JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

The Effect of Solvent Interactions on α-, β-, and γ-Cyclodextrin/Flavor Molecular Inclusion Complexes

TERESA A. REINECCIUS,† GARY A. REINECCIUS,† AND TERRY L. PEPPARD*,‡

Department of Food Science and Nutrition, University of Minnesota, 1334 Eckles Avenue, St. Paul, Minnesota 55108, and Robertet Flavors, Inc., 10 Colonial Drive, Piscataway, New Jersey 08854

Three commonly used flavor industry solvents (propylene glycol, triacetin, and triethyl citrate) were tested for their capacity to interfere with the ability of α-, β-, and γ-cyclodextrin to form molecular inclusion complexes with flavors. Six flavor compounds (ethyl butyrate, ethyl heptanoate, L-menthol, methyl anthranilate, neral, and geranial) were measured by headspace gas chromatography above 2:1 water/ethanol containing appropriate additions of cyclodextrin and flavor solvent. The smallest and most polar solvent molecule represented by propylene glycol had the least effect on cyclodextrin/flavorant complex formation. In contrast, triacetin, intermediate in size among the three flavor diluents studied, had the greatest effect, even though, based on at least some computed molecular parameters, it appears to be more polar than triethyl citrate. The explanation for this apparent anomaly may lie in differences in the extent to which triacetin and triethyl citrate are able to interact with cyclodextrins by means of partial interaction with the hydrophobic cavities of the latter.

KEYWORDS: Cyclodextrin; flavor compound; flavor diluent; molecular inclusion complex; solvent interaction

INTRODUCTION

The process of encapsulation is widely employed in the flavor industry to protect volatile and/or labile flavoring materials during storage (*1*−*3*). A variety of commercial practices are currently followed, but those involving the formation of flavor/cyclodextrin (CyD) molecular inclusion complexes afford some of the greatest potential for increased product shelf life (*4*−*8*). We have previously reviewed the use of CyDs in this respect and have described the results of our studies to evaluate the usefulness of α-, β-, and γ-CyDs in protecting a range of different flavor compounds (*9*). We have also reported our findings following an investigation into the ease with which a number of flavor molecules are subsequently released from CyD inclusion complexes in 3.6% aqueous ethanol over the temperature range 5−85 °C (*10*). Finally, we published the results of studies utilizing CyDs in a number of real world food and beverage applications (*11*, *12*).

As part of our continuing research into the value of CyDs for the protection of flavoring materials to achieve increased product shelf life, we now report the results of a study designed to evaluate the effects of several commonly employed flavor industry solvents (diluents) on CyD efficacy. Specifically, the study determined the effects of propylene glycol, triacetin, and triethyl citrate on the molecular inclusion of selected flavor compounds in α-, β-, and γ-CyD. The primary purpose of the research was to determine whether these flavor diluents compete with the various flavoring agents for inclusion in CyDs and thereby influence the effectiveness of the flavor compounds' inclusion. The flavor compounds studied were as follows: ethyl butyrate and heptanoate, L-menthol, methyl anthranilate, and both geometric isomers of citral, namely, neral and geranial.

MATERIALS AND METHODS

Materials. CyDs (pharmaceutical grade) were purchased from Wacker Biochemical Corp. (Munich, Germany). Individual flavor compounds and flavor solvents were purchased from Sigma Aldrich Chemical Co. (Milwaukee, WI).

CyD/Solvent/Flavor Systems. Solutions were prepared containing individual flavor compounds (ethyl butyrate, ethyl heptanoate, citral, L-menthol, or methyl anthranilate) dissolved in 1 mL of ethanol, propylene glycol, triacetin, or triethyl citrate. Flavor compounds were used at concentrations delivering an equimolar amount as compared to 1 g of α-, β-, or γ-CyD, respectively. Four different samples were then prepared (in duplicate) for each CyD/solvent/flavor combination.

Samples were prepared in 25 mL headspace vials. Water/ethanol (2:1 v/v, 10 mL) was added first to each vial, and then, the remaining components were added to complete the four samples (1−4) detailed in **Table 1**. The sample vials were sealed with Teflon-faced septa and aluminum crimp caps. The sealed vials were next heated in a water bath (55 °C) until the CyD had dissolved; they were then removed from the water bath and allowed to cool to room temperature (22 °C). Vials were finally refrigerated overnight (4 °C) and analyzed by gas chromatography (GC) the following day.

Instrumental Analysis. *GC.* Samples were analyzed using an MPS2 Gerstel Multipurpose Sampler (Gerstel, Inc., Baltimore, MD) equipped

* To whom correspondence should be addressed. Tel: 732-756-3521. Fax: 732-981-3461. E-mail: TPeppard@RobertetUSA.com.
† University of Minnesota.
‡ Robertet Flavors, Inc.

10.1021/jf0488716 CCC: $30.25
Published on Web 12/21/2004

Table 1. Sample Formulations for Each CyD/Solvent/Flavor Combination

sample	water/ethanol (2:1) (mL)	flavor[a] + ethanol (mL)	flavor[a] + solvent[b] (mL)	CyD[c] (g)
1	10	1		
2	10		1	
3	10	1		1
4	10		1	1

[a] Ethyl butyrate, ethyl heptanoate, citral (ca. 1:1 mixture of neral and geranial), L-menthol, or methyl anthranilate (equimolar basis as compared with amount of CyD used). [b] Propylene glycol, triacetin, or triethyl citrate. [c] α-, β-, or γ-CyD.

Table 2. Molecular Parameters of Flavor Solvents from Computer Modeling

calculated molecular parameters	propylene glycol	triethyl citrate	triacetin
molecular volume (Å^3)[a]	46.5	162.5	117.7
global molecular dimensions (Å)[a]			
X	7.7	16.2	13.8
Y	5.9	10.5	9.0
Z	5.0	6.3	5.5
Log P[b]	–0.78	0.33	0.36
hydrophilic surface area (%)[a]	62.8	53.4	62.0
Hansen 3D solubility parameter[a]	30.2	17.9	27.2
Hansen 3D dispersion parameter[a]	16.8	13.9	18.1
Hansen 3D polarity parameter[a]	9.3	5.8	6.8
Hansen 3D hydrogen-bonding parameter[a]	23.3	9.7	19.1

[a] Molecular Designer, V: 5.1.9 (NorGwyn Montgomery). [b] LogKow, V: 1.66 (Syracuse Research Corp.).

with a 2.5 mL gastight syringe. Prior to GC injection, samples were equilibrated for at least 2 h at room temperature and then at 25 °C with continuous shaking (500 rpm) for 30 min. A 2.5 mL pulsed splitless headspace injection was made into the injection port (200 °C) of an Agilent 6890 gas chromatograph (Agilent, Inc., Palo Alto, CA) at a rate of 250 μL/s. Chromatography was performed using a DB-Wax column (30 m, 0.25 mm i.d., 0.5 μm film thickness) (J&W Scientific, Inc., Rancho Cordova, CA). The compounds were detected by flame ionization detection (225 °C).

Prediction of Flavorants' and Flavor Diluents' Molecular Parameters. Molecular structures were drawn in Molecular Designer V:5.1.9 (NorGwyn Montgomery Software, Inc., North Wales, PA), and the most energetically favorable conformation of each was determined using the program's conformational analysis and/or minimization procedures. Log P values were obtained using LogKow V:1.66 via the Internet site of Syracuse Research Corp. Molecular parameters predicted by these means for the flavorants and flavor diluents under investigation are listed in **Tables 2** and **3**.

RESULTS AND DISCUSSION

Whether used directly in liquid form or subsequently converted to a powder, flavors are commonly formulated with diluents such as ethanol, propylene glycol, triacetin, or triethyl citrate; in many cases, such flavor solvents comprise 50−90% or more of the liquid part of the flavor. For a liquid flavor containing a high proportion of diluent, it is important to know the effect that this may have on attempts to make CyD inclusion complexes. For example, if the flavor solvent competes more effectively for the hydrophobic CyD cavity than do some of the flavor compounds, then the latter may be poorly included. In the present study, the effects of three flavor diluents (propylene glycol, triacetin, and triethyl citrate) on the inclusion of a number of flavorants individually in α-, β-, and γ-CyD were investigated. The flavor chemicals ethyl butyrate, ethyl heptanoate, L-menthol, methyl anthranilate, and citral (comprising a ca. 1:1 mixture of neral and geranial) were chosen based on trying to achieve a representative range of molecular dimensions and physicochemical properties. Headspace concentrations of the six flavoring ingredients were determined above solutions of (i) water/ethanol, (ii) water/ethanol with added flavor solvent, (iii) water/ethanol containing either α-, β-, or γ-CyD, and (iv) water/ethanol with added flavor solvent and also containing the CyD corresponding to (iii) above. A 2:1 water/ethanol solution was chosen as the control, since this system is often used in the ethanol precipitation method of making insoluble CyD inclusion complexes (*4*). If there is significant interaction (binding) between a CyD and a flavor solvent, then one would expect such CyD/solvent complex formation to result in a greater proportion of flavorant remaining free (that is, uncomplexed) in the solvent system. Accordingly, this would result in an increased headspace concentration of the flavor compound. The system described above was not intended to simulate a food of any type, since the objective of this study was to determine if solvents interfere with the formation of CyD/flavor complexes, not to investigate their release in food systems.

Effects of CyDs and Solvents on Concentrations of Flavorants in Headspace. The independent effects of the CyDs and flavor solvents, respectively, on changes in headspace concentration of the six flavor volatiles are presented in **Figures 1** and **2**. **Figure 1** is based on measured differences between sample types 1 and 3 in **Table 1**, whereas **Figure 2** is based on differences between sample types 1 and 2. The CyD effects (that is, the effects due to the CyDs in the absence of added propylene glycol, triacetin, or triethyl citrate) are relatively small in the case of α-CyD but become much more marked in the cases of β- and γ-CyD (**Figure 1**). This reflects the tendency of different flavor compounds to form molecular inclusion complexes of varying strengths with α-, β-, and γ-CyD. The unique behavior of methyl anthranilate is worthy of note. The addition of α-CyD actually increased the headspace concentration of this flavorant as compared to that of the corresponding control. This may be due to the high solubility of α-CyD in water, the poor ability of methyl anthranilate to interact with α-CyD (*10*), and

Table 3. Molecular Parameters of Flavor Molecules from Computer Modeling

calculated molecular parameters	ethyl butyrate	ethyl heptanoate	L-menthol	methyl anthranilate	geranial	neral
molecular volume (Å^3)[a]	73.0	102.6	104.9	82.9	100.8	101.0
global molecular dimensions (Å)[a]						
X	10.4	13.6	10.5	10.3	13.2	12.4
Y	6.8	7.4	7.4	7.6	6.5	7.4
Z	5.4	5.5	6.4	5.4	5.6	4.9
Log P[b]	1.85	3.32	3.38	2.26	3.45	3.45
hydrophilic surface area (%)[a]	20.8	15.1	9.8	75.4	16.1	16.5

[a] Molecular Designer, V: 5.1.9 (NorGwyn Montgomery). [b] LogKow, V: 1.66 (Syracuse Research Corp.).

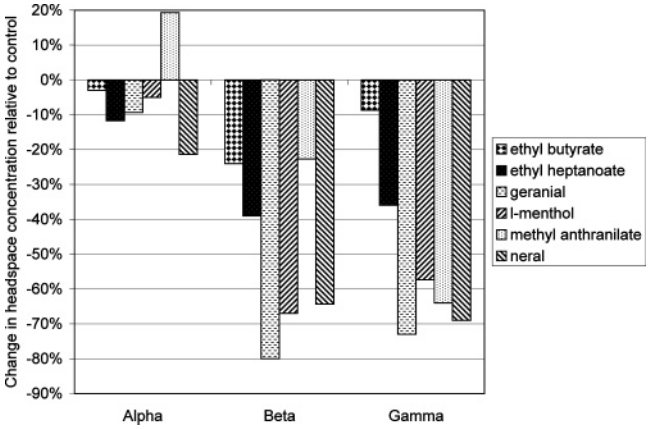

Figure 1. CyD effects in the absence of flavor solvents.

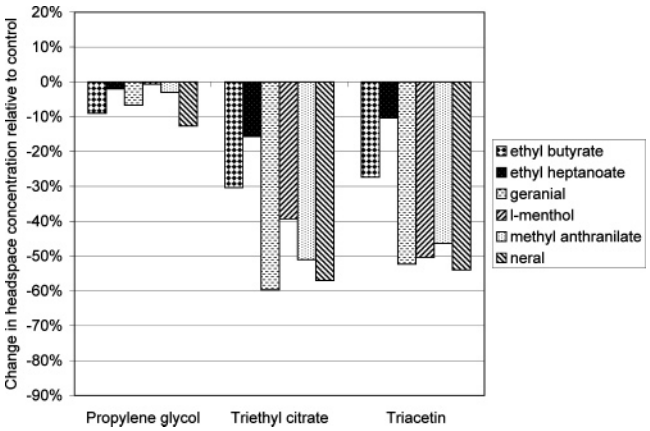

Figure 2. Solvent effects in the absence of CyDs.

consequently a decreased solubility of methyl anthranilate in solution. The effect was not observed for the other compounds studied. As seen in **Figure 2**, the solvent effects (that is, the effects of the solvents in the absence of added CyDs) are relatively weak in the case of propylene glycol, becoming much stronger in the cases of triacetin and triethyl citrate. These results reflect changes (reduction) in flavor compounds' gas/liquid partition coefficients as solvent is added. Solvent and CyD effects are confounded in results involving sample type 4 (**Table 1**) so it was necessary to make adjustments to calculations involving these samples as described in the following section.

Effect of Propylene Glycol on CyD/Flavorant Complex Formation. Figure 3 shows the CyD effect observed with added propylene glycol (*y*-axis, based on sample type 4 vs sample type 2) as compared to the CyD effect determined without added propylene glycol (*x*-axis, based on sample type 3 vs sample type 1). In the absence of interaction (binding) between propylene glycol and CyD, the results should yield a straight line of slope equal to unity passing through the origin. **Figure 3** shows such an idealized line, together with accompanying lines exhibiting deviations of ±10%, respectively (to allow for experimental error, although in practice this was generally less than 7.5%). It can be seen that all data points lie within or very close to the ±10% tramlines. The mean absolute deviation, computed by averaging the absolute differences between *x*/*y*-coordinates, was 3.4% (with a standard deviation of 3.3%) suggesting that propylene glycol has a minimal effect on molecular inclusion of any of the six flavor volatiles in α-, β-, or γ-CyD.

On the basis of the flavor solvents' predicted molecular dimensions listed in **Table 2**, together with the sizes of the CyD

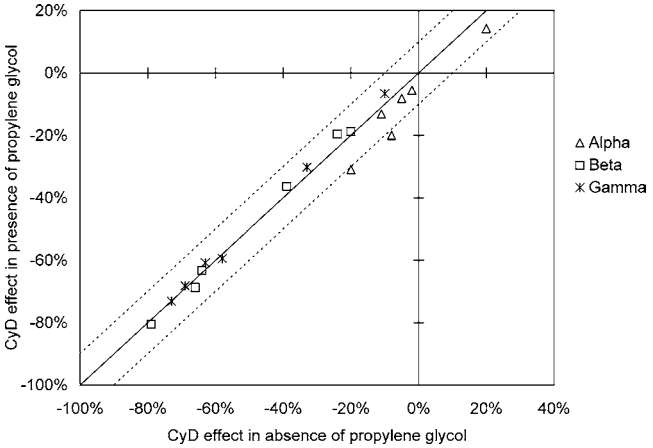

Figure 3. CyD effects with and without added flavor solvent: propylene glycol. Footnote: The mean of absolute deviations (calculated as ordinate – abscissa) is 3.4%, and the standard deviation is 3.3%.

Table 4. Molecular Dimensions of CyDs

CyD	cavity volume (Å^3)[a]	cavity diameter (Å)[b]
α-CyD	174	5.7
β-CyD	262	7.8
γ-CyD	427	9.5

[a] Ref *15*. [b] Ref *14*.

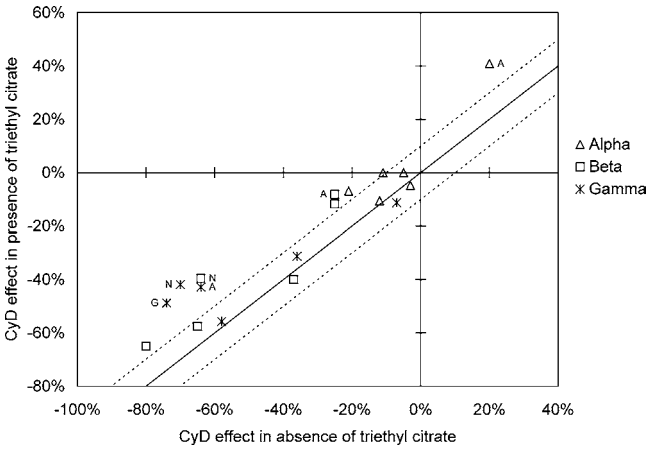

Figure 4. CyD effects with and without added flavor solvent: triethyl citrate. Footnote: The mean of absolute deviations (calculated as ordinate – abscissa) is 12.2%, and the standard deviation is 9.0%. Key: B, ethyl butyrate; H, ethyl heptanoate; M, L-menthol; A, methyl anthranilate; N, neral; and G, geranial.

cavities reported in the literature and collected here in **Table 4**, propylene glycol could fit inside both β- and γ-CyD. However, on the basis of the additional polarity- and solubility-related parameters listed in **Table 2**, propylene glycol is probably too polar to interact well with the hydrophobic CyD cavities, in accordance with the behavior observed in **Figure 3**.

Effect of Triethyl Citrate on CyD/Flavorant Complex Formation. In similar fashion, **Figure 4** shows the CyD effects with and without added triethyl citrate. It also shows the idealized "zero solvent effect" line (together with the accompanying ±10% lines) corresponding to the situation where this solvent has no significant effect on the ability of a CyD to include any of the six flavor volatiles studied. In this case, however, several of the flavorants do fall distinctly outside the +10% line, most notably neral and geranial in the case of β- and/or γ-CyD and methyl anthranilate with all three CyDs.

Directionally, the deviations indicate a greater concentration of flavor volatiles in the headspace when triethyl citrate is present, suggesting a tendency for their displacement from the hydrophobic CyD cavity by this solvent. The mean absolute deviation was 12.2%, with a standard deviation of 9.0%.

Using the flavor diluents' predicted molecular dimensions listed in **Table 2**, together with CyD cavity sizes presented in **Table 4**, triethyl citrate as such would clearly be too large to fit completely within any of the CyD cavities. However, the approximate molecular dimensions of each of the three ethyl ester side chains of triethyl citrate ($Y = 5.5$ Å; $Z = 4.9$ Å) indicate that one such side chain may fit within the cavity of (with increasing ease) α-, β-, and γ-CyD. Moreover, while this solvent would already be appreciably less polar overall than propylene glycol, based on Log P, hydrophilic surface area, Hansen 3D polarity parameter, etc., the ethyl ester side chain considered in isolation may be sufficiently lipophilic to ensure significant interaction with the hydrophobic CyD cavities.

It is not just a question of whether a particular solvent has the inherent ability to form a molecular inclusion complex with a CyD, the competing flavor compound is also important. Thus, under equilibrium conditions, weakly binding flavorants would be expected to be expelled more readily by triethyl citrate than would those that tend to bind more strongly. Clues for identifying which are the potential weakly binding flavor compounds may also be obtained by comparing predicted molecular dimensions (**Table 3**) with those reported for α-, β-, and γ-CyD (**Table 4**), bearing in mind that some flavorants may form CyD complexes by having only part of their molecule located within the hydrophobic cavity. On the basis of size alone, it appears that all six flavor molecules can fit at least partially within the cavity of both β- and γ-CyD. That this may not be the case with α-CyD is also indicated in **Figure 1**. However, it is notable that of the α-CyD/flavor complexes, only the α-CyD/methyl anthranilate combination falls appreciably outside the zero solvent effect line in **Figure 4**. In addition to molecular size, the predicted polarity- and solubility-related parameters listed in **Table 3** should also be taken into account. For example, on the basis of polarity, ethyl butyrate and methyl anthranilate (the most polar of the six flavor compounds in the study) would seem least likely to form strong molecular inclusion complexes, with menthol (based on hydrophilic surface area) and neral/geranial (based on Log P) at the other end of the behavior spectrum. However, while this is borne out in practice for methyl anthranilate (**Figure 4**), neral and geranial are the other two flavorants that were found to deviate most from the zero solvent effect line, behavior which cannot readily be rationalized in terms of molecular dimensions and polarity factors. A clearer picture of what may be happening could likely be achieved using molecular docking software with CyD cavities and both flavorants and flavor diluents. However, this is beyond the scope of the current study.

Effect of Triacetin on CyD/Flavorant Complex Formation. **Figure 5** shows the CyD effects with and without added triacetin. It also shows the idealized zero solvent effect and accompanying ±10% lines corresponding to the situation where this solvent has no consequential effect on the ability of a CyD to include any of the six flavor volatiles investigated in this study. As in the case of triethyl citrate, several of the flavorants fall conspicuously outside the ±10% lines (mostly the +10% line). The greatest deviations occur with menthol (with all three CyDs) and both neral and geranial in the case of γ-CyD. Directionally, the majority of deviations indicate a greater concentration of flavor volatiles in the headspace when triacetin

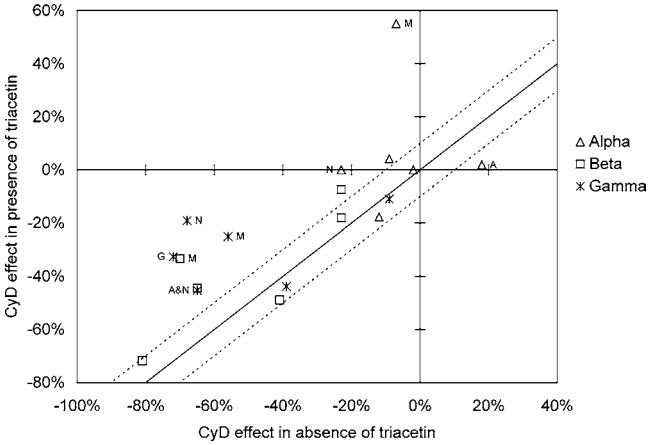

Figure 5. CyD effects with and without added flavor solvent: triacetin. Footnote: The mean of absolute deviations (calculated as ordinate – abscissa) is 20.2%, and the standard deviation is 17.2%. Key: same as indicated in the footnote to **Figure 4**.

is present, suggesting a tendency for their displacement from the hydrophobic CyD cavity by this solvent. The magnitudes of the deviations noted with triacetin were rather greater than those observed in the case of triethyl citrate (most notably for the menthol/α-CyD combination). The mean absolute deviation was 20.2%; the standard deviation was 17.2%.

Using the flavor diluents' predicted molecular dimensions (**Table 2**) and CyD cavity sizes (**Table 4**), triacetin as such would clearly be too large to fit completely within any of the CyD cavities. However, the molecular dimensions of each of the three acetyl ester side chains of triacetin ($Y = 5.5$ Å; $Z = 4.8$ Å) indicate that one such side chain may well fit within the cavity of (with increasing ease) α-, β-, and γ-CyD. Moreover, the acetyl ester side chain considered in isolation may be sufficiently lipophilic to ensure significant interaction with the hydrophobic CyD cavities.

As discussed above in the case of triethyl citrate, weakly binding flavorants will under equilibrium conditions be expelled by triacetin more readily than those that tend to bind more strongly. It was pointed out that, on the basis of size alone, all six of the flavor molecules studied would be expected to fit within the cavity of β- and γ-CyD whereas, in the case of α-CyD, only a partial fit would be possible. On this occasion, however, of the α-CyD/flavor complexes, the α-CyD/menthol, nerol, and methyl anthranilate combinations all fall markedly outside the zero solvent effect line in **Figure 5**. On the basis of polarity, ethyl butyrate and methyl anthranilate (the most polar of the six flavor compounds studied) were those cited as being least likely to form strong molecular inclusion complexes, whereas menthol and neral/geranial were at the other end of the behavior spectrum. However, menthol, neral, and geranial were among the flavorants found to deviate most from the zero solvent effect line, behavior which once again cannot readily be rationalized in terms of molecular dimensions and polarity factors.

In conclusion, on the basis of the various systems studied involving the three CyDs, six flavorants, and three flavor diluents listed above, it appears the smallest and most polar solvent molecule represented by propylene glycol has the least effect on CyD/flavorant complex formation. Triacetin, intermediate in size among the three flavor diluents investigated, has the greatest effect, even though, based on at least some of the computed molecular parameters, it is apparently more polar than triethyl citrate. Presumably the explanation for this anomaly

may lie in differences in the extent to which triacetin and triethyl citrate are able to interact with CyDs through partial interaction with the hydrophobic cavities. Confirmation of this phenomenon would likely be achieved using molecular docking software with both flavorants and flavor diluents and the various CyD cavities. In addition, further studies should be performed using additional flavor chemicals covering a wider range of molecular dimensions and physicochemical properties that would be more representative of the large palette of flavoring materials typically available to flavor developers. The results of such studies should bolster those of the present work and enable flavorists to more readily develop successful flavor/CyD complexes involving optimal choices of flavor diluents.

ACKNOWLEDGMENT

We thank Dr. James Quinn of NorGwyn Montgomery Software, Inc. for helpful discussions on the subject of molecular modeling and the officers of Robertet Flavors, Inc. for permission to publish this paper.

LITERATURE CITED

(1) Risch, S. J., Reineccius, G. A., Eds. *Encapsulation and Controlled Release of Food Ingredients*; ACS Symposium Series 590; American Chemical Society: Washington, DC, 1995; 214 pp.

(2) Gibbs, B. F.; Kermasha, S.; Alli, I.; Mulligan, C. N. Encapsulation in the food industry: A review. *Int. J. Food Sci. Nutr.* **1999**, *50*, 213−224.

(3) Uhlemann, J.; Schleifenbaum, B.; Bertram, H.-J. Flavor encapsulation technologies: An overview including recent developments. *Perfum. Flavor.* **2002**, *27* (5), 52−61.

(4) Reineccius, G. A.; Risch, S. J. Encapsulation of artificial flavors by β-cyclodextrin. *Perfum. Flavor.* **1986**, *11* (4), 1−6.

(5) Pagington, J. S. Beta-cyclodextrin. *Perfum. Flavor.* **1986**, *11* (1), 49−58.

(6) Qi, Z. H.; Hedges, A. R. Use of cyclodextrins for flavors. In *Flavor Technology: Physical Chemistry, Modification and Process*; ACS Symposium Series 610; Ho, C.-T., Tan, C.-T., Tong, C.-H., Eds.; American Chemical Society: Washington, DC, 1995; pp 231−243.

(7) Hedges, A.; McBride, C. Utilization of β-cyclodextrin in food. *Cereal Foods World* **1999**, *44*, 700−704.

(8) Szente, L.; Szejtli, J. Cyclodextrins as food ingredients. *Trends Food Sci. Technol.* **2004**, *15* (3−4), 137−142.

(9) Reineccius, T. A.; Reineccius, G. A.; Peppard, T. L. Encapsulation of flavors using cyclodextrins: Comparison of flavor retention in alpha, beta and gamma types. *J. Food Sci.* **2002**, *67*, 3271−3279.

(10) Reineccius, T. A.; Reineccius, G. A.; Peppard, T. L. Flavor release from cyclodextrin complexes: Comparison of alpha, beta and gamma types. *J. Food Sci.* **2003**, *68*, 1234−1239.

(11) Reineccius, T. A.; Reineccius, G. A.; Peppard, T. L. Utilization of beta-cyclodextrin for improved flavor retention in thermally processed foods. *J. Food Sci.* **2004**, *69* (1), C58−C62.

(12) Reineccius, T. A.; Reineccius, G. A.; Peppard, T. L. Potential for beta-cyclodextrin as partial fat replacer in low-fat foods. *J. Food Sci.* **2004**, *69* (4), C334−C341.

(13) Syracuse Research Corporation, North Syracuse, NY. <http://www.syrres.com/esc/est_kowdemo.htm>. Accessed May 24, 2004.

(14) Hedges, A. R.; Shieh, W. J.; Sikorski, C. T. Use of cyclodextrins for encapsulation in the use and treatment of food products. In *Encapsulation and Controlled Release of Food Ingredients*; ACS Symposium Series 590; Risch, S. J., Reineccius, G. A., Eds.; American Chemical Society: Washington, DC, 1995; pp 60−71.

(15) Castro, E. A.; Barbiric, D. J. Current theoretical methods applied to study cyclodextrins and their complexes. <http://preprint.chemweb.com> CPS: physchem/0203005. Submitted March 2, 2002. Accessed June 11, 2004.

Received for review July 8, 2004. Revised manuscript received October 26, 2004. Accepted November 1, 2004.

JF0488716