showed a lower rate of benzoic formation for the non-azide containing sample. The large viable microbial load in the non-azide containing milk sample may have resulted in a reduced oxygen concentration which retarded the oxidation of benzaldehyde.

The observed improved stability of benzaldehyde in fluid milk samples which had been processed under more severe thermal conditions than HTST pasteurization would be anticipated if enzymatic reactions (i.e. degradation by xanthine oxidase) were responsible for the oxidative conversion to benzoic acid; a reaction that has been previously suggested for the observed conversion of vanillin to vanillic acid in select dairy products. Anklam, et al. (1997), Baumgartner and Neukom (1972), Gassenmeier (2003) and Gassenmeier (2004) all reported the degradation of vanillin to vanillic acid in dairy products and suggested xanthine oxidase as the oxidative pathway. Based on the thermal inactivation of xanthine oxidase (7 min at 73oC or ca. 50 second at 80^{o}C; (Walstra, et al., 1999), both the raw and pasteurized milk samples would be expected to have xanthine oxidase activity while both the UHT milk and the pasteurized with was additionally heated to 100oC, would not. In the current study, however, addition of 40mU/ml xanthine oxidase activity in UHT milk, as previously reported in pasteurized milk (Cerbulis and Ferrell, 1977), was not found to degrade benzaldehyde to benzoic acid at 5oC over 6 day period (data not shown). Possibly, the enzymatic degradation of benzaldehyde via xanthine oxidase is dependant on other matrix parameters such as the availability of transition metals (Allen and Wrieden, 1982a). Milk proteins are known to have relatively strong antioxidant properties, presumably due to their protein-metal chelating activity (Allen and Wrieden, 1982b, Cervato, et al., 1999). Possibly, the metal binding properties of milk proteins increased with higher protein denaturation and therefore may have correlated to the extent of thermal treatment. To suggest if the thermal treatment had an affect on metal availability, PAH milk samples containing XO (40mU/ml) and spiked with ferrous sulfate (100 mg/l) were analyzed over a period of 6

days. Results show no formation of benzoic acid during this time period (Table 2.). Also UHT milk samples containing xanthine oxidase (40mU/ml) when spiked with ferrous sulfate (100 mg/l) similarly showed no changes in benzaldehyde concentration (see Table 2), although it should be noted that the 'available' iron may still be negligible. This implied that xanthine oxidase may not be the direct mode of benzaldehyde oxidation to benzoic acid in milk. Perhaps other pro-oxidative enzymes (either singularly or in combination) or chemical pathways may responsible for benzaldehyde instability in pasteurized milk products.

Historically, a major drawback of high temperature thermal treatment of milk products (i.e. UHT) is the simultaneous generation of thermally generated flavor/off-flavor compounds that ultimately result in the development of negative product traits such as cooked flavor (Beck and Hicks, 1980, Perkins and Deeth, 2001). Recently, Colahan-Sederstrom and Peterson (2004) found the addition of a flavonoid, such as epicatechin, to milk prior to UHT processing inhibited the formation of thermally generated "cooked" aroma compounds (or was similar to pasteurized milk flavor attributes).

4. Conclusions

Fluid milk processed under more severe thermal conditions than HTST processing (i.e. UHT) resulted in improved stability of benzaldehyde. However, the addition of xanthine oxidase (with and without ferrous sulfate) to UHT or PAH milk did not result in benzaldehyde degradation that was observed for the pasteurized or raw milk samples.

While, stability of benzaldehyde was influenced by heat treatment the presence of xanthine oxidase activity was not sufficient to degrade benzaldehyde in milk products as has been previously associated with vanillin degradation in milk products.

Legends to Figures

Figure 1: Degradation of benzaldehyde and formation of benzoic acid in raw milk (▲ legend follow left Y-axis; ■ legend follow right Y-axis

Figure 2: Degradation of benzaldehyde and formation of benzoic acid in pasteurized

milk (with and without azide) (▲legend follow left Y-axis; ■ legend follow right Y-axis;

filled legends represent non-azide samples and open legends azide samples)

Figure 3: Degradation of benzaldehyde and formation of benzoic acid in pasteurized milk heated to 100ºC (no benzoic acid formation) (▲legend follow left Y axis; ■ legend follow right Y-axis)

Figure 4: Degradation of benzaldehyde and formation of benzoic acid in UHT milk (no benzoic acid formation) (▲legend follow left Y axis; ■ legend follow right Y-axis)

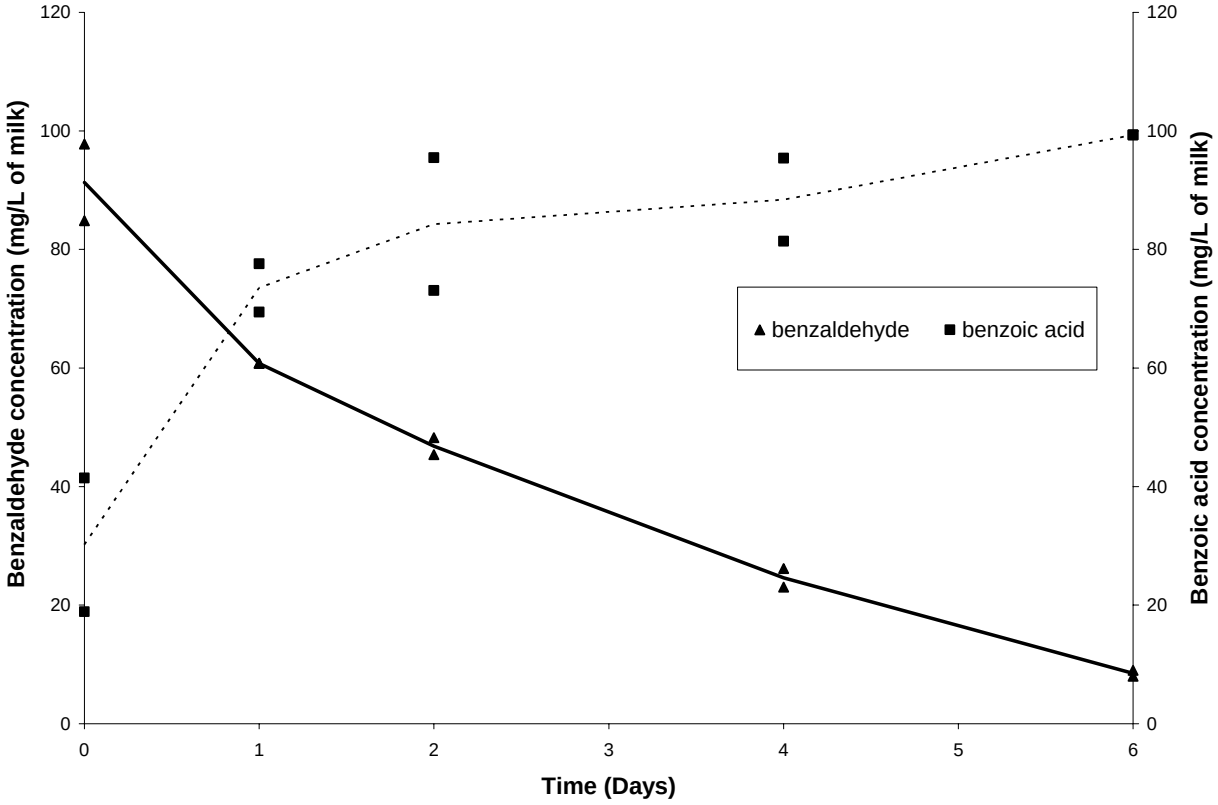
120
100
80
60
40
20
0
Benzaldehyde concentration (mg/L of milk)
Benzoic acid concentration (mg/L of milk)
0
1
2
3
4
5
6
Time (Days)
benzaldehyde
benzoic acid

Figure 1.

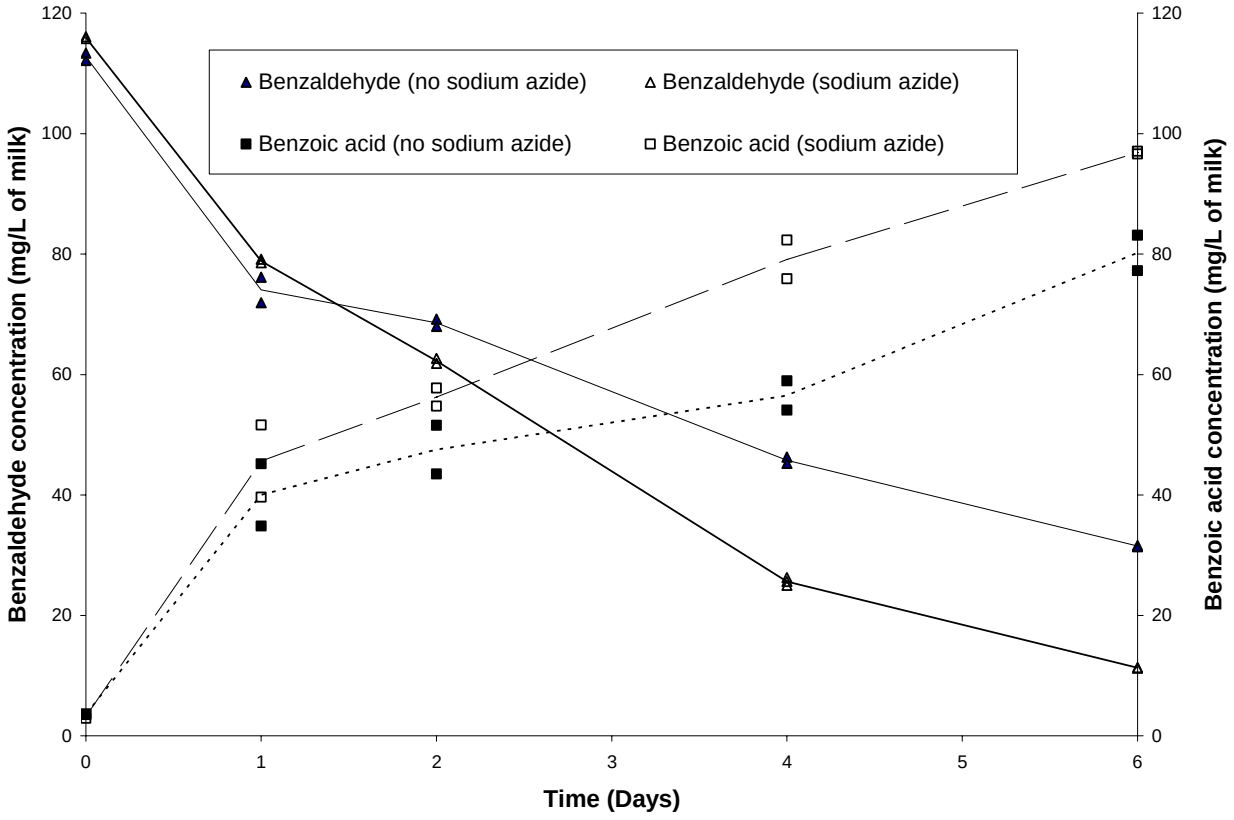
Benzaldehyde (no sodium azide)
Benzaldehyde (sodium azide)
Benzoic acid (no sodium azide)
Benzoic acid (sodium azide)
Benzaldehyde concentration (mg/L of milk)
Benzoic acid concentration (mg/L of milk)
Time (Days)
0
20
40
60
80
100
120
0
1
2
3
4
5
6

Figure 2.

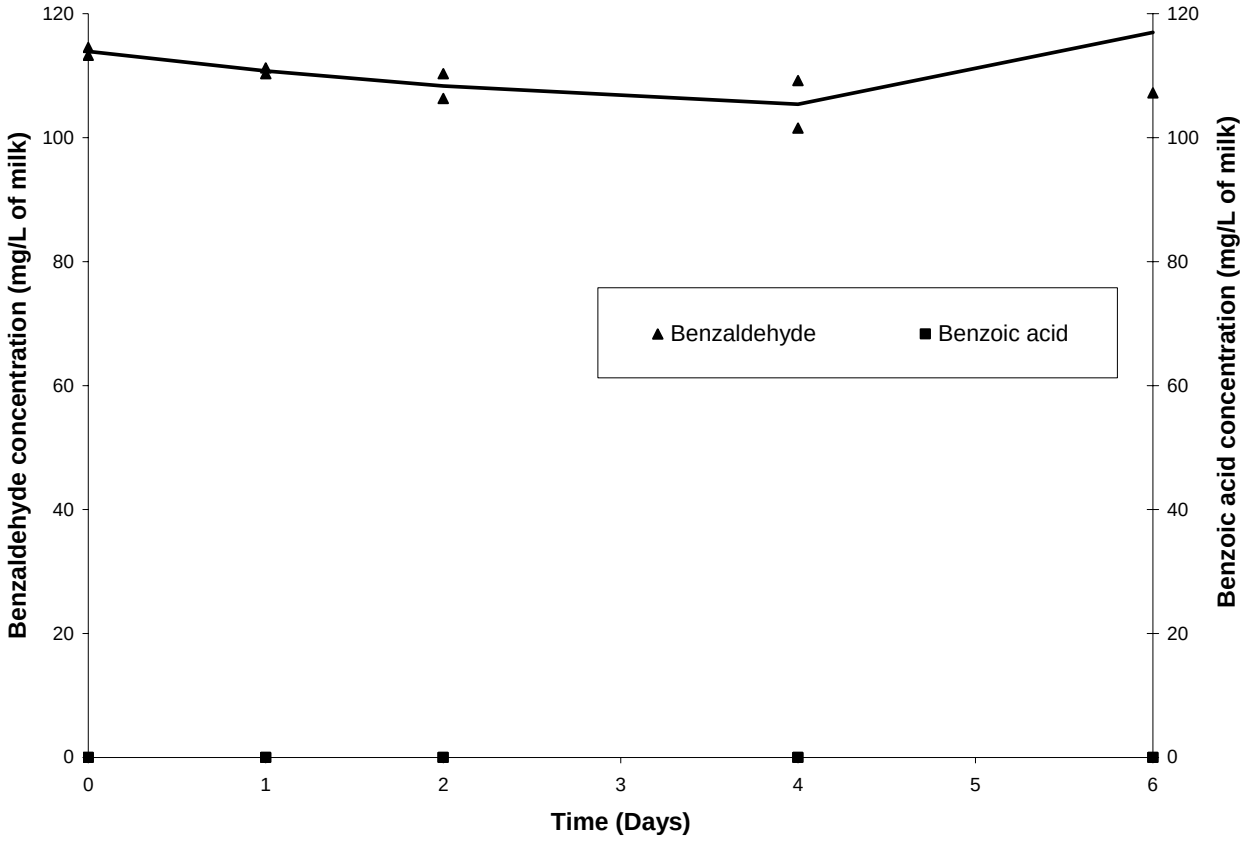
120
100
80
60
40
20
0
Benzaldehyde concentration (mg/L of milk)
Benzoic acid concentration (mg/L of milk)
Benzaldehyde
Benzoic acid
0
1
2
3
4
5
6
Time (Days)

Figure 3.

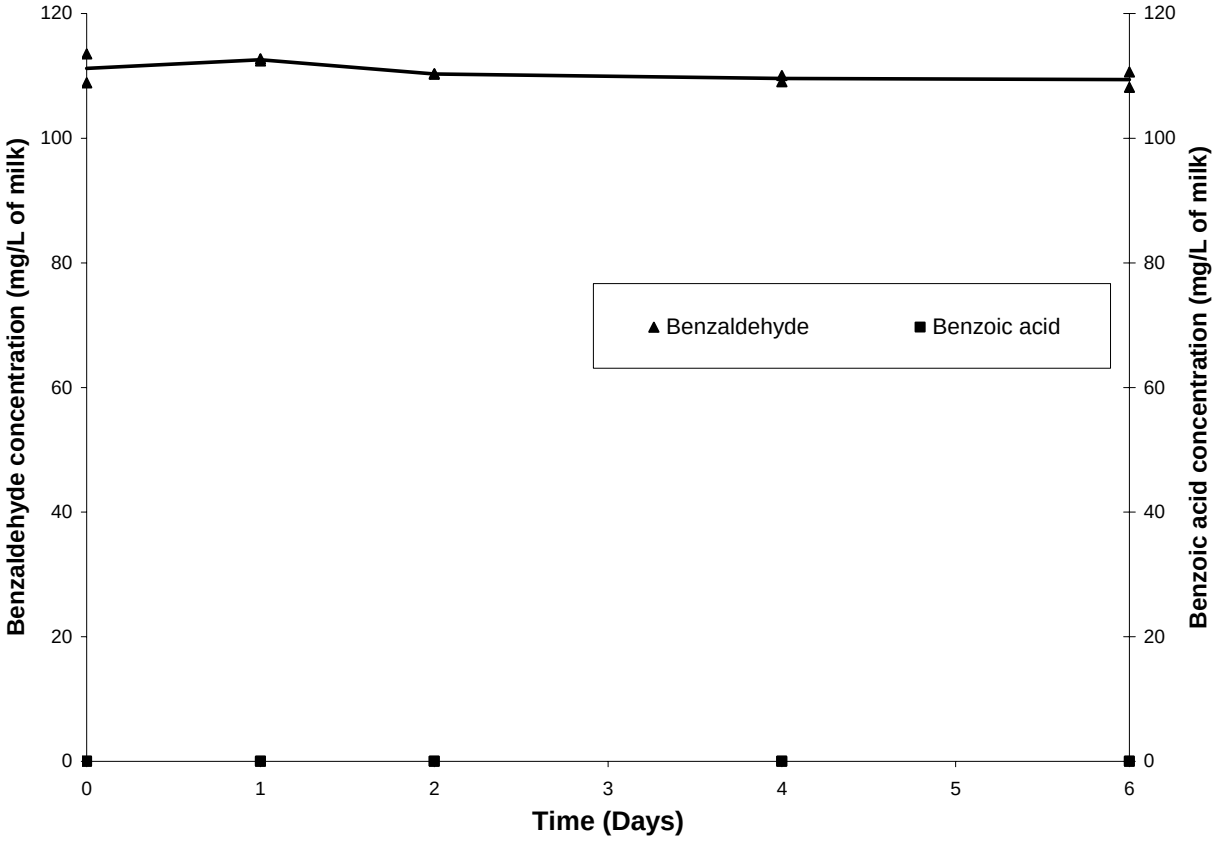
120
100
80
60
40
20
0
Benzaldehyde concentration (mg/L of milk)
Benzoic acid concentration (mg/L of milk)
▲ Benzaldehyde
■ Benzoic acid
0
1
2
3
4
5
6
Time (Days)

Figure 4.

Table 1: Microbial count (Total plate count) for different thermally treated milk at day 0, day 2 and day 6.

Time (days)	Raw milk (With azide) log(cfu)/ml	Pasteurized (No Azide) log(cfu)/ml	Pasteurized (With azide) log(cfu)/ml	Pasteurized Additionally Heated to 100ºC (With azide) log(cfu)/ml	UHT milk (With azide) log(cfu)/ml
0	3.16	2.72	2.20est	ND	ND
2	2.36 ^{est}	2.66	ND	ND	ND
6	1.38^{est}	TNTC	ND	ND	ND

TNTC –too numerous to count; ND- not detected; ^{est}-estimated

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EDUCATION

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PROFILE:

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- Creative experience in product and process development; and sensory evaluation. Awarded first and second prize in NASA FTCSC product development competition in 2 consecutive years (2004, 2003).
- Excellent communication and team work skills. Collectively guided more than 250 people in a variety of departmental short-courses. Published 4 journal articles (2 in progress) and presented at 5 IFT nationwide presentations.
- Strong technical knowledge and operational experience in analytical techniques and instruments (Mass spectroscopy, HPLC, GC, GC-MS, FTIR etc) as well as software programming (C++, JAVA, etc).

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