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Mechanisms of Flavor Release in Chewing Gum: Cinnamaldehyde

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Recently we reported that the release profile of cinnamaldehyde from a sugar-free chewing gum was correlated to the release of the sugar alcohol phase or was not in agreement with the log P model. The objective of this study was therefore to investigate mechanisms of cinnamaldehyde release from a sugar-free chewing gum; *p*-cresol (similar log P value) was also analyzed for comparison. Breath analysis of the chewing gum samples over an 8 min consumption period reported that the maximum concentration of cinnamaldehyde was 2- to 3-fold higher during the initial phase of mastication in comparison to the later phase, whereas the concentration of *p*-cresol was relatively constant over these two time periods. By contrast the release profile of cinnamaldehyde from a flavored gum base (no sugar alcohol phase) was constant over the 8 min consumption period and similar to the release of cresol from the flavored gum base. On the basis of tandem mass spectrometry, cinnamaldehyde was reported to react with sorbitol and generate hemiacetal reaction products that were not stable under slight alkaline conditions; it was suggested to revert back to free cinnamaldehyde and sugar alcohol in the oral cavity. The increased polarity of these transient cinnamaldehyde-sorbitol hemiacetal reaction products would result in a more rapid release rate of cinnamaldehyde than would be typically predicted based on the affinity of cinnamaldehyde for the gum base.

KEYWORDS: Flavor release; flavor perception; breath analysis; chewing gum; time-intensity; cinnamaldehyde; *p*-cresol; hemiacetal reactions

INTRODUCTION

Chewing gum can generally be defined as a two-phase product consisting of a water-insoluble gum base continuous phase and a water-soluble sugar or sugar alcohol discontinuous phase that is typically formulated at a ratio of 1:3 parts, respectively, and contains a flavor load of approximately 1 g/100 g. The distribution of the flavor compounds between the two phases depends on the compound affinity for each phase and historically has been related to the compound hydrophobicity. According to the prior literature, compounds that are more hydrophobic would be predicted to interact more with the gum base, resulting in a relatively lower release rate during mastication (water extraction).

de Roos et al. (*1*) investigated mechanisms of flavor release from chewing gum for a wide range of hydrophobic compounds using a nonequilibrium partition model. According to this 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 weaker relationship with the flavor release measured (*1*). On the basis of this observation, they suggested flavor release after 5 min was diffusion controlled and relied more on mastication efficiency.

Harrison et al. (*2*) also investigated mechanisms of flavor release for various flavor compounds based on gum-to-saliva partitioning coefficient (log cP) in chewing gum model systems. They applied the stagnant layer theory with the interfacial mass transfer from chewing gum to saliva as a rate limiting step for their study. These authors also considered the interaction of flavor compounds with the olfactory epithelium as a controlling factor in the model system. Overall, they concluded that flavor compounds with a lower chewing gum-to-saliva partitioning coefficient were found to release faster and deplete more quickly then those with a higher coefficient value (*2*). 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 (*1, 2*).

Inverse phase chromatography (IGC) has also been applied to understand the interactions between different gum bases and flavor compounds (*3, 4*). Niederer et al. (*3*) 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. On the basis of the thermodynamic data, these authors could predict flavor release. They indicated that a higher affinity between the gum base and flavor molecule leads to slower

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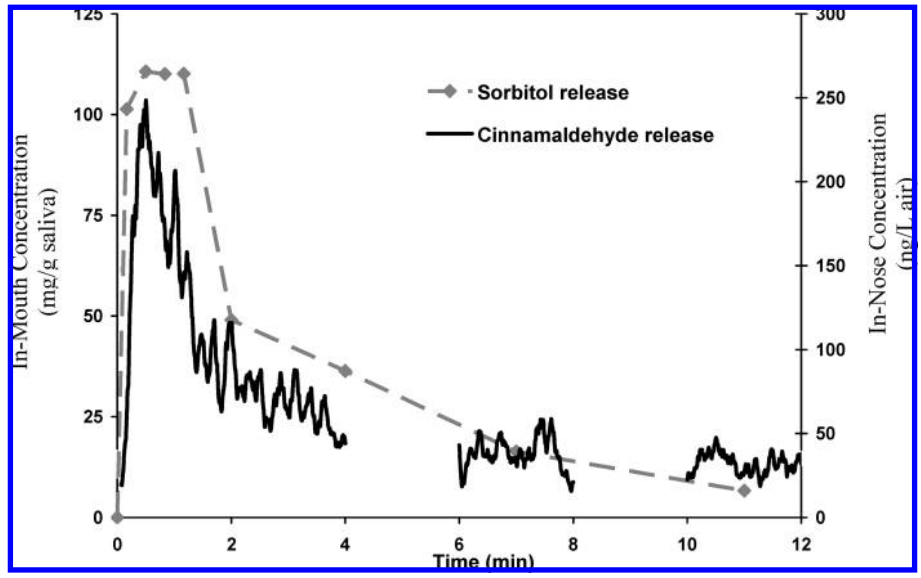

Figure 1. Release profile of cinnamaldehyde and sorbitol from chewing gum; adapted from Potineni and Peterson (*8*).

Table 1. Concentration of Volatile Flavor Compounds in Sugar-Free Chewing Gum Models

	compound concentration[a] (μg/g of chewing gum)					
volatile flavor compound	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)

[a] $n = 5$; average ± 95% confidence interval.

release or long lastingness during mastication and vice versa (*3*). Similarly, Sostmann et al. (*4*) 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 (e.g., pentanol, linalool, benzaldehdye, ethyl benzoate, and eugenol); (2) higher binding to paraffin waxes/lower binding to PVAc (e.g., limonene, ethyl nonanoate, and *p*-cymene); and (3) medium polar compounds with high affinity toward styrene butylene rubber (SBR) (e.g., octanone, tran-2-hexyl acetate, isopropyl-pyridine, octanal, and anethole).

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. In contradiction to these prediction methods, Potineni and Peterson (*5*) reported, however, that the release profile of cinnamaldehyde from chewing gum during mastication was correlated to the sorbitol release rate and was not, as would be predicted, based on the calculated log P value of 1.90 for this compound. Our previously observed release profile for cinnamaldehyde and sorbitol from chewing gum is illustrated in **Figure 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).

MATERIALS AND METHODS

Materials. Cinnamaldehyde, anisaldehyde, and L-carvone were 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), and acesulfame-K was from Wintersun Chemical (Ontario, CA).

Chewing Gum Models. Chewing gum was manufactured according to the procedure previously reported by Potineni and Peterson (*5*). The chewing gum ingredient formulation consists of gum base (PALOJA 30 g/100 g), sorbitol crystals (22.7 g/100 g), saturated sorbitol solution (15 g/100 g), xylitol (15 g/100 g), mannitol (11 g/100 g), glycerine (4 g/100 g), flavor mixture (see **Table 1**), flavor solvent (1 g/100 g; MCT), Lecithin (0.10 g/100 g), aspartame (1 g/100 g), and acesulfame-K (0.2 g/100 g). In brevity, the gum base was heated to approximately 100 °C in a gum mixer (Littleford Day gum mixer; Florence, KY), the heat was turned off, and under constant mixing the emulsifiers/Lecithin was added, followed by 50% of the sugar alcohol phase, at 75 °C; the flavor compounds were added followed by the remaining sugar alcohol, and finally the rest of glycerin, MCT, aspartame, and acesulfame-K were added. The resultant chewing gum was rolled and sheeted, conditioned at 45% humidity for 12 h, and cut into commercial sized sticks. The chewing gum samples were wrapped in aluminum foil and stored at 21 °C at 35% (±10) relative humidity prior to analysis (<4 months). Different chewing gum samples were made by varying the concentration of cinnamaldehyde or *p*-cresol (shown in **Table 1**).

Flavored Gum Base Model. Fifty grams of Paloja gum base was softened by heating on a gas stove (Empire comfort systems, Belleville, IL) to 75 °C. Two flavored gum base models were made [gum base model 1: 7524 (±173) μg of cinnamaldehyde/g gum base; gum base model 2: 784 (±108) μg of cresol /g gum base)]; the amount of flavor solvent (Medium chain triglycerides or MCT), glycerin, cinnamaldehyde, and cresol were added at the equivalent concentration as chewing gum model 1 (cinnamaldehyde) and model 2 (cresol) (shown in **Table 1**). Molten gum base along with added ingredients were stirred (5 min) and then poured on a slab covered with a parchment paper (Alcoa Inc.,

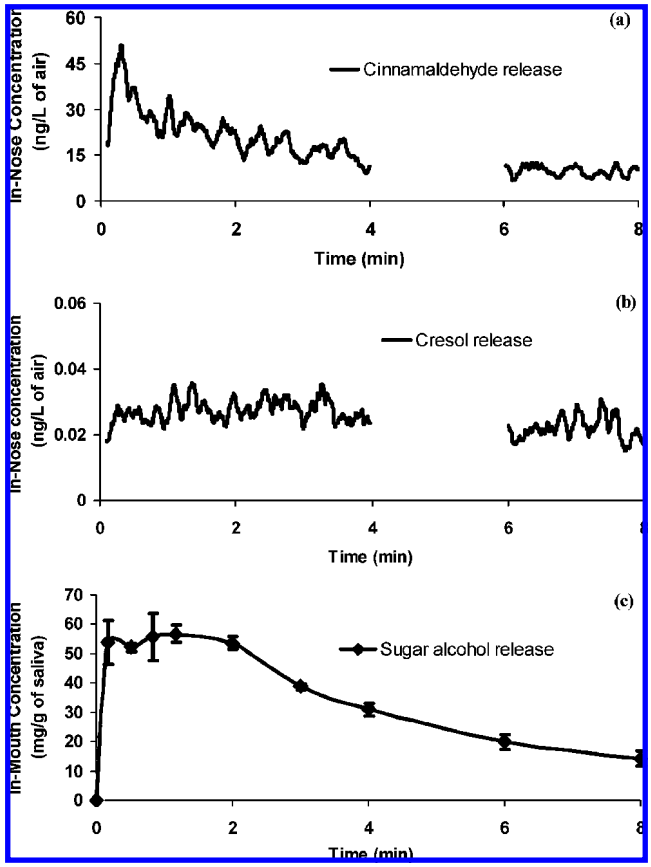

Figure 2. Cinnamaldehyde, cresol, and total sugar alcohol release profile from chewing gum sample for one panelist; **(a)** and **(b)** each curve represents the mean of three replicates subsequently smoothed by a 6 s moving average trendline, **(c)** curve represents the mean of three replicates ±95% confidence intervals.

Richmond, VA) to an approximate thickness of 0.25 cm by gently shaking the slab. After cooling, the flavored gum base samples were stored in glass bottles with Teflon lined lids.

Quantification of Cinnamaldehyde/Cresol in Chewing Gum or Flavored Gum Base. For the chewing gum, 5 gum pieces per sample were analyzed from a box of 50 (every 10th piece of gum was sampled) whereas for the gum base samples, 3 pieces were randomly selected for analysis. The quantification procedure used was as previously reported by Potineni and Peterson (*5*). In brevity, samples were dissolved in hexane, centrifuged, the supernatant was mixed with methanol, recentrifuged, and the supernatant of this methanol−hexane mixture was analyzed by gas chromatography.

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 15 psi (He); 1 μL of sample was injected in split mode (1:20).

Log P Analysis. The log P values for cinnamaldehyde and *p*-cresol were determined by a shake flask method as previously reported by Griffin et al. (*6*). Equivalent amounts of octanol containing each flavor compound (100 mg/L) and distilled water were added together in a volumetric flask. The resulting two-phase system was shaken gently for 1 h on an orbital shaker (Laboratory-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 reported above with one exception, the initial temperature was 100 °C.

Log cP Analysis. One hundred grams of gum base were ground in a blender (Waring blender, Torrington, CT) and subsequently sieved using a sieve shaker (W.S. Taylor Ltd., Gastonia, NC) with sieve number 40− 70 to obtain a 212−425 μm particles size subsample. All cP analyses were conducted with this sieved sample fraction.

Gum Base-to-Water Partitioning Coefficient (Log cP). Gum base (9 g) was suspended in 100 mL of water containing cinnamaldehyde or *p*-cresol 0.1 g/100 g in a 125 mL flat bottomed flask with glass stopper. Flasks were shaken gently in a water bath set at 38 °C, and at regular intervals (0, 1, 4, 8, 12, 24, 48, and 60 h), 350 μL of the solution was 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

Table 2. Log P and Log cP Values for Cinnamaldehyde and *p*-Cresol

	log P value			log cP value	
				aqueous phase	
	predicted[a]	experimental[b,c]	water[b]	sugar alcohol[b,d] (6.6%)	sugar alcohol + glycerine[b,d] (6.6 + 0.4%)
cinnamaldehyde	1.90	1.00 (±0.08)	1.22 (±0.03)	1.22 (±0.02)	1.24 (±0.03)
p-cresol	1.94	1.20 (±0.11)	0.90 (±0.05)	1.02 (±0.07)	0.73 (±0.10)

[a] Estimated values from Hansch et al. (*6*). [b] Average of triplicate ± 95% confidence interval. [c] Shake-flask method (octanol/water) (*9*). [d] Average max concentrate reported in the saliva for chewing gum samples in this study for panelist 1.

Table 3. Maximum Average Concentration of Cinnamaldehyde and Cresol Monitored from the Nose at 0–4 and 6–8 min from Chewing Gum and Gum Base during Mastication

	in-nose compound concentration[a] (ng/L air)								
	panelist 1			panelist 2			panelist 3		
sample	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)
				Chewing gum					
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)

[a] Average of triplicate ± 95% confidence intervals.

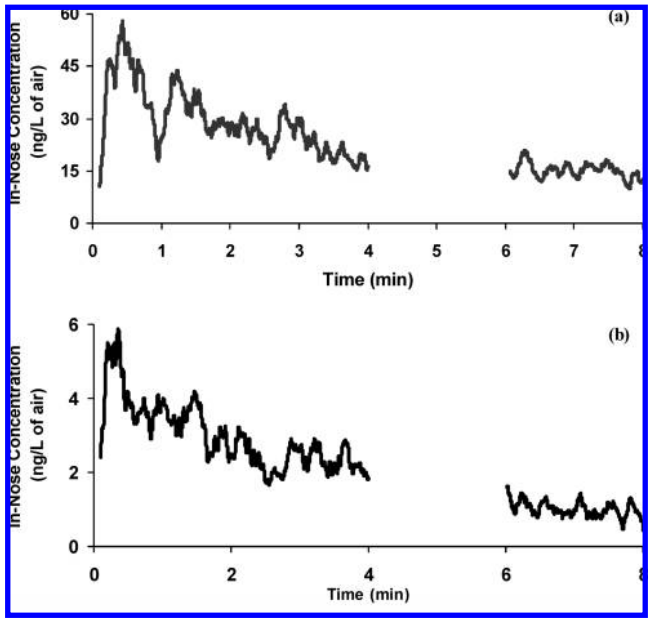

Figure 3. Release profile of cinnamaldehyde from chewing gum at two different cinnamaldehyde concentrations, **(a)** 2860 μg/g chewing gum and **(b)** 288 μg/g chewing gum, for one panelist; each curve represents the mean of three replicates subsequently smoothed by a 6 s moving average trendline.

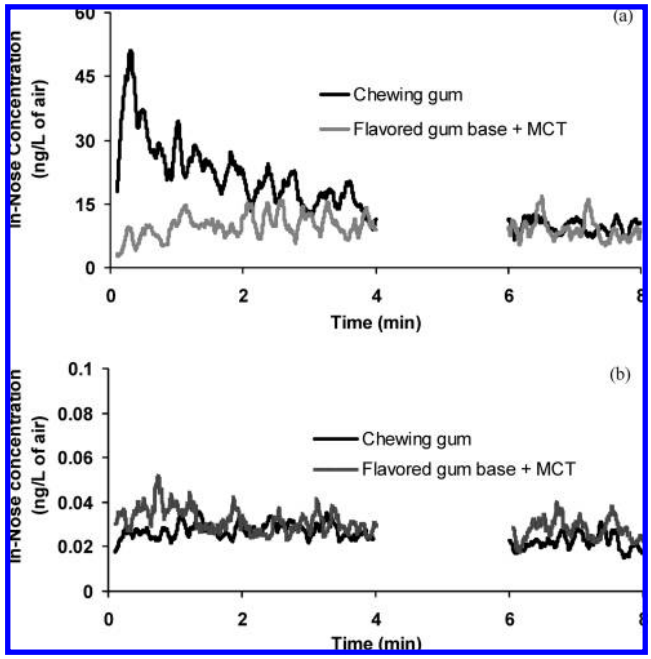

Figure 4. Release profile of **(a)** cinnamaldehyde and **(b)** cresol release from gum base with MCT for one panelist; **(c)** cinnamaldehyde or cresol release profile from chewing gum (**Figure 2**) was also illustrated for comparison; each curve represents the mean of three replicates subsequently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

100% over 25 min. The flow rate was 1 mL/min and the injection volume was 20 μL. Partition coefficients were determined from the data after 60 h of equilibrium equilibration (previous analysis of selected time points between 0 and 60 h indicated that a steady state was reached). The log cP value was calculated using the following equation:

$$\log \mathrm{cP} = \log\left(\frac{\text{mg flavor/g gumbase}}{\text{mg flavor/g aqueous solution}}\right)$$

Gum Base-to-Sugar Alcohol Solution or -Sugar Alcohol/Glycerine Solution Partitioning Coefficient. The same procedure as described for

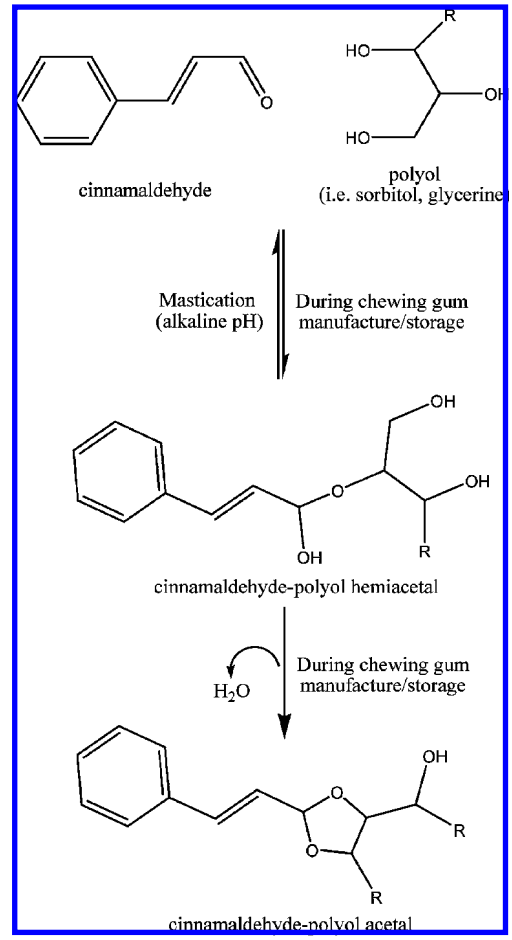

Figure 5. Mechanisms of cinnamaldehyde reactivity in chewing gum during manufacture/storage as well as during consumption (mastication).

the "Gum base to water partitioning coefficient" method was used with the following exception: the 100 mL aqueous phase also consisted of sorbitol (3.5 g/100 g), xylitol (1.8 g/100 g), and mannitol (1.3 g/100 g) with or without glycine (0.4 g/100 g). The proportion of sugar alcohol mixture or glycine was based on the maximum concentration determined in the expectorant saliva, data not shown (at retention time 70 s, **Figure 2c**).

Breath Analysis. The release profile of the exhaled aroma compounds during sample mastication were determined with a modified atmospheric pressure chemical ionization-mass spectrometer (APcI-MS) as previously described by Schober and Peterson (*7*). 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 (*5*). In brevity, chewing gum samples (2.5 g) or gum bases (1 g) were masticated at the rate of 60 chews/min by 3 panelists (1 male and 2 female), while breathing normally and keeping their mouths closed. The breath from the nose was directly and continuously sampled via an interface set at 80 °C into the ZMD 4000 Micromass–mass spectrometer (Waters, Milford MA) at time intervals of 0–4 and 6–8 min. The APcI operating conditions are as follows: SIM mode; breath sampling flow rate was 200 mL/min; block temperature is 100 °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 and 30 V 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 Potineni and Peterson (*5*). Quantification of cinnamaldehyde and cresol were determined via standard calibration curve; 0.5, 1, 5, 10, 30, 48 μL of a 0.02 g cinnamaldehyde/ml pentane and 0.1, 0.5, 1, 5, 10 μL of 0.005 g cresol/mL pentane was injected into a airtight water-jacketed 1.1 L deactivated glass vessel (*7*) 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 as described above.

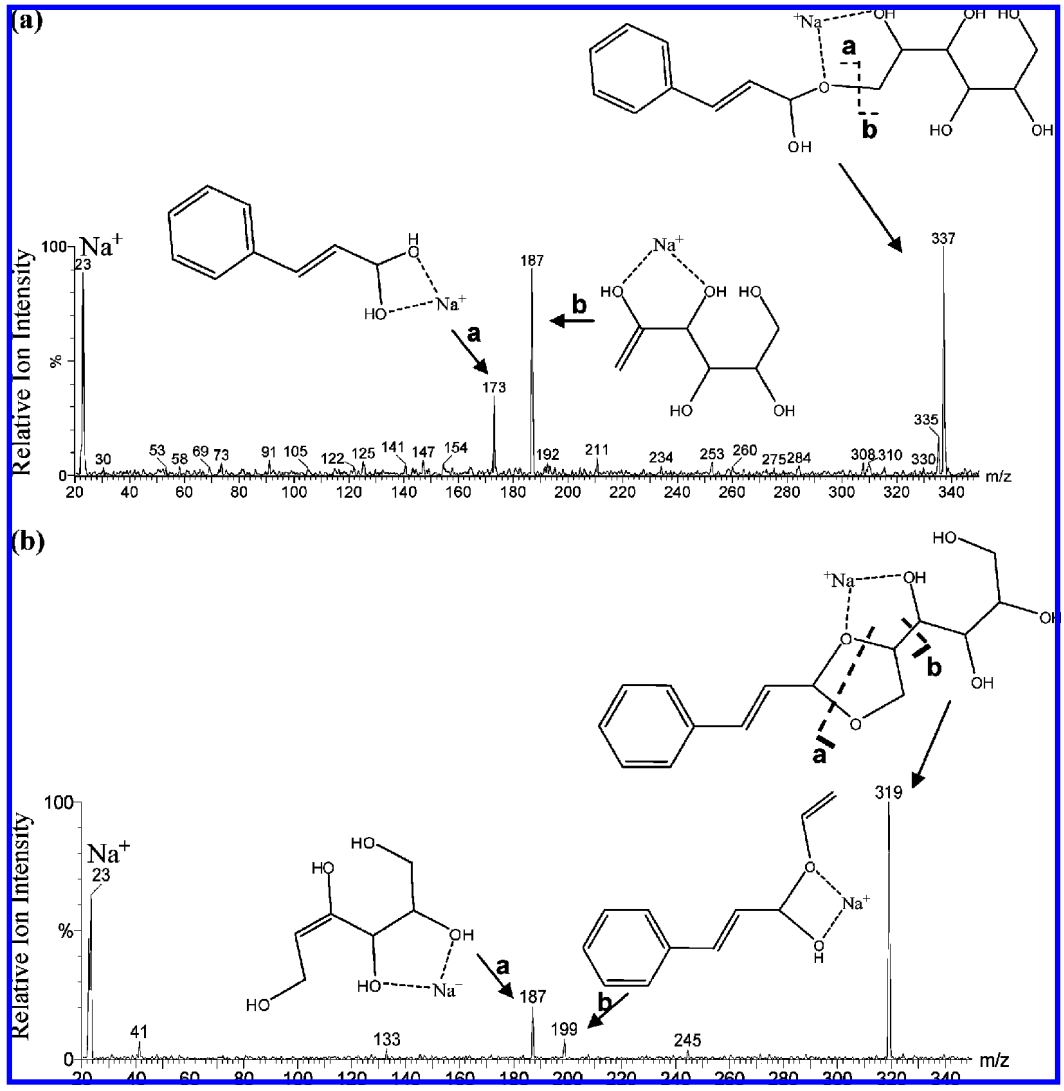

Figure 6. Sibling ion spectrum of the cinnamaldehyde-sorbitol hemiacetal *m/z* ion 337 **(a)** and the cinnamaldehyde-sorbitol acetal *m/z* ion of 319 **(b)** extracted from sorbitol/cinnamaldehyde chewing gum model.

The peak height (ion intensity) versus μg weight of each compound per L of air was plotted (all compounds reported an $r^2 > 0.99$).

For the breath analysis data, evaluations were also conducted on the maximum average aroma concentration in the breath for each panelist based on a 6 s moving average trendline from 0 to 4 min (termed "max1") and from 6 to 8 min (termed "max2"), and the ratio (termed "max1/max2") for chewing gum and gum base made with cinnamaldehyde or cresol.

Sugar Alcohol Release and Glycerine Analyses. The concentration of sorbitol, xylitol, mannitol, and glycerine was determined in expectorated saliva of three panelists while chewing a 2.5 g piece of chewing gum sample over an 8 min time period. The same chew/swallow protocol (*5*) was used for saliva collection. In brevity, three panelists expectorated saliva at regular intervals at 0, 10, 30, 50, 70, 110, 180, 240, 360, and 480 s which were collected into spit cups with lids. Saliva (0.5 g) was immediately transferred into a centrifuge tube containing 1 mL of 0.1 g/100 g of formic acid, centrifuged at 11 750 rcf for 3 min before the supernatant was transferred into 2 mL amber bottles. All analyses were conducted in triplicate. The sugar alcohol concentration was determined by HPLC analysis using an external standard curve at 6, 13, 25, 38, 50 g/L for sorbitol or mannitol or xylitol, and 0.02, 0.04, 0.09, 0.2, 0.4, 0.7 g/L for glycerine plotted versus peak area ($r^2 > 0.99$). HPLC analysis was performed on a LC column Supelcogel-H (5 μm, 250 × 4.6 mm i.d.,) using an isocratic run with 0.1 g/100 g formic acid in water as the mobile phase maintained at 40 °C. The flow rate was 0.17 mL/min and the injection volume 10 μL.

Analysis of Sorbitol-Cinnamaldehyde Reaction Products in Model and Chewing Gum Samples. A simplified chewing gum model system consisting of 90 g of sorbitol and 20 g of a sorbitol solution (70 g/100 g water) was heated to 75 °C; immediately, the heat was removed and 6 mg of cinnamaldehyde/g of sugar alcohol mixer was added and mixed for 5 min. A duplicate sample without cinnamaldehyde was also manufactured. The samples were immediately stored in a glass jar with Teflon-lined lid. Prior to mass spectrometry (MS) analysis, 0.1 g of the model mixture was added to 1 g of acetonitrile (with added anhydrous Na_2SO_4) and the liquid layer was directly analyzed.

For chewing gum, 5 g of sample was crushed under liquid N_2 with a mortar and pestle and added to 10 g of acetonitrile (with anhydrous Na_2SO_4). The decanted extracts were filtered; anhydrous Na_2SO_4 was added and stored in 2 mL glass vials prior to MS analysis.

High Performance Liquid Chromatography (HPLC). 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-10A).

Mass Spectrometry (MS). Analyses were conducted with a Waters Quattro Micro triple quadrupole instrument (Milford, MA) equipped with an electrospray probe. Samples were directly injected with an integrated Rheodyne injector (10 μL loop), and the mobile was 75% acetonitrile and 25% water at 200 μL/min using a Shimadzu LC-10ADvp pump. The MS conditions were as follows: positive ion mode, capillary voltage (3.5 kV); source temperature (100 °C); probe temperature (250 °C). For samples analyzed in scan mode the scan

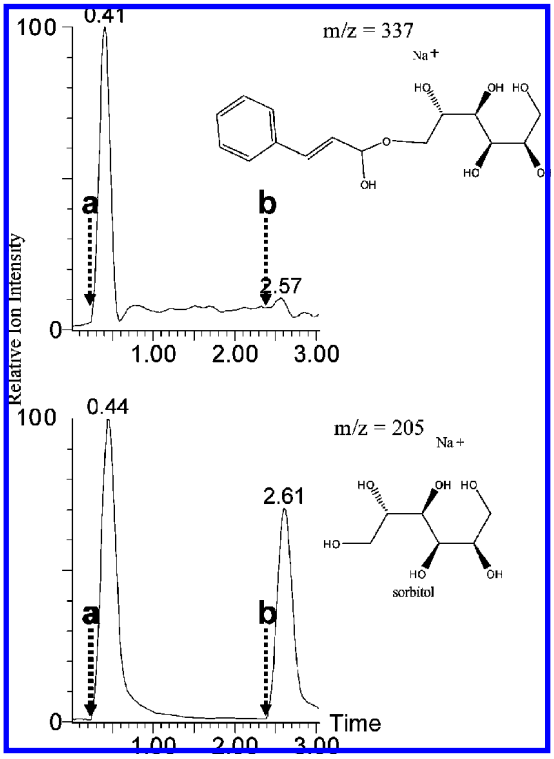

Figure 7. Ion chromatogram of the cinnamaldehyde-sorbitol adduct (M + Na, *m/z* 337, **top**) and sorbitol (M + Na, *m/z* = 205, **bottom**) from an acetonitrile extract ("a" = injection time) and an acetonitrile extract plus the addition of 25% by volume of simulated saliva ("b" = injection time; 15 mM sodium bicarbonate, pH 7.4); analyzed by direct injection.

range was 100–1000 Da, while for sibling ion analysis of *m/z* 327 or 319, the CID was $3.4e^{-4}$, collision voltage was 21 V, and the sibling ions were scanned over a range *m/z* 20–500.

RESULTS AND DISCUSSION

The release properties of flavor compounds from chewing gum are commonly predicted by log P values. These hydrophobicity values can be derived from either computational chemistry techniques such as the quantitative structure–activity relationship (QSAR) method (*8*) or by experimental determination (*6*). Both the predicted and experimental log P values for cinnamaldehyde and *p*-cresol are listed in **Table 2**. Although the experimental values were found to be lower than the predicted values, both methods indicated that these two flavor compounds would be of similar hydrophobicity. However, considering that the gum base is not octanol, perhaps a better prediction method would measure the binding affinity of the flavor compounds to the gum base as reported previously (*1, 2*) (known as log cP; distribution between the gum base and aqueous phase). The log cP values for both compounds were also determined and are reported in **Table 2**. 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 2**), implying that the release of these compounds from chewing gum would be comparable if dictated by gum base interactions. It was furthermore considered that the use of water and gum base as a model to predict flavor release may also be too simplistic as the saliva phase would contain other water soluble compounds (i.e., sugar alcohols, glycerin) from the chewing gum which may alter the affinity of aroma compounds 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 aqueous phase contained sugar alcohol or sugar alcohol plus glycerine at levels reported in the saliva phase during mastication (**Table 2**). The log cP value of cinnamaldehyde was not found to be influenced by the addition of sugar alcohol or sugar alcohol and glycerin, whereas the affinity of *p*-cresol for the gum base was lowest for the aqueous model containing sugar alcohol and glycerin. Consequently, based on log P or log cP values determined (**Table 2**) for cinnamaldehyde and cresol and previous flavor release prediction models used for chewing gum (*1, 2*), it would be anticipated that the release of cinnamaldehyde during the mastication of chewing gum would be comparable or even relatively slower than that for cresol.

To test these prediction models, the release profile of cinnamaldehyde and cresol as well as the sugar alcohols (sorbitol, xylitol, and mannitol) from chewing gum models 1 and 2 (**Table 1**) over an 8 min consumption time interval was determined; the data for one panelist is shown in **Figure 2**. Similar to our previous findings (**Figure 1**), the release of cinnamaldehyde correlated to the release profile of the sugar alcohol; both showed a more rapid release rate initially and subsequently decreased to approximately 20–30% of the initial concentration maximum over the 8 min consumption time period (**Figure 2**; calculation not shown). The correlation between cinnamaldehyde and the sugar alcohol phase was, however, observed to be greater for the chewing gum samples we previously investigated (**Figure 1**) in comparison to those in this study (**Figure 2**). In the current study, chewing gum was formulated with three sugar alcohol compounds (sorbitol, xylitol, and mannitol) and Paloja gum base, whereas the previous samples contained only one sugar alcohol, sorbitol, and VHI gum base. Perhaps the change in the sample formulation may have related to the different degrees of correlation between cinnamaldehyde and the sugar alcohol phase in these two studies. Nonetheless, for both of these chewing gum samples, the release profile of cinnamaldehyde was similar to that of the sugar alcohol phase.

The average maximum cinnamaldehyde and cresol concentration as measured from the exhaled breath from the nose from 0 to 4 min (max1) and from 6 to 8 min (max2) as well as a ratio of max1/max2 are also presented in **Table 3** for all three panelists. The max1/max2 ratio was used as an indication of the flavor compound release profile; a number greater than 1 indicated that the compound has decreased in concentration for the second time period, indicating it was released more rapidly initially, whereas a number of approximately 1 indicates a the release rate was stable or consistent over time. Notably, the max1/max2 ratio values for cinnamaldehyde were approximately 2–3 and higher than for cresol (approximately 1) for all 3 panelists, which indicated the release of cinnamaldehyde was more rapid than cresol during the initial consumption period. These findings indicated that log P or cP value (thermodynamic model) was not accurate in predicting the release of cinnamaldehyde in comparison to cresol.

Because the concentration of cinnamaldehyde was approximately 16-fold higher than cresol in the chewing gum model analyzed in **Figure 2** (see **Table 1** – model 1 and model 2; simulated a commercially flavored product), the influence of cinnamaldehyde concentration on flavor release was further investigated. The release profile of cinnamaldehyde from chewing gum samples containing 0.2880 mg and 2.860 mg/g chewing gum (**Table 1** – model 3 and 4) are illustrated in **Figure 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

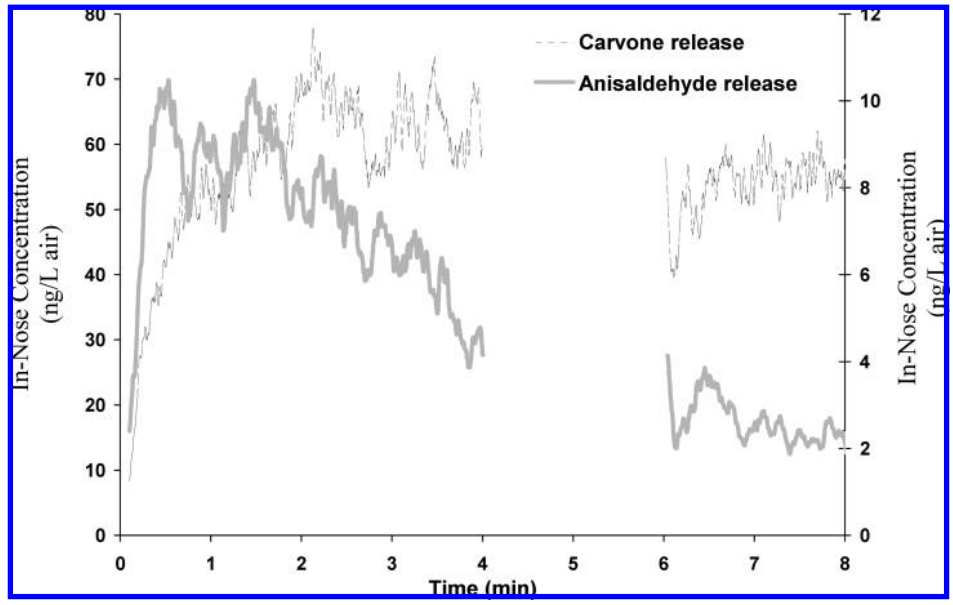

Figure 8. Release of anisaldehyde (right axis) and carvone (left axis) in chewing gum made with PALOJA gum base for panelist one; each curve represents the mean of three replicates subsequently smoothed by a 6 s moving average trendline.

for all 3 panelists (data not shown). The lowest concentration of cinnamaldehyde in chewing gum (**Table 1** – model 3) was at a similar concentration level as cresol in chewing gum model 2 (**Table 1**; between 0.1 and 0.3 mg/g chewing gum) and further supported that the different release properties reported for these two compounds in **Figure 2** were not due to any concentration effects.

To further validate that cinnamaldehyde release from chewing gum was not adequately predicted by log cP or its affinity for the gum base, the release profile of cinnamaldehyde or cresol from just the gum base with MCT, (model 1: 7524 (±173) μg of cinnamaldehyde/g gum base, model 2: 784 (±108) μg of cresol/g gum base) was determined and reported in **Figure 4a,b** (the release of these compounds from chewing gum is also shown for direct comparison). The maximum breath concentrations for both of these compounds at 0–4 and 6–8 min and the ratio of these values are reported for these flavored gum base samples in **Table 3**. Notably, the release of cinnamaldehyde from the gum base was relatively constant over the 8 min consumption time period compared to when in chewing gum, whereas for cresol both the gum base and chewing gum reported very similar release profiles (**Figure 4a**; **Table 3**). Consequently, the release properties of cinnamaldehyde from the gum base were very similar to cresol, as would be predicted based on the log P or cP values.

On the basis of these observations, it was proposed that cinnamaldehyde generated transient hemiacetals with the sugar alcohol phase during chewing gum manufacture or storage which during mastication were converted back to cinnamaldehyde and the corresponding alcohol (under alkaline pH conditions of the oral cavity); the hypothesized mechanism is illustrated in **Figure 5**. In theory, any alcoholic compound such as glycerin (4.0 g/100 g of the chewing gum composition) may also be involved in this hemiacetal reaction. However, based on the predicted log P values using the software program Chemdraw (Ultra 10, Cambridgesoft, Cambridge, MA), the hemiacetals formed with glycerin (log P = 1.21) were likely not hydrophilic enough in comparison to the hemiacetals formed with sorbitol (log P = −0.4) to be correlated to the release profile of sugar alcohol phase (sorbitol, log P = −2.94). The sugar alcohol phase was also the most abundant alcohol precursor in these samples.

To analytically investigate the reaction products in **Figure 5**, simplified chewing gum models consisting of only sorbitol with and without cinnamaldehyde were used for analysis. These models systems were processed under similar heating/mixing conditions as the chewing gum samples made in the current study. Extracts of these samples were subsequently directly analyzed by mass spectrometry. Sorbitol was easily detected as a monosodiated adduct ion at *m/z* of 205 [$(M + Na)^+$, 100% relative abundance)] and a monosodiated cluster ion at *m/z* of 387 [$(2\ M + Na)^+$, 14% relative abundance] by mass spectrometry for both models with and without cinnamaldehyde addition. For the model system with cinnamaldehyde, both the predicated pseudomolecular ion for the sorbitol-cinnamaldehyde hemiacetal [*m/z* 337, $(M + Na)^+$, 15% of the relative abundant of *m/z* 205] as well as the sorbitol-cinnamaldehyde acetal [*m/z* 319, $(M + Na)^+$, 50% of the relative abundant of *m/z* 205] were also observed. Each of these predicted hemiacetals and acetals reaction products were subsequently analyzed by tandem mass spectrometry and the sibling ion scan of *m/z* 337 and 319 are shown in **Figure 6**. The fragments generated for both of these ions (*m/z* 337 and 319) were in agreement the structures proposed in **Figure 5**. Acetal reaction products between cinnamaldehyde and propylene glycol have been previously reported (*9*). To further support the reversibility of the hemiacetal reaction product during mastication (**Figure 5**), the stability of this product (*m/z* 337) under alkaline pH conditions (pH 7.4) previously reported in the oral cavity (*10*) was determined by mass spectrometry (shown in **Figure 7**). The addition of a simulated saliva to the extract resulted in a loss of the *m/z* 337 $(M + Na)^+$ hemiacetal sorbitol-cinnamaldehyde reaction product while the monosodiated sorbitol adduct ions at *m/z* of 205 was only suppressed in height by approximately 25% (due to sample dilution). The sorbitol-cinnamaldehyde acetal product (*m/z* 319) was also stable after the addition of the simulated salvia as would be anticipated (was the same as the sorbitol response illustrated in **Figure 7**; data not shown). Both of these reaction products were also identified in the

extracts of the chewing gum used in this study based on matching the daughter ion scan of both *m*/*z* 319 and 327 shown in **Figure 6**.

To further explore the reactivity of hemiacetal reactions between flavor compounds containing a carbonyl group with the sugar alcohol phase of chewing gum, the breath release properties from chewing gum made with an aldehyde (anisaldehyde – model 5, **Table 1**) and a ketone (L-carvone; model 6, **Table 1**) were determined, see **Figure 8**. As predicted, anisaldehyde had a similar release profile as cinnamaldehyde. However, L-carvone did not follow the release pattern of cinnamaldehyde and suggested this was due to the lower reactivity of ketones, due to methyl inductive effects, to form hemiacetals in the sorbitol phase. Furthermore, this would also suggest that similar carbonyl-containing flavor compounds could be added to chewing gum in a hemiacetal state with a specific hydrophobicity by altering alcoholic hemiacetal moiety ultimately to tailor their release properties.

In summary, 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 (dominant mechanism during the initial stage of mastication) and (2) the release from the gum base as predicted by the log cP value (after the dissolution of the sugar alcohol phase).

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