Competitive Binding of Aroma Compounds by β-Cyclodextrin

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Retention of six aroma compounds has been studied after dehydration of ternary mixtures of aroma water and β-cyclodextrin. A maximal retention of a mole of aroma per mole of β-cyclodextrin has been observed for five of the aroma compounds, whereas retention of benzyl alcohol can be twice as high. Retention of a mixture of aroma compounds has also been studied. It has been noted that when volatile compounds compete for the same binding sites on β-cyclodextrin, ethyl hexanoate, 2-methylbutyric acid, and benzyl alcohol are, respectively, better retained than ethyl propionate, hexanoic acid, and hexanol. Preferential retention observed with esters can be simply explained by their difference of physicochemical properties, but for the acids and alcohols a study at the molecular scale has been necessary. The better retention of 2-methylbutyric acid can be explained by differences in the nature of interaction between the acids and their carrier. At least selectivity of retention noted for the alcohol could be due to a difference in the location of the guest and also a difference in the number of aroma molecules that can be bound per polysaccharide molecule.

Keywords: *Aroma; encapsulation; competition; β-cyclodextrinn; interaction; NMR*

INTRODUCTION

Microencapsulation is a process by which a substance or a mixture is coated or entrapped in another material (*1*). It is widely used in the food industry to protect flavor compounds and to control their release, polysaccharides being the most common carriers (*2*−*6*). Among them, maltodextrins and modified starch are the most frequently used, but β-cyclodextrins can be used, too (*7*). The main advantage of β-cyclodextrins is their ability to protect efficiently aroma compounds against thermal or chemical degradation and especially against oxidation (*8*−*10*). Their main drawback is a problem encountered with any carrier material, that, their selectivity toward the numerous volatile compounds that constitute an aroma. As a consequence, depending on the composition of the aroma to be encapsulated, some molecules are poorly retained, whereas others are strongly trapped, resulting in the production of an unbalanced flavor (*11*). Because most aromas are complex mixtures of flavor compounds, this problem is frequently encountered in the food industry and still empirically resolved. Studying mechanisms involved in the selective retention of volatiles by polysaccharides would help to reduce this empiricism.

The study of mechanisms involved in the retention of aroma compounds by polysaccharides has concerned binary or ternary systems (water, polysaccharide, and a single aroma compound) (*12*−*15*) and some papers have focused on retention of flavor mixtures (*16*−*19*). Encapsulation deals with retention of flavor initially added to polysaccharides and water at very high concentrations (10−25% w/w), and except for papers dealing with operating conditions or choice of carrier (*20*−*23*), few data are available on the retention of concentrated aromas (*24*). Papers published on the retention of mixtures have mostly concerned the retention of diluted aroma, reflecting concentrations encountered in foods.

The present work focuses on the mechanisms involved in the retention of high amounts of volatile mixtures by β-cyclodextrins. This polysaccharide was chosen as model carrier because it is used for flavor encapsulation and is not a mixture of polymers such as starch hydrolysates or modified starches. β-Cyclodextrins are indeed cyclic oligosaccharides made of seven glucopyranose units. Their inner hydrophobic cavity is torus shaped, and its molecular dimensions allow total or partial inclusion of a wide range of aroma compounds (*25*). The purpose of this study is to investigate the effect of the physicochemical characteristics of aroma compounds on their retention when they compete for the same carrier.

MATERIALS AND METHODS

Materials. β-Cyclodextrins (β-CD) were provided by Roquette (Lestrem, France) with a certified minimum purity of no less than 99 wt %. Six aroma compounds were selected among strawberry natural flavor compounds. Their physicochemical characteristics are given in Table 1. They were provided by International Flavor and Fragrance (Longvic, France) (purity > 98%).

Preparation of Complexes with a Single Aroma. Retention of a single aroma compound was studied by the addition of increasing amounts of aroma to a paste composed of β-CD and osmosed water (75% w/w). The initial amount of aroma compound in the mixture, prior to dehydration, ranged from 1 to 4 mol of flavor compound/mol of β-CD.

All of the mixtures were done in triplicate in hermetically closed flasks, shaken for 24 h at 25 °C, and dehydrated using a USIFROID SMJ freeze-dryer. The operating conditions were as follows: samples were frozen for 2 h at −50 °C and then

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10.1021/jf0101049 CCC: $20.00
Published on Web 11/22/2001

Table 1. Physicochemical Properties of Aroma Compounds

aroma compound	mol wt	saturated vapor pressure at 25 °C (Pa)	solubility in water at 25 °C (g/L)	hydrophobicity (log P)[b]
hexanol	102	197	6.0 (*27*)	1.94
benzyl alcohol	108	13	40.0 (*28*)	1.03
ethyl hexanoate	144	238	0.52 (*29*)	2.83
ethyl propionate	102	5374	12.4 (*29*)	1.24
hexanoic acid	116	20	7.8	1.84
2-methylbutyric acid	102	114	20.0	1.12

[a] Saturated vapor pressures were estimated using the Gomez Thodos model (*26*). [b] Log P is a hydrophobic constant, calculated using Rekker's method (*30*).

freeze-dried. During freeze-drying the temperature of the shelves was set at −25 °C for 10 h and then at 30 °C for 19 h.

The amount of aroma compound necessary for saturation of the binding sites of the carrier was deduced from these experiments.

Preparation of Complexes with Mixture of Aromas. The aim of this paper is to better understand the effect of physicochemical characteristics of aroma compounds on their behavior when they compete for the same carrier. In the food industry aromas are very often made of numerous compounds. Because the effect of the characteristics of each volatile is difficult to put into evidence in such a mixture, we chose to work with mixtures of two aroma compounds to be able to explain the effects observed. Competitions for the binding on β-cyclodextrin were also performed between ethyl propionate and ethyl hexanoate, between 2-methylbutyric acid and hexanoic acid, and between hexanol and benzyl alcohol. Binding sites of the carrier were first saturated by the addition of a sufficient amount of one of the aromas to a mixture of 25 wt % β-CD and 75 wt % water. Rising amounts of the second aroma were then added.

All of the mixtures were done in triplicate in hermetically closed flasks. They were shaken and freeze-dried as previously described for the complexes prepared with single aroma compounds.

Retention Measurements. Retention of aroma, expressed as moles of aroma retained per mole of β-CD, was measured by dilution of 0.1 wt % of dried complexes in osmosed water and injection of portions (1 μL) of this solution on the gas chromatographic column of a 5710A Hewlett-Packard chromatograph equipped with a flame ionization detector. Gas chromatographic conditions were the following: stainless steel column (3 m × 3.15 mm) packed with 100/120 mesh Carbowax 20M−10%, maintained at 110 °C for analysis of esters and at 150 °C for the analysis of alcohols. The injector and detector temperatures were 250 and 220 °C. Nitrogen, hydrogen, and air flow rates were, respectively, 19, 25, and 250 mL/min. For determination of hexanoic acid and 2-methylbutyric acid retention, a 30 min CH_2Cl_2 extraction (1:1 v/v) was performed on the aqueous solution, prior to injection of aliquots (1 μL) of the organic phase on an EC1000 capillary column (30 m × 0.32 mm, 0.25 μm film thickness) (Alltech) on a GC14 Shimadzu chromatograph equipped with a flame ionization detector. Heptanoic acid was used as internal standard. Gas chromatographic conditions were the following: capillary column temperature, 130 °C; injector and detector temperature, 250 °C; helium, hydrogen, and air entry pressures, 60, 50, and 35 kPa, respectively.

Measurements obtained with this method were checked by analysis of the same samples by ^{13}C NMR in the inverse gate mode.

^{13}C NMR and ^{1}H NMR Measurements. Spatial conformation of the complexes obtained with benzyl alcohol or 2-methylbutyric acid was deduced from the comparison of the spectra of the aroma (70 g/L) and of the corresponding complexes (70 g/L) diluted in deuterated dimethyl sulfoxide. Matsui and Tokunaga (*31*) have shown that use of an internal standard avoided the solvent effect, so tetramethylsilane (11 g/L) was used as internal standard. The NMR spectra were obtained on a Bruker Avance 500 FT-NMR spectrometer operating at 500 MHz for ^{1}H and at 125.8 MHz for ^{13}C. ^{1}H NMR spectra were obtained after four scans with 90° pulses. ^{13}C NMR spectra were recorded in the inverse gate mode and obtained after 1024 scans using 30° pulses and 3 s relaxation delay.

A two-dimensional NOESY spectrum was acquired using the standard Bruker NOESY pulse sequence on benzyl alcohol complexes (70 g/L) diluted in deuterated dimethyl sulfoxide.

All of the NMR spectra were obtained with the samples maintained at 27 °C.

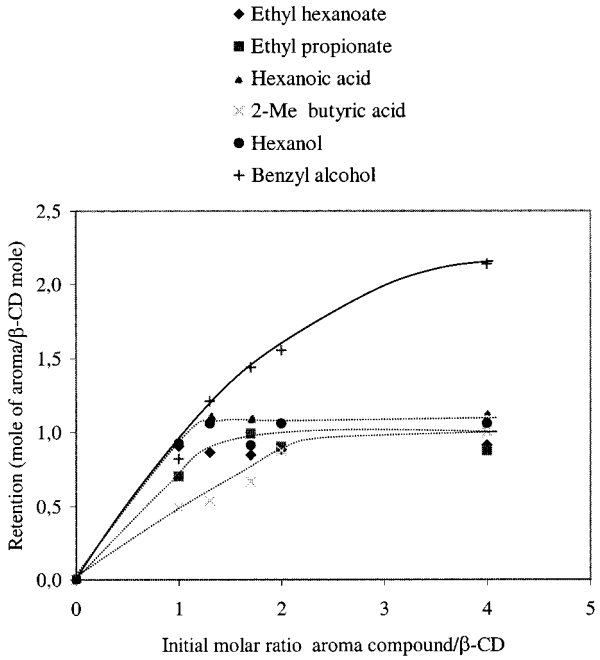


Figure 1. Retention after freeze-drying of ternary mixtures of aroma compound, β-cyclodextrin, and water.

RESULTS AND DISCUSSION

Figure 1 reports retention of each of the six aroma compounds after dehydration of a mixture of water and β-cyclodextrin in which increasing amounts of a single compound were added.

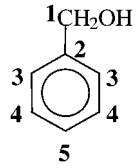
It can be stated that for five of the volatile compounds a maximal retention of 1 mol of aroma is reached even in the presence of a large excess of aroma before freeze-drying (4 mol/mol of β-CD). For ethyl propionate, ethyl hexanoate, hexanoic acid, and hexanol this maximal retention is in agreement with NMR results published on the structure of their β-CD complexes showing that their carbon chain is included in the β-CD cavity (*32*, *33*). It seems also that the only aroma molecules retained during freeze-drying are those encapsulated into the β-CD cavity, whereas the others are unable to interact in a sufficient way with the carrier. According to this saturation experiment it seems that only one molecule of 2-methylbutyric acid can be bound per molecule of β-CD, but no data were available on its β-CD complex to fully confirm this result. In the case of the five aliphatic aroma compounds selected for the study, saturation of the binding sites is achieved as soon as two molecules of aroma are initially added per β-CD molecule to the mixture of water and carrier.

In contrast, retention of benzyl alcohol can reach 2 mol/mol of β-CD when four molecules of this aroma are added to the paste of β-CD prior to dehydration. Reten-

tion of benzyl alcohol can also be twice that of hexanol. Sanemasa et al. (*15*) have already reported a retention rate >1 mol/mol of β-CD in the case of aromatic compounds. They showed that up to 1.9 mol of benzene or fluorobenzene can be retained per mole of β-CD, whereas retention of pentane and heptane, respectively, reached 1.1 and 0.88 mol/mol of β-CD in the same conditions. Therefore, it seems that the presence of an aromatic ring can influence the number of molecules that can be bound per β-CD molecule. Moreover, Sanemasa et al. (*15*) noticed that compounds such as *p*-dichlorobenzene and iodobenzene can be, respectively, retained up to 1.4 and 1.6 mol/molof β-CD. They also observed a fractional guest/cyclodextrin ratio for those compounds, but they did not explain it or determine the location of the guest. Retention of 2 mol of benzyl alcohol/mol of β-CD could be explained by the inclusion of more than one molecule of aroma per cavity of β-CD or by the retention of alcohol molecules between carrier molecules. If the molecular dimension of benzyl alcohol is considered, it appears that the only way to include two molecules into a β-CD cavity is to insert one at each extremity of the cavity. Indeed, an aromatic ring is 0.43 nm large and as the inner diameter of β-CD is 0.60 nm at the primary hydroxyl side and 0.64 nm at the secondary hydroxyl side (*34*), an aromatic ring can be inserted at an extremity and fill it. The tear formed by β-CD is 0.79 nm deep, so it can be supposed that at least part of two aromatic rings can be inserted at both extremities of β-CD. This hypothesis is reinforced by the results obtained by Hirai et al. (*35*) on the structure of 4-biphenylcarboxylate complexes. They showed that this compound can be included into the cavity of β-CD and that its two aromatic rings are inserted into the cavity. It can also be supposed that two aromatic molecules can at least be partly included at each extremity of the β-CD cavity. The hypothesis of flavor retention on the outer surface of the carrier molecules can nevertheless not be excluded, provided these molecules interact in a sufficient way with β-CD to be retained during dehydration. β-CD molecules are lined at both their extremities by hydroxyl groups, so it can also be imagined that they are able to interact with alcohol molecules through hydrogen bonds.

Spatial conformation of benzyl alcohol complexes has also been further studied by ^{13}C and ^{1}H NMR. Inoue et al. (*36*) have shown that the inclusion of a ^{13}C nucleus into the apolar medium of the β-CD cavity induce a decrease of its resonance frequency. Resonance frequencies of carbon nuclei of encapsulated benzyl alcohol were also compared to that of the free aroma compound. In the presence of β-cyclodextrins, resonance frequencies of all carbon nuclei of benzyl alcohol decrease but that of the hydroxylated carbon is more affected than those of the aromatic ring (Table 2). Moreover, shifts in resonance frequencies of carbon nuclei decrease in the ortho > meta, para order. Similar results have been observed with ^{1}H resonance frequencies. When encapsulated, the proton resonance frequency of the alcohol function is much more decreased (−26 Hz) than that of CH_2 protons (−10 Hz). To the contrary, the resonance frequency of the aromatic ring protons is only slightly decreased (−3 Hz). Benzyl alcohol seems also to include its alcohol function into the β-CD cavity. Moreover, this result is reinforced by the observation of the NOESY spectrum of benzyl alcohol complexes. No cross-peak has been observed connecting the resonance signal of the aromatic ring protons to the resonance signal of the H-3 and H-5 internal protons of β-CD, meaning that the aromatic ring is not included in the cavity. Chang et al. (*37*) have observed a similar inclusion orientation for benzaldehyde into the α-cyclodextrin cavity, in agreement with the antioxidant protection of that carrier. From these results it seems that benzyl alcohol is retained by β-cyclodextrins by inclusion of its alcohol function into the β-CD cavity and that several benzyl alcohol molecules interact in this way with the carrier, which explains retention of this aroma compound in a high amount. It cannot be excluded that some benzylic alcohol functions might hydrogen bond with each other within the cavity. The differences observed in the retention of single aroma compounds can also be explained by a difference in the location of the guest and also in the nature of interaction with the carrier. The five aliphatic compounds seem to be able to fully include their carbon chain into the β-CD cavity, whereas only the hydrophilic part of benzyl alcohol molecules seems to be included and interact with the hydroxyl groups lining β-CD cavity.

Table 2. Variation of the Resonance Frequency of Carbon Nuclei (^{13}C) of Benzyl Alcohol after Encapsulation by β-Cyclodextrin



carbon atom	variation of resonance frequency (Hz)	carbon atom	variation of resonance frequency (Hz)
C1	−20.2	C4	−14.4
C2	−16.3	C5	−14.4
C3	−16.3		

Competition between aromas was then studied by observation of retention after addition of increasing amounts of aroma to a paste of carrier initially saturated with one of the aromas. In the case of esters or acids the binding sites were saturated by the addition of 2 mol of one of the esters or the acids and increasing amounts of the second one were added. In the case of alcohols the binding sites were initially saturated by the addition of either 2 mol of hexanol/mol of β-CD or 4 mol of benzyl alcohol/mol of β-CD and increasing amounts of the second alcohol were then added.

Competition between Esters for Their Binding on β-Cyclodextrin. Figure 2 reports the retention of esters when ethyl hexanoate is added to a paste initially saturated with 2 mol of ethyl propionate/mol of β-CD. Retention of ethyl hexanoate strongly increases, whereas that of ethyl propionate decreases. When competitions are done in the reverse order, ethyl propionate retention slightly increases with the amount added to a paste saturated with ethyl hexanoate (Figure 3). Ethyl hexanoate and ethyl propionate have the same number of binding sites on β-CD, and the better retention of ethyl hexanoate compared to that of ethyl propionate can be explained only by their difference of affinity for the binding site, closely linked to the physicochemical properties of these aroma compounds. The hydrophobicity of ethyl hexanoate is higher (log $P = 2.83$) than that of ethyl propionate (log $P = 1.24$), and the affinity constant of ethyl hexanoate for the hydrophobic cavity of β-CD is 8.3 times higher ($K_a = 318\ M^{-1}$) than that of

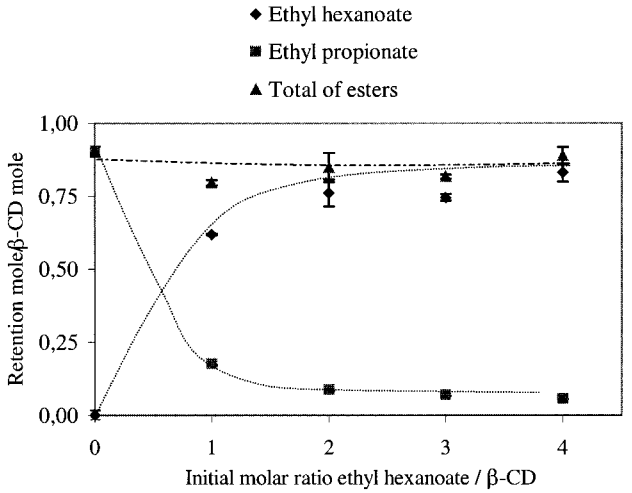


Figure 2. Retention of esters after freeze-drying of β-cyclodextrins initially saturated with 2 mol of ethyl propionate/mole of β-CD, in which increasing amounts of ethyl hexanoate have been added.

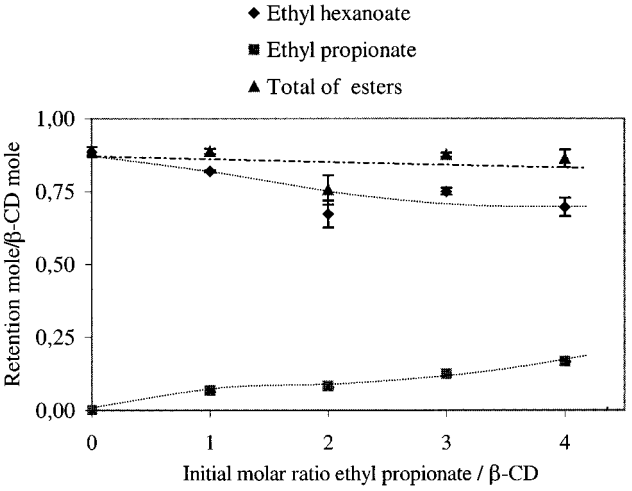


Figure 3. Retention of esters after freeze-drying of β-cyclodextrins initially saturated with 2 mol of ethyl hexanoate/mol of β-CD, in which increasing amounts of ethyl propionate have been added.

ethyl propionate ($K_a = 38\ M^{-1}$) (*33*). During the mixing of aroma with the paste of β-CD and water, ethyl hexanoate molecules are therefore easily complexed in the hydrophobic cavity of β-CD, whereas part of the ethyl propionate molecules remain in the aqueous phase. Moreover, the saturated vapor pressure of ethyl propionate is much more higher than that of ethyl hexanoate (5374 vs 238 Pa at 25 °C), and during freeze-drying ethyl propionate is also more easily removed than ethyl hexanoate. This also clearly shows that the presence of aroma compounds with high differences of volatility and affinity for the carrier strongly affect the composition of the encapsulated flavor and that in some simple cases preferential retention can be predicted from the physicochemical properties of the pure aroma.

Competition between Acids for Their Binding on β-Cyclodextrin. Figures 4 and 5 show the results obtained with acids. It can be stated that when the carrier is saturated with hexanoic acid and increasing amounts of 2-methylbutyric acid are added, retention of the latter strongly increases, whereas that of hexanoic acid decreases, clearly demonstrating the preferential retention of 2-methylbutyric acid. When the carrier is

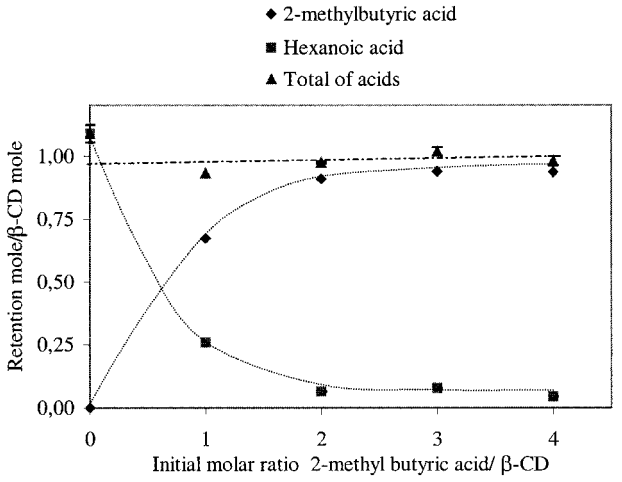


Figure 4. Retention of acids after freeze-drying of β-cyclodextrins initially saturated with 2 mol of hexanoic acid/mol of β-CD, in which increasing amounts of 2-methylbutyric acid have been added.

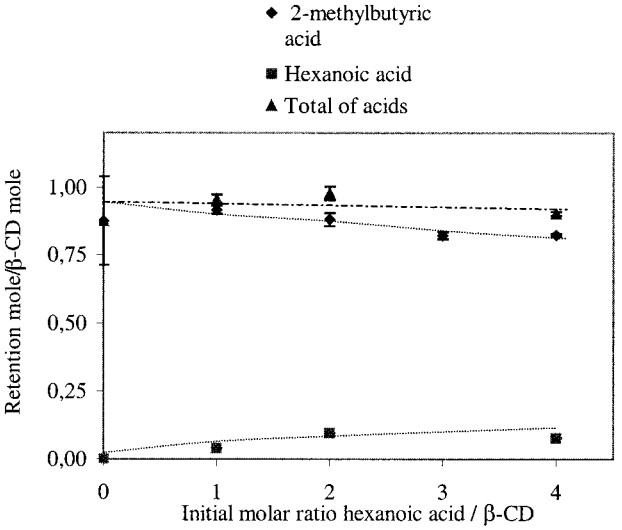
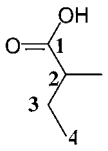


Figure 5. Retention of acids after freeze-drying of β-cyclodextrins initially saturated with 2 mol of 2-methylbutyric acid/mol of β-CD, in which increasing amounts of hexanoic acid have been added.

initially saturated with 2-methylbutyric acid and increasing amounts of hexanoic acid are added, retention of the latter slightly increases, whereas that of 2-methylbutyric acid decreases. In agreement with results obtained during the saturation study by a single aroma (Figure 1), retention does not exceed 1 mol/mol of β-CD even when six molecules of aroma are initially added per molecule of β-CD. For these two acids it seems also that the retention rate is limited by the number of available binding sites and that the preferential retention of 2-methylbutyric acid is induced by a stronger affinity for the cavity of β-CD. Nevertheless, the preferential retention of 2-methylbutyric acid could not be predicted from the physicochemical properties of pure aroma. Indeed, 2-methylbutyric acid is less hydrophobic (log $P = 1.12$) than hexanoic acid (log $P = 1.84$) and is more volatile (Table 1). In this case the physicochemical properties of pure aroma are not sufficient to predict preferential retention in the hydrophobic cavity of β-CD. The preferential retention of 2-methylbutyric acid could be explained by differences in the conformation of the carbon chain. Hexanoic acid has a linear carbon chain,

Table 3. Variation of the Resonance Frequency of Carbon Nuclei (^{13}C) of 2-Methylbutyric Acid after Encapsulation by β-Cyclodextrin



carbon atom	variation of resonance frequency (Hz)	carbon atom	variation of resonance frequency (Hz)
C1	−6.71	C4	−16.3
C2	−18.2	2-methyl group	−16.3
C3	−23.0		

for which contact with the apolar walls of the β-CD cavity should be lower than that of 2-methylbutyric acid. The nature of interactions involved in the retention of these two compounds could also be different and explain the differences in affinity for the carrier. Kano et al. (*38*) proposed that the nature of interactions involved in the retention of guest by β-cyclodextrins depends on the molecular volume of the guest. They hypothesized that molecules with a small molecular volume are mainly retained by hydrophobic interactions, whereas big molecules have a better contact with the apolar inner wall of β-cyclodextrins. Dispersion forces would also be the main contribution to their retention.

To understand the differences of behavior observed between these two acids, the spatial conformation of 2-methylbutyric acid complexes has been investigated at a molecular scale by ^{13}C NMR and compared to that of hexanoic acid complexes, previously reported (*33*). Results are reported in Table 3. The resonance frequency of all carbon nuclei is decreased upon encapsulation but that of the carboxylic function is the less affected, meaning that 2-methylbutyric acid is included in the β-CD cavity and that its carboxylic function is located near the extremity of the β-CD cavity. Variations of resonance frequency of the methyl carbon nuclei are relatively low, meaning that the methyl groups are surrounded by a relatively polar medium and are also close to the extremity of β-CD. Moreover, the resonance frequency of the C3 carbon nucleus is more greatly affected by encapsulation, meaning that this part of the acid molecule is more deeply included in β-CD cavity. The carbon chain of 2-methylbutyric acid seems also not to be included along the axis of the β-CD cavity but in an inclinational way, as proposed in Figure 6. This orientation allows methyl groups to interact through dispersion forces with the apolar inner walls of β-cyclodextrins. It has already been reported that hexanoic acid includes its linear carbon chain along the axis of the β-CD cavity. It can be also proposed that the better retention of 2-methylbutyric acid compared to hexanoic acid is due to a difference in the nature of interactions that occur between the guest and its host. Because hexanoic acid is more hydrophobic (log $P = 1.84$) than 2-methylbutyric acid (log $P = 1.12$) and because the hexanoic acid carbon chain is included along the axis of the β-CD cavity, it can be supposed that removing these molecules from water during the encapsulation process is more favorable than removing 2-methylbutyric acid ones and also that the entropic contribution of hydrophobic interactions is more strongly involved in the retention of hexanoic acid molecules. If steric hindrance is considered, including the carbon chain of

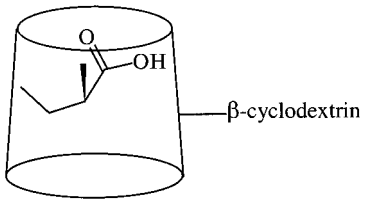


Figure 6. Configuration of β-cyclodextrin/2-methylbutyric acid complexes.

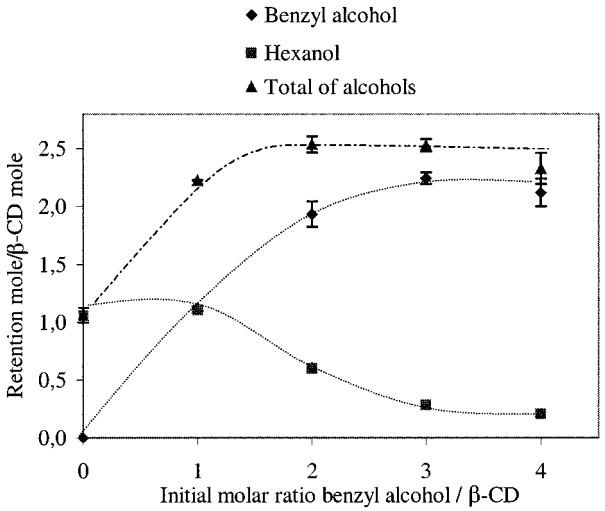


Figure 7. Retention of alcohols after freeze-drying of β-cyclodextrins initially saturated with 2 mol of hexanol/mol of β-CD, in which increasing amounts of benzyl alcohol have been added.

2-methylbutyric acid into the β-CD cavity provides a closer contact with the apolar walls than including the linear chain of hexanoic acid. Dispersion forces should also be more strongly involved in the retention of 2-methylbutyric acid. The balance of the two kinds of interaction would also be different for the retention of these two acids, and a stronger contribution of dispersion forces in the retention of 2-methylbutyric acid could explain its preferential retention.

Competition between Alcohols for Their Binding on β-Cyclodextrin. Figure 7 reports retention of the alcohols measured after dehydration of the carrier paste initially saturated with hexanol and in which increasing amounts of benzyl alcohol have been added. Retention of benzyl alcohol increases with the initial ratio of benzyl alcohol per carrier molecule. To the contrary, retention of hexanol decreases. When competitions are done in the reverse order, retention of benzyl alcohol decreases slightly with the amount of hexanol added, whereas retention of hexanol increases (Figure 8). Retentions obtained in the two mixing orders are not significantly different. Indeed, when benzyl alcohol and hexanol are initially present in 4:1 and 2:1 respective ratios with the carrier, 2.20 (± 0.26) mol of benzyl alcohol are retained when this compound has been introduced first in the mixture, whereas its retention reaches 2.12 (± 0.12) mol/mol of carrier when it has been introduced after saturation by hexanol. Retention of hexanol is 0.21 (± 0.016) mol/mol of β-CD when it is introduced first and 0.26 (± 0.034) mol/mol when it is introduced in second place into the carrier paste. This result can be explained by the fact that aroma molecules are mixed with the carrier during 24 h prior to dehydration and that an equilibrium independent of mixing order is reached. Moreover, when results obtained by

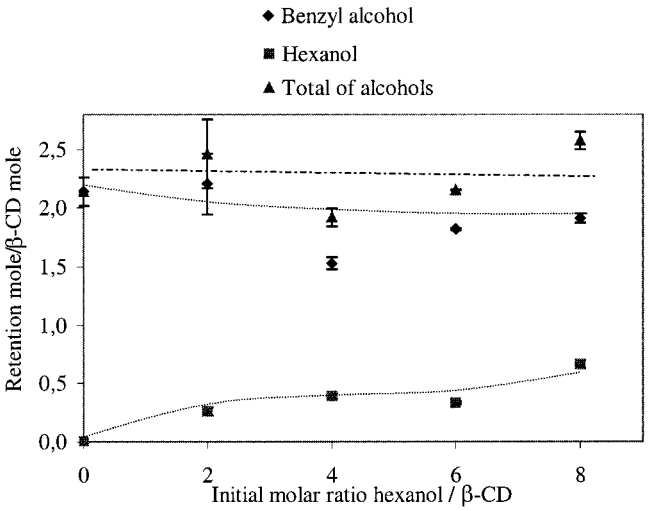


Figure 8. Retention of alcohols after freeze-drying of β-cyclodextrins initially saturated with 4 mol of benzyl alcohol/mol of β-CD, in which increasing amounts of hexanol have been added.

GC analysis of the complexes are compared to that obtained by ^{13}C NMR in the inverse gate mode, no significant difference ($p < 0.05$) is observed.

In agreement with previous results, benzyl alcohol is better retained than hexanol. When two molecules of each alcohol are initially present per molecule of carrier, retention of benzyl alcohol is 3 times higher than that of hexanol. Moreover, retention of the mixture of these two alcohols can exceed 2 mol of aroma/carrier molecule. As an example, when two molecules of each alcohol are initially present per molecule of carrier, retention of the total of alcohols after dehydration reaches 2.4 mol of alcohol/mol of β-CD. Indeed, 0.6 mol of hexanol and 1.8 mol of benzyl alcohol are retained per mole of β-CD. Retention of hexanol can be explained by the inclusion of 0.6 mol of hexanol/mol of β-CD as it has been observed previously that excess molecules are removed during the dehydration process. The better retention of benzyl alcohol compared to that of hexanol is explained by the difference of alcohol molecule that can be bound per β-CD molecule and by a different location of the guest. Hexanol molecules are indeed located in the β-CD cavity, whereas benzyl alcohol can be partly included.

From the above result, it can also be seen that differences in retention between volatiles competing for the same binding sites can be due either to differences in the affinity for the binding sites or to the number of molecules that can be bound per carrier molecule. Differences in retention of ethyl hexanoate and ethyl propionate as well as a difference in retention between hexanoic and 2-methylbutyric acids could be related to their difference of affinity for their binding site. In the first case the behavior of aroma compound could be predicted by the hydrophobicity and volatility of the pure compounds, but in the second case the study of the molecular conformation of the complexes has been necessary to fully understand the difference in the nature of interactions that explain the difference of retention. The study of interaction on a molecular scale in the case of competitions between hexanol and benzyl alcohol provided evidence that in this case a difference in the number of molecules of volatile retained per β-CD molecule is involved in the difference of retention observed during competitions for the binding on the same carrier. In these three cases study on a molecular scale also proved to be useful to put into evidence the nature of the interaction involved in the retention of each aroma compound and also in the explanation of selectivities of retention. This study provides some information on the mechanisms involved in preferential retention, but a lot of work remains to be done to explain the behavior of aroma in much more complex systems such as those encountered in flavor encapsulation. This study must be taken as a model to understand which are the parameters that have an influence and is a step in understanding more complex systems.

ACKNOWLEDGMENT

We thank Roquette and International Flavors and Fragrances companies for providing us, respectively, β-cyclodextrins and aroma compounds.

LITERATURE CITED

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Received for review January 24, 2001. Revised manuscript received September 25, 2001. Accepted September 26, 2001. This work was supported by a grant from the French Science and Technology Ministry.

JF0101049