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Effect of β-Cyclodextrin on Aroma Release and Flavor Perception

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Binding and release of volatile compounds to and from β-cyclodextrin were measured in model aqueous systems using static equilibrium headspace and dynamic headspace dilution. β-Cyclodextrin decreased the static equilibrium headspace for some volatiles (e.g., ethyl octanoate and decanone) due to binding, but dilution studies demonstrated that binding was readily reversible. Dynamic release of hydrophobic volatile compounds was similar to that observed from emulsions. When β-cyclodextrin was added to fat free yogurt, the release of a commercial lemon flavoring was modified and was similar to release from a regular fat yogurt. Sensory difference testing confirmed the release results. The data demonstrate that β-cyclodextrin can be used to modify flavor delivery in both model and real systems; the effects in the latter are sensorially significant.

KEYWORDS: Nosespace; binding; APCI-MS; yogurt; inclusion complex

INTRODUCTION

Controlling flavor retention in foods during manufacture and flavor release during consumption is of major interest to food manufacturers. The use of encapsulated flavors to achieve this goal is well-established. Much is known about the encapsulation process and the materials available (see, for example, refs *1*−*3*). Less is known about the dynamics of flavor release from encapsulated systems. Cyclodextrins represent one of the simplest encapsulant systems. They consist of circular chains of α-1,4-glycosidically linked glucopyranose units that form a hollow truncated cone and are classified on the basis of the number of glucopyranose units in the circular structure (α, 6; β, 7; and γ, 8) (*4*). β-Cyclodextrin (β-CD) is the most commonly studied form. Work in the past with β-CD in the area of flavor binding has looked at selectivity (*5*, *6*), separation (*7*), retention (*8*−*11*), and stability (*12*) of volatile compounds. Computer-aided molecular modeling has also been used to understand the spatial orientation of guest compound complex inclusions with β-CD (*13*−*15*).

Binding of aroma (guest) compounds to the β-CD molecule (host) leads to the formation of an inclusion complex. The nature of the interactions leading to complexation has been discussed elsewhere (*16*). Essentially, complexation is dependent primarily on guest compound hydrophobicity as well as molecular size and geometry. Compounds usually complex with β-CD on a 1:1 stoichiometric basis, due to the limited amount of physical space available for guest inclusion, although other compositions have also been observed in some cases (*17*). Using NMR analysis, the arrangement of several guest molecules in the β-CD host has been proposed (*8*).

In the preparation of encapsulated flavors using β-CD, the aroma compound is mixed in excess with β-CD and solvent and then dried to remove any unbound aroma and solvent. When the inclusion complex is placed in excess water, release of the guest molecule proceeds by reversal of the thermodynamic equilibrium. Investigation into the release of compounds from β-CD has been mainly based on formal (and informal) sensory observations (*18*). Dynamic release of aroma compounds can be studied in model systems to provide fundamental information like the mass transfer behavior and the physicochemical parameters that control release. Aroma release can also be monitored in vivo using direct mass spectrometry methods such as atmospheric pressure chemical ionization mass spectrometry (APCI-MS) (*19*) and PTR-MS (*20*). Typically, model systems involve measuring the partition between the air (a) and the solution (s) to give the partition coefficient (K_{as}). Comparison of the K_{as} values for water and for the test solution indicates the degree of binding that occurs (*21*). Some indication of dynamic release can be obtained by dynamic headspace dilution analysis as described by Marin (*22*) who showed that the dynamic release behavior was dependent on the K_{as} values. The data obtained from the model systems can then be compared with the aroma release behavior in vivo to study potential mechanisms governing in vivo release.

The reason for monitoring aroma release is that the rate at which release occurs affects aroma perception (*23*). If release is too slow, perception will be diminished; if release is too fast, only a brief burst of flavor will be experienced. A similar situation is noted in regular and low fat foods. For the same

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aroma content, reduced fat foods release hydrophobic compounds to a greater extent and more rapidly than their regular fat counterparts (*24*, *25*). While the increase in release can be addressed by reformulating the flavor (to adjust the content of hydrophobic volatile compounds), sensory analysis of reduced fat foods often reports a "thin" perceived flavor, which may be due to the different aroma release profile. Previous work from our laboratory showed this effect in yogurt and in biscuits with different fat contents (*24*, *25*). In this paper, the release of aromas from β-CD in model systems is measured and the use of β-CD to manipulate the temporal release of aroma from reduced fat yogurt (and its effect on perceived flavor) is also studied.

MATERIALS AND METHODS

Preparation of Solutions. Stock solutions each containing two or three volatile compounds (Sigma-Aldrich, Poole, Dorset, U.K., min 98% purity) were prepared in distilled water to give final concentrations as follows: ethanol (40 mg/L), butanol (40 mg/L), methyl acetate (1 mg/L), ethyl butyrate (1 mg/L), ethyl hexanoate (1 mg/L), ethyl octanoate (1 mg/L), heptanone (1 mg/L) octanone (1 mg/L), and decanone (1 mg/L). Compounds were selected so that they had unique ions, which could be monitored by APCI-MS analysis; there was no interference from fragment ions.

β-CD (min 98% purity, Sigma-Aldrich) was dissolved in distilled water or in solutions of the volatiles to form a range of β-CD concentrations (in g/L: 0.03, 0.06, 0.3, 0.6, 3 and 6). Solutions of the volatile compounds in water were used as the control sample. All solutions were gently agitated on an orbital shaker OS21 (Chiltern Scientific, Wendover, U.K.) to ensure complete dissolution of the volatile compounds. The solutions were sealed in bottles (solution volume, 50 mL; headspace volume, 73 mL). Samples were left to equilibrate for 2 h at room temperature (22 °C) and at 37 °C by holding the bottle in a temperature-controlled water bath (Grant Instruments, Cambridge, U.K.) during measurements under static and dynamic conditions.

APCI-MS Analysis. Headspace or breath was sampled into a Platform LCZ mass spectrometer fitted with the MS Nose interface (Micromass, Manchester, U.K.) at a flow rate of 5, 10, and 35 mL/min during dynamic headspace dilution, static equilibrium, and in vivo (nosespace) analyses, respectively. The volatile compounds in the gas phase were ionized by a 4 kV corona discharge before passing into the quadrupole analyzer region of the mass spectrometer. The APCI-MS compound ionization parameters were set to minimize fragmentation of MH^+ ions to enable clear interpretation of measurements of both headspace and nosespace. Different cone voltages were used for the volatiles studied as follows: decanone, 20 V; ethyl octanoate, 23 V; all others, 18 V. Acquisition times were 0.2 (headspace), 2 (dynamic headspace dilution), and 0.02 s (in vivo).

Static Equilibrium Headspace Analysis. Sealed bottles were left to equilibrate for 2 h before the static headspace at equilibrium was measured using the APCI-MS. A sample of headspace was drawn out upon removing a plug from the lid of the sample flask. Headspace values (three replicates) were obtained by measuring the ion intensity once a steady state signal was obtained from the ion trace using Masslynx 3.2 software (Micromass Ltd., Manchester, U.K.).

Dynamic Headspace Dilution Method. Dynamic headspace dilution analysis was performed using the methods and principles developed previously (*22*, *23*). Solutions (100 mL) of decanone and ethyl octanoate (1 mg/L) were prepared with and without 6 g/L β-CD samples in 80 mL Schott bottles fitted with specially adapted lids for dynamic headspace dilution work (*26*). The lid had three ports, one for introducing a constant flow of nitrogen (70 mL/min) into the headspace, a second for sampling the headspace for analytes to the APCI-MS (at 5 mL/min), and the third to allow the excess flow from the headspace to escape. Three replicate measurements were taken for each sample.

Nosespace/In Vivo Aroma Release from Solutions. Six panelists were instructed to inhale a normal breath of air, and then, a 10 mL aliquot of sample solution (containing a volatile compound with or without β-CD) was pipetted into the mouth. They were asked to swallow the solution immediately and then exhale through their nose into the nosepiece connected to the APCI-MS. Nosespace volatile intensity data were collected during a 30 s period of steady and relaxed breathing. Sufficient time was allowed between samples to avoid carryover of volatile before reusing the panelists. Decanone and ethyl octanoate were added at 10 mg/L instead of 1 mg/L to increase sensitivity for the in vivo measurements. Three replicate measurements were taken for each of the six panelists.

From the breath by breath trace of aroma release, the height of the first peak was measured to determine the maximum nosespace volatile intensity. Data were expressed relative to the in vivo release values obtained from an aqueous solution of the volatile compounds. The percentage values for any particular volatile compound were averaged across the assessors to provide an overall panel mean. Persistence of aroma release was calculated by comparing the second peak height of aroma release with the first and expressing the difference as a percentage.

Yogurt Samples. Natural yogurt containing 5.1 g/100 g of fat content was obtained from a supermarket (Tesco), and this sample was used as the regular fat yogurt. A fat free natural yogurt with 0.1 g/100 g fat content (Yeo Valley's Organic) was used as the reduced fat yogurt. A commercial lemon flavor (502136 T, Firmenich, Geneva, Switzerland) was added to both of the yogurts at 250 mg/kg.

Dispersion of lemon flavor in yogurts was achieved using a high speed mixer (Ultra Turrax; Janke & Kunkel Gmbh & Co., Germany) set at 9500 revolutions/min for 3 min initially and 100 rpm for the next 7 min. A batch of fat free yogurt had β-CD added prior to the addition of lemon aroma solution to give a concentration of 8 g/kg. Sucrose was added to all yogurt samples at 10 g/kg (w/w) to compensate for any differences in sourness between the yogurts. The yogurts were left to rest at 4 °C for 48 h, which enabled reformation of network structure thereby restoring the viscosity close to the original level.

Headspace Analysis of Lemon Flavored Yogurts. Lemon-flavored yogurt samples (50 mL) were placed into 80 mL glass flasks (Schott, Fisher Scientific, Loughborough, U.K.) and sealed. After a 2 h equilibration period, the headspace was measured using the APCI-MS. Headspace values (three replicates) were obtained by recording peak ion intensities of the steady state signal obtained from the ion trace. Analysis of the headspace above pure lemon aroma found six significant *m*/*z* values: 81, 136, 137, 155, 166, and 229 from full scan operation.

In Vivo Analysis. Samples of yogurt were consumed using a standard protocol of the panelist first breathing in, placing 15 mL of yogurt (by spoon) into the mouth, and placing a nostril onto a sampling tube prior to the first exhalation and chew. This allowed real-time aroma release monitoring of the exhaled breath using the APCI-MS as described by Taylor et al (*27*) for a period of 1 min. The sample was consumed using normal mastication until swallowing, after which no further mouth movements were made, to measure the persistence of aroma release during normal breathing. The sampling rate to the APCI-MS was set at 30 mL/min, and the acquisition time was set to detect selected *m*/*z* values every 0.02 s. Compounds corresponding to the following *m*/*z* values, 81, 136, 137, 155, 166, and 229, were monitored at a cone voltage of 18. One panelist was used to obtain three replicate measurements from which the average release profile of each yogurt was plotted.

Sensory Difference Tests. Thirty untrained assessors, consisting of staff and students, were recruited from within the Division of Food Sciences (University of Nottingham, U.K.) on the basis of availability. Two triangle tests (BS 5929-3:1984; ISO 4120:1983) were performed by the panel; the first, compared fat free and regular fat, and the second compared fat free with 8 g/L β-CD and regular fat. The presentation of samples (20 mL) was balanced across assessors for all possible combinations of two samples, and all tests were performed in well lit and ventilated sensory booths. Sensory data were collected using Fizz Sensory Analysis software (Courternon, France). Assessors were instructed to identify the odd sample on the basis of lemon flavor by taste only. In addition, assessors were required to indicate their degree of certainty of sample choice on a scale of 1 (not sure) to 10 (sure). A comment box was also available to state the basis of their choice. Assessors were instructed to use a palate cleanser of crackers and water between samples and observed a preprogrammed rest of 1 min between

Table 1. Relationship between Physicochemical Properties and Static Equilibrium Headspace Intensity of Compounds in 6 g/L β-CD Solutions (22 °C) Relative to an Aqueous Control

volatile compd	molecular mass (Da)	LogP[a]	static headspace intensity (%) relative to control[b]	retention (%)
methyl acetate	74	–0.13	99 (2)	1
ethanol	46	0.077	129 (2)	0
butanol	74	1.013	117 (2)	0
ethyl butyrate	116	1.23	68 (4)	32
ethyl hexanoate	144	2.02	45 (5)	55
heptanone	114	2.19	56 (3)	44
octanone	128	2.59	31 (2)	69
ethyl octanoate	172	2.81	9 (2)	91
decanone	156	3.38	9 (1)	91

[a] Calculated using CAChe 3.2 (Oxford Molecular, Beaverton, OR). [b] Values in brackets indicate %CV ($n = 3$).

the two triangle tests. Each triangle test was analyzed to determine if a significant difference ($p = 0.05$) existed using Fizz Calculation software (Courternon). Degree of certainty data were recorded as "not sure" (1−5) and "sure" (6−10) and subsequently cross-tabulated with the assessors' ability to perceive a difference.

RESULTS AND DISCUSSION

Effect of Compound on Binding to β-CD. Although the key factors governing binding of compounds to β-CD are established, most of the data refer to concentrated encapsulation systems, not interactions in dilute aqueous solutions. Therefore, the degree of binding under these conditions was investigated for this particular sample of β-CD. Nine volatile compounds (**Table 1**) were selected from ester, ketone, and alcohol groups to represent different physicochemical parameters such as Log P (hydrophobicity), Log *p*L (vapor pressure) (*28*), and different molecular weights. The headspace concentrations of the compounds above 6 g/L β-CD solutions and control solutions were measured at equilibrium and expressed on a relative basis. A value of 100% indicates no change in headspace concentration and therefore no binding of the volatile compound to β-CD. Values less than 100% indicate binding; values above 100% indicate an increase in headspace concentration (the "salting out" effect). To ensure that equilibrium had been reached after the 2 h equilibrium time, the same solutions were reanalyzed after 6 and 18 h equilibration but no changes in headspace concentration as compared to the 2 h values were found.

The data in **Table 1** show a trend of increasing binding to the β-CD host molecule as hydrophobicity of the guest molecule increased, but it was clear that the hydrophobicity index (Log P) was not the only factor involved. For the hydrophilic compounds, ethanol and butanol, there was some evidence of salting out in the presence of β-CD whereas for hydrophobic compounds such as ethyl octanoate and decanone, over 90% of the compounds added were bound to β-CD. Other authors have discussed the factors governing binding in detail (*29*, *30*), but the purpose of these results was to provide a measure of binding for these compounds in this particular system.

The salting out of ethanol and butanol in the presence of 6 g/L β-CD is difficult to explain on the basis of the mole fraction of β-CD in solution. Salting out with sugars (e.g., sucrose) occurs at concentrations of over 600 mM (*31*), whereas β-CD was present at around 6 mM. Another factor to consider is the use of a relatively high ethanol and butanol concentration (40 mg/L) in volatile mixtures. Over control solutions, competition for ionization between volatile compound mixtures could have occurred, favoring those compounds with a greater proton affinity (*32*). Above β-CD solutions, the reduced headspace concentration of those volatile compounds bound to β-CD could have reduced "suppression" of the higher concentration of ethanol and butanol ions and thus ionized a greater number of these ions resulting in a false salting out effect. A concentration of 40 mg/L was required to overcome the low partition coefficients of ethanol and butanol in order to obtain headspace signals within a working range. In contrast, the polar compound methyl acetate (1 mg/L) showed no significant changes in the headspace above control or β-CD solutions.

The remaining six compounds were retained in the following order: ethyl butyrate < heptanone < ethyl hexanoate < octanone < decanone = ethyl octanoate. The compounds that were bound to the greatest extent to β-CD were the most hydrophobic. For the homologous esters and ketones, the headspace concentration decreased over β-CD solutions of volatiles as carbon chain length increased, implicating LogP and molecular size as important factors for β-CD binding as previously reported (*16*, *33*). Other factors involved in binding to β-CD include van der Waals, dipole−dipole interactions, and Lennard−Jones energy term (*34*) as well as the displacement of high enthalpy water molecules from the cavity by guest compounds resulting in favorable enthalpy change (*33*). From the preliminary investigations, decanone and ethyl octanoate showed the highest affinity for β-CD and these two compounds were used to study complex formation and release under different conditions.

Effect of β-CD Concentration on Binding. For encapsulation purposes, conditions are manipulated to approach the optimum situation where each (and every) β-CD molecule binds one guest molecule. However, when β-CD is used in solution, its limit of solubility is 18 g/L, while the solubility of hydrophobic volatiles lies in the mg/L range and there is a dynamic equilibrium between guest and host, which lies well away from the 1:1 ratio. To study the host−guest interactions further, solutions of β-CD (0.03−6 g/L) were prepared with a constant concentration of guest compound (ethyl octanoate and decanone; both 1 mg/L). The headspace above each solution was measured at equilibrium, and the data are plotted in **Figure 1**. Over the β-CD concentration range measured, the headspace concentration of ethyl octanoate and decanone decreased to nearly 90% as compared to the SEH measured from the control. From these data, the apparent solution−air partition coefficients K_{as} were calculated for the control and 6 g/L concentrations (**Table 2**). The effect of β-CD at 6 g/L was to decrease the apparent partition value by approximately an order of magnitude. From these values, the ratio of "free" β-CD to decanone and ethyl octanoate was calculated on a molar basis. The ratios obtained for decanone and ethyl octanoate were 60 and 73 at 0.03 g/L β-CD and 759 and 902 at 6 g/L β-CD, respectively. Because there is a dynamic equilibrium between the free and the complexed states of the guest molecule and cyclodextrin (*17*), the relative number of uncomplexed β-CD molecules will affect the release behavior when the system moves away from equilibrium. This was later studied using the technique of dynamic headspace dilution analysis.

Effect of Temperature. Because the release of decanone and ethyl octanoate from control and β-CD solutions was to be studied in vivo, the effect of temperature was first measured using an in vitro system. Temperature also affects the dynamic equilibrium of the system and the equilibrium headspace above control and β-CD solutions. Thus, the static equilibrium headspace concentrations of the solutions were measured at 22

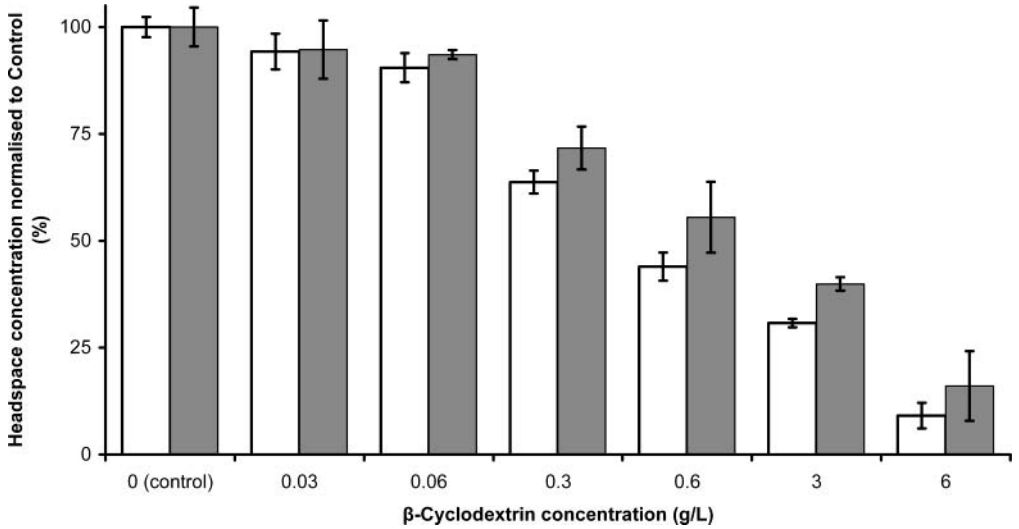

Figure 1. Effect of β-CD concentration on decanone (white bars) and ethyl octanoate (gray bars) headspace partitioning at equilibrium. Data normalized to control solution (containing no β-CD).

Table 2. Effect of β-CD on Gas Phase Concentration and Apparent Air–Solution Partition Coefficient (K_{as}) for Decanone and Ethyl Octanoate at 22 °C

compd	solution	compd added at mg/L	gas phase concn (mg/m^3)	K_{as}
decanone	control	1	9.3	9.29E-03
decanone	6 g/L β-CD	1	0.6	6.22E-04
ethyl octanoate	control	1	38.0	3.80E-02
ethyl octanoate	6 g/L β-CD	1	5.1	5.10E-03

and 37 °C. Changing the temperature from 22 to 37 °C increased the headspace concentrations above water and β-CD solutions by a factor of about 5 for decanone and a factor of about 3 for ethyl octanoate (data not shown). However, the headspace concentration for water and β-CD solutions changed to the same extent, with no significant differences ($P > 0.05$) in the behavior of the two systems. Thus, any changes noted in vivo for release of volatiles from control and β-CD solutions could not be attributed to temperature effects on the equilibrium state of the two solutions.

Dynamic Headspace Dilution. The dynamic headspace dilution technique provides information on the dynamic behavior of a system as the equilibrium headspace is diluted by a gas flow and the system attempts to maintain equilibrium. The rate at which this occurs depends on both the physicochemical properties of the volatile compound and the environmental conditions in the system (*35*).

In terms of absolute headspace concentration, control solutions gave higher values than 6 g/L β-CD solutions (**Figure 1**). To compare the relative performance of the two systems, the initial headspace concentrations were set to 100% to produce relative traces of the headspace dilution process. In **Figure 2**, the initial headspace concentration represents the volatile intensity at static equilibrium conditions. The decrease after 1.5 min occurred as the headspace was diluted with gas, and this continued until a steady state was reached where removal of volatile compounds from the headspace is effectively balanced by partition of volatile from the solution.

The relative rate at which headspace intensity decreased above aqueous control solutions was greater than over β-CD solutions. The presence of β-CD creates a reservoir of bound decanone and ethyl octanoate molecules, which are progressively released as the free molecules become depleted at the interfacial layer.

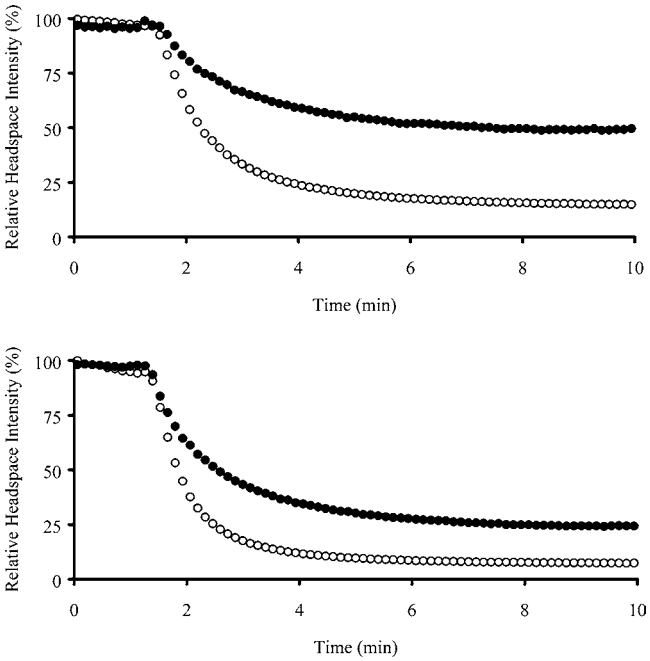

Figure 2. Dynamic headspace dilution curves of volatile headspace intensity normalized to SEH concentration. Top chart: decanone ○ = control solution; ● = 6 g/L β-CD solution. Bottom chart: ethyl octanoate ○ = control solution; ● = 6 g/L β-CD solution.

This effectively enhances the stability of headspace concentration as compared to the control solution. The addition of β-CD decreased the K_{as} of decanone and ethyl octanoate by a factor of 14 and 8, respectively. The lower K_{as} contributes to a more stable headspace concentration during gas phase dilution than compounds with high K_{as} (*35*). The behavior of the β-CD solutions was similar to the effects seen with emulsions under dynamic headspace dilution (*26*, *36*).

Volatile Release In Vivo. During consumption of liquid foods, dilution of the liquid and gas phases occurs, with the extent being dependent on the individual's pattern of breathing, mouth movement, and saliva flow. The release behavior of control and β-CD solutions was measured using the in vivo technique developed in our lab (*27*). Volatile concentration was increased to 10 ppm so that there was sufficient material to overcome any dilution effects in mouth with saliva, tidal air,

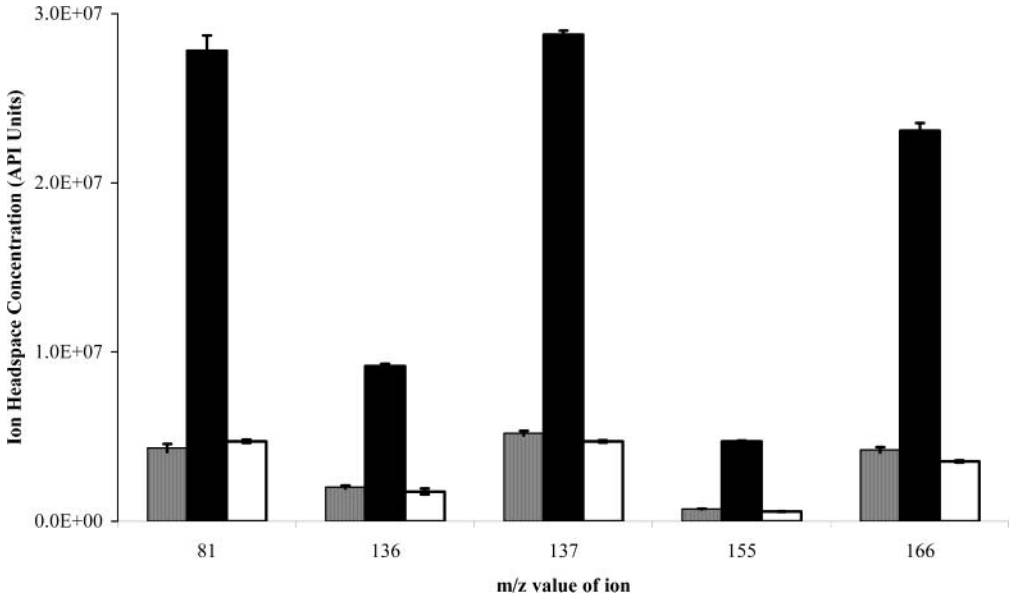

Figure 3. Static equilibrium headspace concentrations of selected ions above lemon-flavored yogurt: regular fat (gray bar), fat free (black bar), and fat free + 8 g/L β-CD (white bar). Error bars indicate ± SD.

Table 3. Comparison of Volatile Release and Persistence Values from 6 g/L β-CD Solutions Relative to the Aqueous Control between SEH Studies (37 °C) and In Vivo Release from Nosespace Measurements

			nosespace persistence	
volatile	static headspace intensity (%)	nosespace intensity (%)	aqueous control	β-CD
decanone	9	42	12	23
ethyl octanoate	16	64	9	12

and volume changes and still ensure compounds were above the detection limits of the APCI-MS technique. Samples were consumed as described in the Materials and Methods, and the maximum concentration in the nosespace was measured. The differences in volatile release between β-CD and control solutions were expressed as a concentration ratio to allow comparison of ethyl octanoate and decanone behavior (**Table 3**). For decanone release in vivo, the maximum nosespace concentration from β-CD solutions was 42% of the release from control solutions, and for ethyl octanoate, the value was 64%. For comparison, the same ratios calculated from equilibrium headspace gave values of 9 and 16%, respectively, which are in good agreement with the values calculated from **Table 2**, 6 and 13%, respectively. The in vivo data show that release was greater than expected on the basis of static equilibrium data. This suggests that the dynamic conditions in vivo facilitate mass transfer from the β-CD solutions (*19*). The dynamic nature of the interaction was also reported for menthol encapsulated in β-CD where the dry product was virtually odorless but elicited a strong menthol flavor when placed in the mouth (*37*). Dilution by saliva would cause the shift in the equilibrium toward release.

In addition, the persistence was also measured (as described in the Materials and Methods). β-CD solutions showed greater persistence, thereby strengthening the hypothesis that the conditions in vivo result in release of some of the bound volatile as well as the free volatile (**Table 3**). This ultimately changes the shape of the aroma release profile. This effect can be attributed to the rapid dynamic reversibility of the guest/host complex in solution (*17*). This type of behavior has also been noted in studies on ester release from lipid emulsions (*26*).

Effect of β-CD on Flavor Release in a Yogurt System. Previous work on volatile release from low and regular fat yogurt demonstrated that volatile release is grossly affected at lower fat values (*25*). At lower fat contents, volatile release from yogurt is more intense (and of short duration) and this release behavior has been associated with the "thin" flavor reported from sensory analysis of such low fat products. Experiments were performed to determine whether β-CD could change the volatile release from commercial fat free yogurt and how that change would be perceived by a sensory panel. Commercial, fat free, and regular fat yogurts were flavored with a commercial lemon flavoring, which contained a range of compounds, some of which would be expected to interact with β-CD (*38*).

Headspace Analysis of Yogurts. Headspace analysis of the lemon flavoring identified the ions associated with the major lemon volatile compounds, namely, at *m*/*z* 81, 136, 137, 155, and 166. Complete identifications were not important as the ions were used simply as markers for the effect of β-CD on volatile release from yogurt. Equilibrium headspace analysis of the yogurt samples showed a significant reduction ($P < 0.01$) in all of the ions monitored over the regular fat yogurt as compared with the fat free yogurt (**Figure 3**). The ion concentrations were 5−10 times greater in the headspace above fat free yogurt (**Figure 3**). The fat free yogurt with 8 g/L β-cyclodextrin had a similar headspace concentration to the headspace above regular fat yogurt. This showed that β-CD could function in a complex food matrix in the same way it did in water by binding hydrophobic volatile molecules. By adding 8 g/L β-CD to fat free yogurt, the headspace above it was modified to mimic that of a regular fat yogurt. However, we have seen from the in vitro experiments above that there can be significant changes in release in vitro and in vivo.

In Vivo. Samples of the different yogurts were eaten, and the release of lemon aroma in the nose was measured with time using APCI-MS. **Figure 4** shows the typical nosespace concentration (sum of all of the ions listed above) of lemon aroma from all three yogurt samples over 1 min. Although they all contained the same amount of lemon flavoring, the release from the fat free yogurt was much greater than for the regular yogurt and also for the fat free yogurt containing β-CD. Close

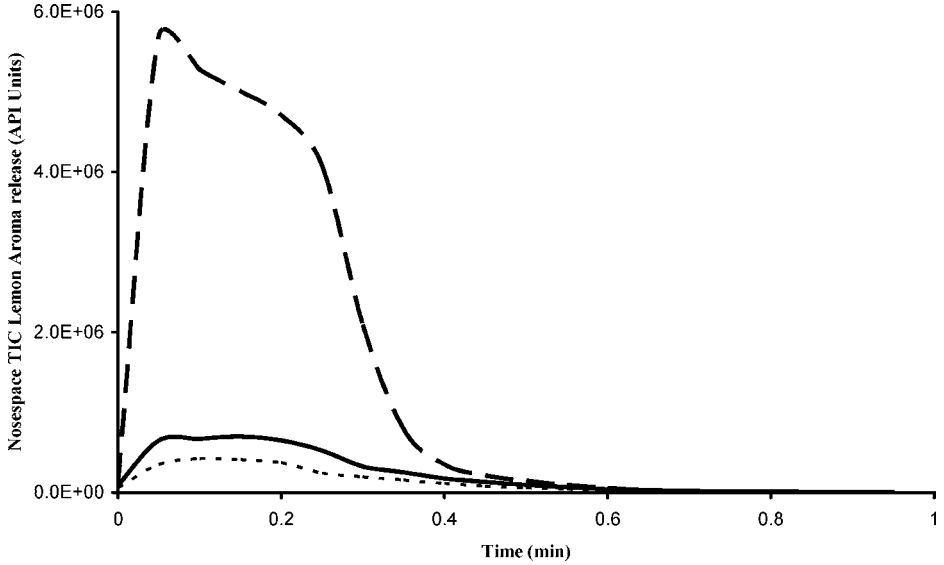

Figure 4. Nosespace concentration of lemon aroma release from fat free (– –), fat free + β-CD (—), and regular fat (- - -) yogurt. Average of three profiles from one panelist (10% coefficient of variation).

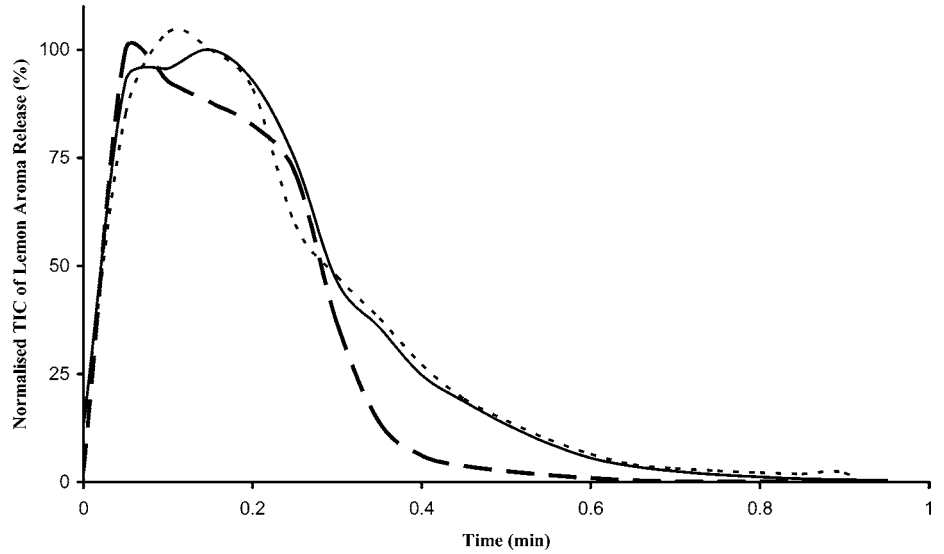

Figure 5. Normalized concentration of lemon aroma release in the nosespace from fat free (– –), fat free + β-CD (—), and regular fat (- - -) yogurt. Average release profile from three nosespace measurements from one panelist (10% coefficient variance).

inspection of the traces in **Figure 4** shows some minor differences, which can be more clearly seen in **Figure 5**, where the intensity of release has been adjusted to 100 for all samples so that the difference in timing of release can be more clearly seen. All three yogurts showed maximum release at about the same time, but the area under the peak was different for fat free yogurt and regular fat and β-CD/fat free yogurt. Both β-CD/fat free and regular fat yogurts had a larger area, which resulted in an extended period of aroma release. Thus, release from the β-CD/fat free yogurt was modified to bring it closer to the regular fat sample with most notably the reduction in intensity and extension of volatile release. The sensory significance of these changes in volatile release was tested using sensory analysis.

Sensory Analysis. Because β-CD had affected the release of the lemon flavor components in different ways, it was likely that the flavor quality of each sample was different. This could prejudice sensory testing based on a rating system, so instead, difference testing was used to determine whether the fat free/β-CD yogurt was different from regular fat or the fat free yogurt. Twenty-two assessors out of 30 noted a difference between the fat free and the regular fat yogurt ($P < 0.0001$) on the basis of lemon flavor (**Table 4**). Twenty assessors were sure of their choice. In the second test, 15 assessors identified a difference between the fat free/β-CD and the regular fat ($P < 0.0435$). However, of the 15 assessors, only eight were positively sure with their choice, two of whom indicated creaminess as the factor for their selection, not lemon flavor.

Table 4. Triangle Test Results of Lemon-Flavored Yogurt from 30 Assessors

test	assessors perceiving difference	significance level of P	assessors positively sure of choice	assessors unsure of choice
fat free vs higher fat	22	<0.0001	20	2
fat free + 8 g/L β-CD vs higher fat	15	0.0435	8	7

Although sensory analysis indicated assessors could differentiate the fat free/β-CD sample from the regular fat yogurt, the significance was much reduced to approaching $P = 0.05$.

Furthermore, assessors indicated greater uncertainty in their ability to differentiate between samples, suggesting an element of guessing. This early study clearly demonstrates the ability of β-CD to bring the lemon flavor profile of fat free yogurt closer to regular fat yogurt based on the substantial reduction in significance of difference. There are other factors that should be considered for future studies. Despite instructions to choose samples based on lemon flavor, it is possible that assessors used other attributes to select a particular sample. Because the regular fat yogurt contained 5% fat as compared to 0.1% fat (and no added hydrocolloids) in the fat free yogurt, there were also textural differences between the samples. Given the multimodal nature of flavor perception (*39*), these textural changes may affect a person's ability to judge purely on flavor. However, the experiments do suggest that compounds similar to β-CD, which have a flavor reservoir effect, may play a part in the design of low fat foods with flavor characteristics of the regular fat analogues.

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