JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Influence of Strawberry Yogurt Composition on Aroma Release

JENNIFER B. MEI,[†] GARY A. REINECCIUS,*[,†] W. BERK KNIGHTON,[§] AND ERIC P. GRIMSRUD[§]

Department of Food Science and Nutrition, University of Minnesota, St. Paul, Minnesota 55108, and Department of Chemistry, Montana State University, Bozeman, Montana 59717

The primary objective of this study was to determine how yogurt ingredients affect aroma release in the mouth during eating. A model strawberry flavor consisting of ethyl butanoate, ethyl 3-methylbutanoate, (*Z*)-hex-3-enol, 2-methylbutanoic acid, 5-hexylhydro-2(3*H*)-furanone, and 3-methyl-3-phenylglycidic acid ethyl ester was added to unflavored, unsweetened yogurt that had different added sweeteners and hydrocolloids. In all, 12 yogurt formulations were examined to determine the effects of gelatin, modified food starch, pectin, sucrose, high-fructose corn syrup, and aspartame on aroma release. Aroma release was monitored by breath-by-breath analysis (proton-transfer reaction−mass spectrometry) during eating of the test yogurts. Results showed aroma release of the ethyl butanoate, (*Z*)-hex-3-enol, and ethyl 3-methylbutanoate to be suppressed by sweeteners, with 55 DE high-fructose corn syrup having the greatest effect. Addition of thickening agents had no significant effect on the aroma release profiles of the compounds under study.

KEYWORDS: Proton-transfer reaction−mass spectrometry; yogurt; high-fructose corn syrup; aroma release; flavor release; breath-by-breath

INTRODUCTION

Aroma release is crucial in the perception of flavor as aroma molecules must reach the olfactory epithelium in order to be sensed by the consumer. Volatiles can reach the olfactory receptors via the retronasal or orthonasal pathway (*1*). The retronasal pathway delivers the aroma during chewing and swallowing. By monitoring the air expired from the nose, one obtains a better understanding of the volatile profile passing over the olfactory epithelium.

In 1983, Benoit and colleagues acknowledged the importance of studying expired breath and described how atmospheric pressure ionization−mass spectrometry (API-MS) could be used to study volatiles in the breath (*2*). Advances in API-MS and similar mass spectrometric techniques, such as proton-transfer reaction−mass spectrometry (PTR-MS), allow the real-time monitoring of volatile aroma compounds during eating (*3*, *4*). These mass spectrometric techniques have the ability to detect aroma compounds at parts per billion (ppb) concentrations in air (*4*). PTR-MS is a direct inlet mass spectrometric technique and can have a linear response over several orders (*5*).

Breath analysis has been used to study how ingredients affect volatile release in numerous food products. For example, chewing gum sweeteners, sorbitol and xylitol, will affect the quantity and rate of volatile release differently (*6*). In pectin gels, it has been shown that corn syrup resulted in lower volatile release than a mix of sucrose and glucose (*7*). The release of lipophilic compounds is suppressed by raising fat levels in milk (*8*). In yogurt, higher fat contents allowed longer volatile persistence, whereas volatiles reached maximum breath concentration much more quickly in low-fat yogurt (*9*).

A large number of yogurts are available in the marketplace. Each product varies slightly in fat content as well as types and amounts of thickeners and sweeteners used. Fruit pectin, modified food starch, and gelatin are among the thickening agents used in commercial yogurts. Depending on the type of yogurt and the target market, manufacturers may add sucrose, high-fructose corn syrup (HFCS), aspartame, or a combination of sweeteners. Using real-time monitoring by PTR-MS, this study aims to determine how various thickening agents and sweeteners affect the release of select aroma compounds from yogurt during eating.

MATERIALS AND METHODS

Flavor Compounds. A simple artificial strawberry flavor was studied. A stock solution of six volatile compounds was prepared according to mass percentages outlined in **Table 1**. All flavor chemicals were obtained from Robertet Flavors, Inc. (Piscataway, NJ). This stock solution was diluted to 1% w/w in propylene glycol (Sigma Aldrich, Milwaukee, WI) by adding 0.1 g of stock solution to 9.9 g of propylene glycol; 0.4 g of the 1% w/w solution was added to 200 g of yogurt to achieve a final concentration of strawberry flavor at 20 ppm (20 mg/kg). Consequently, the final concentrations of each individual aroma were as follows: 3-methyl-3-phenylglycidic acid ethyl ester (6.0 mg/kg), 5-hexylhydro-2(5*H*)-furanone (6.0 mg/kg), (*Z*)-hex-3-enol (2.8 mg/kg), ethyl butanoate (2.0 mg/kg), 2-methylbutanoic acid (1.6 mg/kg), and ethyl 3-methylbutanoate (1.6 mg/kg).

* Author to whom correspondence should be addressed [e-mail greinecc@umn.edu; telephone (612) 624-7717; fax (612) 625-5272].
[†] University of Minnesota.
[§] Montana State University.

10.1021/jf049787t CCC: $27.50
Published on Web 09/08/2004

Table 1. Composition of the Model Strawberry Aroma Used in This Study

compound	CAS Registry No.[a]	% by mass
3-methyl-3-phenylglycidic acid ethyl ester	077-83-8	30
5-hexylhydro-2(3*H*)-furanone	706-14-9	30
(*Z*)-hex-3-enol	928-96-1	14
ethyl butanoate	105-54-4	10
2-methylbutanoic acid	116-53-0	8
ethyl 3-methylbutanoate	108-64-5	8

[a] Provided by the author.

Subjects. Two subjects (one male and one female, ages 25 and 22, respectively) were recruited from the Department of Chemistry at Montana State University (Bozeman, MT) for the in-breath analysis. Proper human subject procedures were followed to ensure panelists' safety.

Preparation of Yogurt Samples. Old Home Plain Yogurt (St. Paul, MN; code date, 12/04/02) was used as a commercial yogurt base to which a sweetener [granulated sucrose (bought locally, Roseville, MN), aspartame (Nutrasweet, Chicago, IL), or 55 DE high-fructose corn syrup (Cargill, Ville, IA)] and/or a thickener [pectin (CPKelco LM pectin YA-100, Wilmington, DE), gelatin (Knox, Parsippany, NJ), or Thermtex modified food starch (National Starch Corp., Bridgewater, NJ)] was added. Samples with thickeners all contained 8% sucrose. To each formulation was added 0.2% w/w diluted flavoring. **Table 2** gives the compositions and designations for each yogurt prepared and tested. To create stirred yogurt, sweeteners and flavor were added directly to the yogurt. Modified starch and pectin were added, and the sucrose and flavor then mixed with the yogurt base. The 8% sucrose sample was used as the control for the modified starch and pectin trials. Gelatin was hydrated with warm water to make a 10% gelatin solution and then added to sweetened/flavored yogurt. Gelatin controls contained the same amount of water as gelatin sample but no gelatin. Each type of yogurt was prepared in 200 g batches.

Real-Time Nosespace Analysis. Nose-space analysis was performed at Montana State University, Bozeman, MT, using a PTR-MS (Ionicon Analytik, Innsbruck, Austria) with a modified inlet. The modified inlet consisted of a critical orifice made by crimping Teflon tubing with a caliper. The caliper reduced the opening so that 2 mbar pressure was constantly maintained in the drift tube of the PTR-MS. Breath was sampled through a nosepiece. The nosepiece was a plastic tube affixed perpendicularly to the Teflon tube. Panelists rested one nostril on the top end of the nosepiece. As the panelist exhaled, 15 mL/min of breath was drawn into the PTR-MS sample line by the inlet vacuum. The panelist was asked to breathe for 1 min, eat the yogurt sample (5 g), and continue breathing for 2 min following swallowing. (*Z*)-Hex-3-enol, ethyl butanoate, and ethyl 3-methylbutanoate were monitored by masses 83, 117, and 131, respectively. The dwell times for these masses were 0.5, 0.1, and 0.1 s, respectively. Masses 19 (H_3O^+) and 37 ($H_3O^+ \cdot H_2O$) had dwell times of 20 ms each and were monitored for calibration purposes (*4*, *10*). Mass 59 (acetone) was monitored as an indicator of breathing pattern. The dwell time for mass 59 was 50 ms. Yogurt samples were presented in random order, and subjects were instructed to drink water between samples. The subjects rested for a minimum of 5 min between samples. Subject 1 (female) performed the test in triplicate, whereas subject 2 (male) did the test in duplicate. To determine the intraperson variability of this analysis, subject 1 consumed a control sample (0.2% diluted flavor, no additional ingredients) eight times.

Data Analysis. Raw PTR-MS data were converted to concentrations in parts per billion (ppb) (*4*, *10*). The data from the five test sets (three replicates from subject 1 and two replicates from subject 2) were studied by analysis of variance (ANOVA). ANOVA was used to determine significant differences in the maximum concentration in the first, second, and third exhaled breaths separately. Significance was determined at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Volatile Release Profiles. Of the six compounds in the strawberry flavor, three had measurable signals on the PTR-MS at the levels found in our yogurt and in the exhaled breath. 3-Methyl-3-phenylglycidic acid ethyl ester could not be detected in its pure form due to its low vapor pressure. 5-Hexylhydro-2(3*H*)-furanone was undetectable at the levels in yogurt and exhaled breath due to both its low vapor pressure and its high solubility in the yogurt matrix. 2-Methylbutanoic acid could not be detected at the concentration found in yogurt. Thus, only ethyl butanoate, ethyl 3-methylbutanoate, and (*Z*)-hex-3-enol showed adequate instrumental response. Although each panelist differed in expired volatile concentrations, similar release patterns, that is, maximum volatile concentration in the first breath and a sharp drop in concentration in subsequent breaths, were seen for both participants (**Figure 1**). This pattern was especially pronounced for the two esters. In addition, the ethyl butanoate concentration was consistently higher than that of ethyl 3-methylbutanoate. (*Z*)-Hex-3-enol had the weakest signal of the three volatiles and had the slowest decay. Roberts et al. also documented large concentration differences between panelists, noting that release values often differed by a factor of 2, even though panelists consumed equivalent milk samples (*11*).

Intraperson Variability. The highest variability was seen in the compound with the greatest in-nose concentration, ethyl butanoate (**Figure 2**). In a study of milks varying in fat, Roberts et al. found ~41% CV (100 × SD/mean) within panelists for ethyl butanoate release (*11*). The current study found that the ethyl butanoate had a 48% CV for the eight identical yogurt samples eaten by one panelist, which is in agreement with the

Table 2. Composition of Yogurt Samples and Their Designations

	sweetener		thickener			
control	type	g	type	g	yogurt, g	diluted flavor, g
control		0		0.0	200.0	0
sweet control		0		0.0	200.0	0.4
8% sugar	sucrose	16		0.0	184.0	0.4
12% sugar	sucrose	24		0.0	176.0	0.4
aspartame	aspartame	0.08		0.0	199.9	0.4
55 DE HFCS	55 DE HFCS	20		0.0	180.0	0.4
gelatin control, low	sucrose	16	water	1.4	182.6	0.4
gelatin control, high	sucrose	16	water	2.7	181.3	0.4
low pectin	sucrose	16	CPKelco LM-Pectin YA-100	1.6	182.4	0.4
high pectin	sucrose	16	CPKelco LM-Pectin YA-100	3.0	181.0	0.4
low gelatin	sucrose	16	10% Knox solution	1.6	182.4	0.4
high gelatin	sucrose	16	10% Knox solution	3.0	181.0	0.4
modified starch	sucrose	16	National Starch Thermtex	2.0	182.0	0.4

Table 3. Nosespace Results for Three Volatile Compounds Based on Formulation[a]

formula	ethyl butanoate	ethyl 3-methylbutanoate	(*Z*)-hex-3-enol
8% sugar	– (27%), –1, –48	NS	NS
12% sugar	– (19%), –7, –42	NS	– (14%), +11, –34
400 ppm of aspartame	– (20%), –2, –48	NS	NS
10% 55 DE HFCS	– (53%), –34, –72	– (55%), –31, –79	– (36%), –18, –48
0.8% pectin[b]	NS	NS	NS
1.5% pectin[b]	NS	+ (20%), –2, +71	NS
1% modified starch[b]	NS	NS	NS
low gelatin	+ (24%), –20, +72	+ (34%), +2, +51	+ (23%), –13, +71
high gelatin	NS	NS	NS

[a] NS= not significantly different from control; – = decreased released from the control; + = increased release from the control. [b] The control sample for these formulations was the 8% sugar trial. Numbers in parentheses indicate the average percentage change in concentration seen in the first breath. The range in percentage change for each formulation and compound combination is represented as the smallest change, largest change from zero.

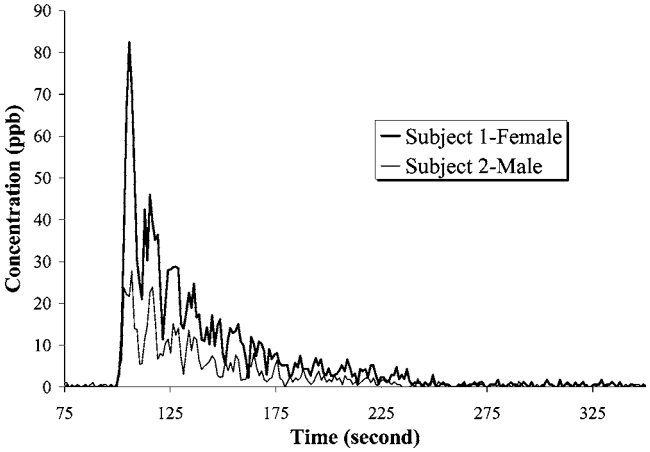

Figure 1. Comparison of ethyl butanoate release profiles for two model flavored yogurt samples consumed by two different panelists.

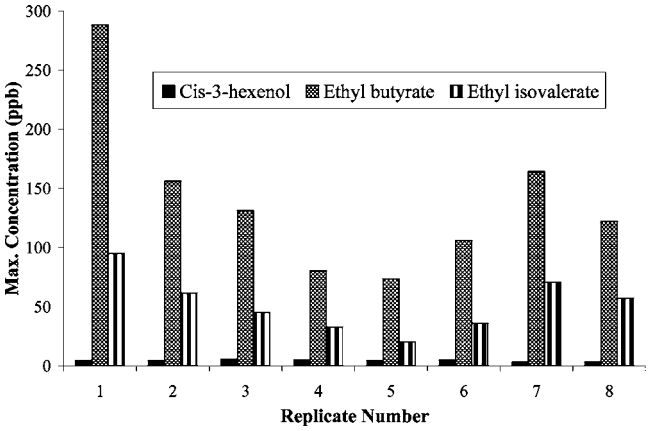

Figure 2. Eight samples of yogurt flavored with 0.2% diluted flavor stock solution consumed by one subject. Maximum concentration of three compounds during the swallow breath is shown.

observations of Roberts et al. (*11*). (*Z*)-Hex-3-enol was less variable. Variations in eating mechanics from one time to another likely account for this large variability.

Effects of Yogurt Composition. The effect of yogurt formulation on volatile release in the breath was determined with resultant data and statistical analysis presented in **Table 3**. All of the sweeteners significantly reduced ethyl butanoate release (19−53% compared to unsweetened control). Using a retronasal aroma stimulator (RAS), Deibler and Acree found that the type of sweetener used in beverage formulation changed the amount of volatile released. For example, it was found that the addition of aspartame to an unsweetened model beverage decreased the release of ethyl valerate by 30% and sucralose

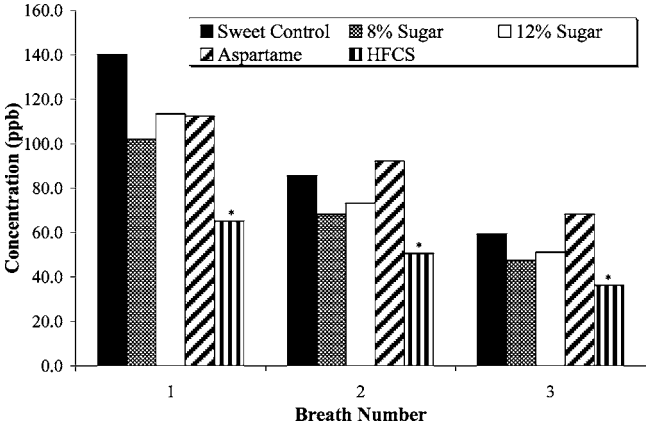

Figure 3. Ethyl butanoate concentrations (ion intensity) of aroma compounds in the breath as a function of breath number. * = significant at $\alpha = 0.05$ level.

decreased it by 60% (*12*). Our results show a comparable 20% decrease in the release of ethyl butanoate when 400 ppm of aspartame is added to yogurt.

In the current yogurt study, only high-fructose corn syrup used at 10% significantly decreased the release of all three volatiles monitored (36−55%). A comparison of how different sweeteners affected the release of ethyl butanoate is shown in **Figure 3**. The effects of the sweeteners used in this study on the in vivo release of ethyl butanoate from yogurt were not significantly different with the exception of high-fructose corn syrup. These results are consistent with those of Lubbers and Guichard, who found corn syrups to reduce flavor release and lower aroma perception in pectin gels (*7*). Although the difference in release is statistically significant, we note that the sensory significance of this analytical difference is unknown. There are no data in the literature on how much the concentration of a volatile in the nose-space must change in order to elicit a noticeable sensory change in intensity.

The thickeners used in this study had less of an effect on volatile release than the sweeteners. Adding a low level of gelatin increased release for all three volatiles. However, this 23−34% increase in release was only marginally significant. The fact that the higher level of gelatin showed no significant change in release makes one question the validity of this difference. Pectin and modified starch had no detectable influence on aroma release. In a study to determine the effect of pectin and viscosity on low-sucrose foods thickened with pectin, it was also seen that pectin added to a low-sugar model food system did not alter aroma release (*13*). When looking at viscous food system thickeners with hydroxypropylmethyl cellulose (HPMC) or λ-carrageenan, Cook and Hollowood found

that in in vivo aroma concentration was not affected by the addition of the thickeners, whereas sensory perception of flavor intensity decreased at high concentrations (*14*, *15*).

This work shows that the PTR-MS technique may be used for the real-time monitoring of aroma release from viscous foods such as yogurt. However, even with the exceptional sensitivity claimed for the PTR-MS instrument (*10*), only three of the six compounds in this model strawberry flavor could be monitored. The inability of this approach to measure differences in aroma release across a greater range of aroma compounds demonstrates a weakness that is common to real-time breath analysis in general. This weakness is often compensated for by selecting aroma compounds that give good responses by PTR-MS or API-MS; however, then one is assuming that the aroma model accurately represents the flavor being modeled. This may be a source of error.

There is a wide person-to-person variation in aroma release. This variability may partially account for differences in sensory thresholds among people. The within-person variation is also large, making it difficult to find statistical differences in release as a function of food composition. This variability suggests that in vitro methods (artificial mouths), which have much lower variability, may have greater utility in the study of the effect of formulation on aroma release than in vivo techniques.

In conclusion, our results show that the three sweeteners studied decrease volatile release. Specifically, addition of 10% high-fructose corn syrup to yogurt suppressed the volatility of the studied compounds by 36−55% in vivo. The thickening agents, pectin, gelatin, and modified starch, had very little effect on aroma release of ethyl butanoate, ethyl 3-methylbutanoate, and (*Z*)-hex-3-enol, suggesting that any observed sensory differences may be due to sensory cues other than aroma release.

LITERATURE CITED

(1) Taylor, A.; Linforth, R. Techniques for Measuring Volatile Release In Vivo during Consumption of Food. In *Flavor Release*; Roberts, D., Taylor, A., Eds.; ACS Symposium Series 763; American Chemical Society: Washington, DC, 2000; pp 8−19.

(2) Benoit, F.; Davidson, W.; Lovett, A.; Nacson, S.; Ngo, A. Breath Analysis by Atmospheric Pressure Ionization Mass Spectrometry. *Anal. Chem.* **1983**, *55*, 805−807.

(3) Linforth, R.; Taylor, A. Apparatus and Methods for the Analysis of Trace Constituents of Gases. Eur. Patent EP 0819 937 A1, 1998.

(4) Hansel, A.; Jordan, A.; Holzinger, R.; Prazeller, P.; Vogel, W.; Lindinger, W. Proton-Transfer Reaction Mass Spectrometry: On-line Trace Gas Analysis at the ppb Level. *Int. J. Mass Spectrom. Ion Processes* **1995**, *149/150*, 609−619.

(5) Yeretzian, C.; Jordan, A.; Brevard, H.; Lindinger, W. Time-Resolved Headspace Analysis by Proton-Transfer Reaction-Mass Spectrometry. In *Flavor Release*; Roberts, D., Taylor, A. Eds.; ACS Symposium Series 763; American Chemical Society: Washington, DC, 2000; pp 58−72.

(6) Haahr, A.; Pilsgaard, C.; Stahnke, L.; Bredie, W.; Refsgaard, H. Effect of Sweetener on Release of Flavour Compounds From Chewing Gum. In *Flavour Research at the Dawn of the Twenty-First Century*; LeQuéré, J., Étiévant, P., Eds.; Laviosier and Intercept: Paris, France, 2003; pp 224−227.

(7) Lubbers, S.; Guichard, E. The Effects of Sugars and Pectin on Flavour Release from a Fruit Pastille Model System. *Food Chem.* **2003**, *81*, 269−273.

(8) Roberts, D. D.; Pollien, P.; Antille, N.; Lindinger, C.; Yeretzian, C. Comparison of Nosespace, Headspace, and Sensory Intensity Ratings for the Evaluation of Flavor Absorption by Fat. *J. Agric. Food Chem.* **2003**, *51*, 3636−3642.

(9) Brauss, M. S.; Linforth, R. S. T.; Cayeux, I.; Harvey, B.; Taylor, A. J. Altering the Fat Content Affects Flavor Release in a Model Yogurt System. *J. Agric. Food Chem.* **1999**, *47*, 2055−2059.

(10) Lindinger, W.; Hansel, A.; Jordan, A. On-line Monitoring of Volatile Organic Compounds at pptv Levels by Means of Proton-Transfer-Reaction Mass Spectrometry (PTR-MS): Medical Applications, Food Control and Environmental Research. *Int. J. Mass Spectrom. Ion Processes* **1998**, *173*, 191−241.

(11) Roberts, D.; Pollien, P.; Yeretzian, C.; Lindinger, C. Nosespace Analysis with Proton-Transfer-Reaction Mass Spectrometry: Intra- and Interpersonal Variability. In *Handbook of Flavor Characterization: Sensory Analysis, Chemistry, and Physiology*; Diebler, K., Delwiche, J., Eds.; Dekker: New York, 2004; pp 151−162.

(12) Deibler, K.; Acree, T. Effect of Beverage Base Conditions on Flavor Release. In *Flavor Release*; Roberts, D., Taylor, A., Eds.; ACS Symposium Series 763; American Chemical Society: Washington, DC, 2000; pp 333−341.

(13) Bylaite, E.; Meyer, A.; Adler-Nissen, J. Changes in Macroscopic Viscosity Do Not Affect the Release of Aroma Aldehydes from a Pectinaceous Food Model System of Low Sucrose Content. *J. Agric. Food Chem.* **2003**, *51*, 8020−8026.

(14) Hollowood, T.; Linforth, R.; Taylor, A. The Effect of Viscosity on the Perception of Flavour. *Chem. Senses* **2002**, *27*, 583−591.

(15) Cook, D.; Linforth, R.; Taylor, A. Effects of Hydrocolloid Thickeners on the Perception of Savory Flavors. *J. Agric. Food Chem.* **2003**, *51*, 3067−3072.

Received for review February 6, 2004. Revised manuscript received July 29, 2004. Accepted August 1, 2004. We thank Robertet Flavors, Inc. (Piscataway, NJ), for their financial support of this project.

JF049787T