

Interaction of Cyclodextrins with Aliphatic Acetate Esters and Aroma Components of La France Pear

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The interaction between aliphatic acetate esters, which are the major flavor components of La France pear (*Pyrus communis* L.), and cyclodextrins (CDs) was studied to obtain fundamental data for preparing powdered pear flavor materials. The complexing abilities of α -, β -, or γ -CD with six kinds of aliphatic acetate esters were compared. α -CD formed the highest numbers of complexes with all of the esters. All of the CDs produced complexes more readily with the more hydrophobic esters. Among our samples of freeze-dried pear juice containing dissolved α -, β -, or γ -CD, the juice with α -CD retained the greatest amount of esters. These data demonstrate that α -CD is an effective material with which to prepare powdered pear flavor materials. The nuclear Overhauser effect, measured by ^1H NMR spectra, of an α -CD–butyl acetate or α -CD–hexyl acetate complex showed that these esters were included in the α -CD cavity.

KEYWORDS: Cyclodextrin; ester; complex; flavor; aroma; pear; La France

INTRODUCTION

Cyclodextrins (CDs) are cyclic oligosaccharides with six to eight α -D-glucopyranose units joined by α -1,4 glucoside bonds. They have a hydrophobic cavity and a hydrophilic outer wall, attributed to primary and secondary hydroxyl groups of α -glucopyranose units facing the outside of the ring. Because of this specific structure, CDs are known to include various organic molecules in the cavity (1–8). Thus, they have many food-processing applications, such as controlling the volatility of flavor components, dissolving poorly soluble substances, and masking bitter tastes by their inclusion action. In the field of food flavoring, many investigations have been performed to prepare powdered flavor materials and analyze the interaction between CDs and flavor components [limonene (9, 10), menthol (10, 11), vanillin (12, 13), lemon oil (13–15), coffee flavoring (16), and orange flavoring (17)].

This study was carried out to obtain basic data for the preparation of powdered flavoring that retains pear flavor components [La France (*Pyrus communis* L.)] in CDs. La France pear flavor components primarily consist of esters, aldehydes, and alcohols (18, 19); significantly, aliphatic acetate esters, for example, butyl acetate and hexyl acetate, are the main components closely related to aroma (18). Many studies have probed the interaction between CDs and various volatile esters [methyl acetate (20), ethyl acetate (21), ethyl propionate (21, 22), ethyl butyrate (11, 20, 21, 23), ethyl hexanoate (20–25, 27), ethyl heptanoate (11, 21), and ethyl octanoate (20)]. However, no research has focused on a comparison of the interaction between butyl acetate, hexyl acetate, and other aliphatic acetate esters and α -, β -, γ -CDs; thus, our paper explores such interactions in an attempt to fill this gap.

MATERIALS AND METHODS

Test Materials. α -CD (α -100, >98%), β -CD (β -100, >98%), and γ -CD (γ -100, >98%) obtained from Bio Research Corp. of Yokohama, Ltd., were dried for 7 days at 6.7 kPa and 70 °C using a temperature-controlled vacuum drier (model DP-61, Yamato Scientific, Ltd.). Maltose and maltoheptaose, used as a control for the CDs, and D_2O and 3-(trimethylsilyl)propionic-2,2,3,3- d_4 acid sodium salt (TMSF), used in ^1H NMR spectroscopy, were purchased from Wako Pure Chemical Industries, Ltd. Esters used for the standards for the GC analysis and diethyl ether and sodium chloride used in the ester extraction were purchased from Kanto Kagaku, Ltd. (extra pure grade). The pulp of La France pear, grown in Yamagata Prefecture in 2003, was homogenized using a homogenizer (model AM-10, Nihonseiki Kaisha, Ltd.) and centrifuged (4220g, 4 °C, 20 min). The supernatant was dispensed into glass screw-top test tubes with a volume of 10 mL as La France pear juice and kept frozen at -80 °C in a freezer. Frozen samples in test tubes were thawed in running tap water for use. The samples used in this paper are the following: (1) an aliphatic acetate ester aqueous solution with CDs, (2) vacuum freeze-dried pear juice prepared by adding CD, and (3) α -CD–aliphatic acetate ester complexes.

Ester Composition Analysis in the Headspace Gas of La France Pear Juice. Ten milliliters of La France pear juice was placed in glass vials with a volume of 25 mL. The vials were kept at 40 °C and stirred for 10 min using a magnetic stirrer. One milliliter of headspace gas was injected via a glass syringe into a Shimadzu Corp., Ltd., model GC-17A gas chromatograph. The column was a CBP20-W25-100 (i.d. 0.53 mm \times L 25 m, 1.0 μm film thickness, Shimadzu Corp., Ltd.). The conditions for the GC analysis were as follows: carrier gas, He at 18 kPa; column temperature, 40 \rightarrow 80 °C (3 °C/min) and 80 \rightarrow 170 °C (5 °C/min); detector, flame ionization detector operated at 250 °C.

Aliphatic Acetate Ester Aqueous Solution with CD. Measurement of Concentration of Aliphatic Acetate Esters in the Headspace Gas. Five milligrams each of ethyl acetate, propyl acetate, butyl acetate, pentyl acetate, hexyl acetate, and heptyl acetate was added to 1 L of distilled water. The ester concentrations of the solution correspond to the following: ethyl acetate, 0.057 mM; propyl acetate, 0.049 mM; butyl

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acetate, 0.043 mM; pentyl acetate, 0.038 mM; hexyl acetate, 0.035 mM; heptyl acetate, 0.032 mM. This mixture was agitated vigorously using a homogenizer (model GLH-115, Yamato Scientific, Ltd.) to prepare an ester aqueous solution. A 0.5 mL aliquot of the ester aqueous solution and the corresponding aqueous CD solution (2.5, 5.0, and 7.5 mM) were added to a glass vial with a volume of 10 mL. These vials were agitated (500 rpm) at 40 °C for 10 min, and 1 mL of headspace gas was injected into the gas chromatograph using an autoinjector (model AOC-5000, Shimadzu Corp., Ltd.). Preliminary examination showed that equilibrium was reached after 5–10 min (data not shown). The conditions for the GC analysis are listed above; the carrier gas pressure and column temperature were changed, respectively, to 20 kPa and 100 °C (constant).

Mathematical Modeling of the Release Behavior of Aliphatic Acetate Esters from CD Solutions. The aliphatic acetate ester concentration in the headspace gas fell due to uptake by the CD according to the first-order rate equation for the current CD concentration. Thus, we quantified the release behavior of ester into the headspace gas using an apparent constant of retention (k) defined by the equation

$$\log I = -kc \log I_0 \quad (1)$$

in which c is the CD concentration (mM), I is the GC peak area of the ester, and I_0 is the peak area at $c = 0$. The apparent constant of retention (k) is a measure of the complexation capacity of CDs. In this experiment six esters were submitted in a mixture to the CD solutions; therefore, some competition between the different esters could occur. Therefore, we approximated the release behavior of each ester into the headspace gas as an "apparent" constant of retention.

Vacuum Freeze-Dried Pear Juice with CD. Preparation of Vacuum Freeze-Dried Juice with CD. A 0.5 g amount of α -, β -, or γ -CD, maltohexaose, or maltoheptaose was added to 2 mL of La France pear juice. The mixture was heated at 45 °C for 5 min, mixed in a water bath (for the β -CD, the warming was at 80 °C for 5 min), and lyophilized.

Analysis of Esters Retained in a Vacuum Freeze-Dried Juice with CD. Two milliliters of distilled water and sufficient sodium chloride to reach saturation were added to vacuum freeze-dried juice containing CD. One milliliter of diethyl ether was added to the solution, the mixture vigorously was shaken for 10 min, centrifuged (1200g, 20 min, at room temperature), and the ether layer was decanted. This extraction process was repeated three times, and the extracts were pooled. The esters dissolved in the ether layer were gas chromatographically analyzed as the ester fraction retained in the vacuum freeze-dried juice. The operating conditions for the GC analysis were the same as described above.

Assessment of Sustained Release of Esters from Vacuum Freeze-Dried Juice with CD. The vacuum freeze-dried juice containing CD and 2 mL of distilled water were placed in a pear-shaped flask with a volume of 100 mL. The flask was kept at 40 °C and purged with nitrogen gas (100 mL/min); esters included in the headspace gas were quantified by purge and trap GC-MS (with a Chrompack Corp. model CP4020 injector) every 15 min for up to 60 min. The instrument conditions were as follows: sample tube temperature, 280 °C; cold trap temperature, -150 °C; the sample tube, with an inner diameter of 4 mm, was filled with three layers of Carboxen-569, Carbotrap, and Carbotrap-C (Supelco Inc.). A gas chromatograph mass spectrometer (model GC-MS-QP5050, Shimadzu Corp., Ltd.) was used for GC-MS analysis. The conditions for the GC-MS analysis were as follows: carrier gas, He at 150 kPa; column, DB-Wax (i.d. 0.32 mm \times L 60 m, 0.5 μ m of film thickness, J&W Scientific); column temperature, 40 °C (5 min) and 40 \rightarrow 200 °C (7 °C/min); detector, electron ionization (70 eV). The peak area of the single-ion chromatogram at 61 m/z was used to quantify the esters.

α -CD-Aliphatic Acetate Ester Complex. Preparation of α -CD-Aliphatic Acetate Ester Complex. An amount of butyl acetate or hexyl acetate equal to 10 times the molar quantity of α -CD used was added to an α -CD-saturated aqueous solution at room temperature (\sim 20 °C). After 24 h of stirring with a magnetic stirrer, the precipitate produced was filtered with filter paper (no. 5A, Advantec Toyo Kaisha, Ltd.). After the moisture from the precipitate was removed with a dry filter paper, it was lyophilized (final temperature of 20 °C, pressure of \sim 15 Pa) to obtain the α -CD-aliphatic acetate ester complex.

Table 1. Ester Composition of La France Pear Juice in Headspace Gas

ester	% (w/w)
methyl acetate	0.35
ethyl acetate	5.03
propyl acetate	0.44
2-methyl-1-propyl acetate	0.01
butyl acetate	35.25
3-methyl-1-butyl acetate	0.03
pentyl acetate	0.54
hexyl acetate	58.34
heptyl acetate	0.01
total	100.00

1 H NMR Spectra of α -CD-Aliphatic Acetate Ester Complex. The α -CD-aliphatic acetate ester complex was dissolved in D₂O at a concentration of \sim 1% (w/w); then, TMS⁺ was added to this solution as the internal standard. This mixture served as the analytical sample. A sample of \sim 0.7 mL was placed in a sample tube (5 mm \times L 203 mm). The Fourier transform NMR spectrometer used (model JNM-A620, JEOL, Ltd.) was operated at 22 °C and a sample tube rotational speed of 12 Hz. The transient nuclear Overhauser effect (NOE) signals observed using rotating frame nuclear Overhauser and exchange spectroscopy [ROESY] in a rotating coordinate system were accumulated 128 times to produce the final spectra.

RESULTS AND DISCUSSION

Interaction between CD and Aliphatic Acetate Esters in Aqueous Solution. Nine kinds of esters were detected in the headspace gas of La France pear juice (Table 1). All of the esters were acetic acid esters, and most of them, accounting for \sim 99.9% (w/w) of all of the ester components, were normal aliphatic acetate esters. From these nine, we used six types of aliphatic acetate esters that are closely associated with aroma (18) as samples in this experiment.

Among the six types of aliphatic acetate esters tested, ethyl acetate, butyl acetate, and hexyl acetate have been reported to form complexes with α - and β -CDs in aqueous solution (26). Moreover, when β -CD was dissolved in aqueous solutions of esters, for example, ethyl octanoate (20) and ethyl hexanoate (27), CD-ester complexes formed, and the volatility of the esters decreased. Thus, in our experiments as well, some of the esters probably formed complexes with the CDs, and their volatility would therefore drop. The amount of esters contained in the headspace gas of an ester aqueous solution depends on the concentration of free esters in solution (those not forming complexes with CDs). Therefore, we can indirectly determine the amount of esters forming complexes with CDs in aqueous solution by measuring the amount of esters in the headspace gas. Our research comparing the interaction between CDs and various aliphatic acetate esters was based on this methodology.

The relative amounts of the esters found in the headspace gas using several CD concentrations in the ester aqueous solution are shown in Figure 1. Values of the apparent constant of retention (k) of the ester concentration in the headspace gas are gathered in Table 2. A larger value for k indicates a higher complexing ability of the aliphatic acetate ester per unit amount of CD. The k value rose with a more hydrophobic aliphatic acetate ester with a longer alkyl chain. This change occurs because the inside of the CD cavity is hydrophobic; a highly hydrophobic aliphatic acetate ester is more easily included in the cavity of the CD molecule. Table 2 shows that in a comparison of the type of dissolved CD with k , the k for α -CD was the largest for all of the aliphatic acetate esters, followed by that for β -CD. The value for γ -CD was markedly smaller than that for the others. This result means that α -CD forms the highest number of complexes with aliphatic acetate esters

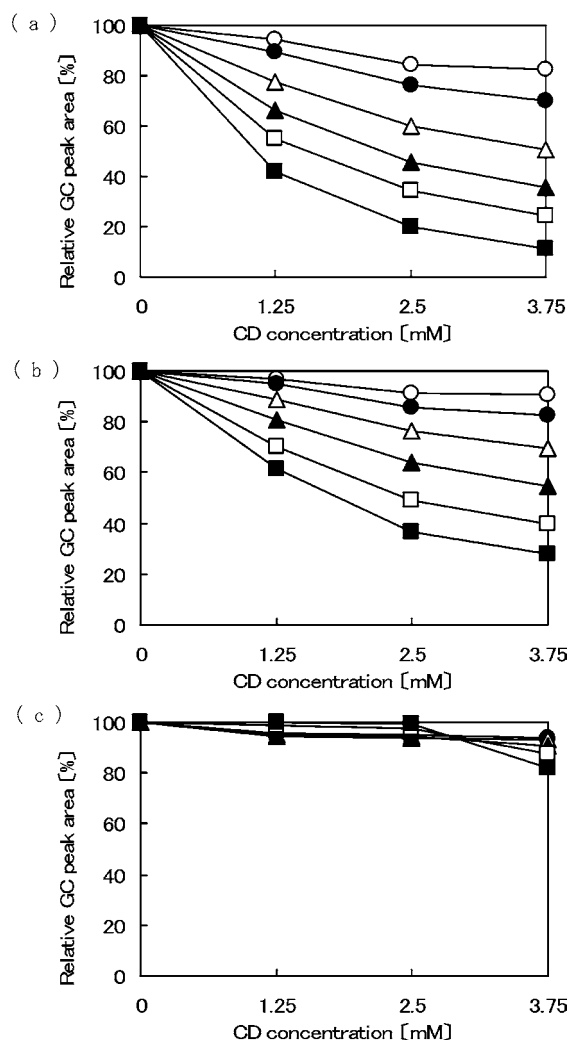


Figure 1. Relative quantity of esters in the headspace gas and the CD concentration: (a) α -CD; (b) β -CD; (c) γ -CD; \circ , ethyl acetate; \bullet , propyl acetate; \triangle , butyl acetate; \blacktriangle , pentyl acetate; \square , hexyl acetate; \blacksquare , heptyl acetate. The concentrations of the ester aqueous solution were as follows: ethyl acetate, 0.057 mM; propyl acetate, 0.049 mM; butyl acetate, 0.043 mM; pentyl acetate, 0.038 mM; hexyl acetate, 0.035 mM; heptyl acetate, 0.032 mM.

Table 2. Apparent Constant of Retention (k) of Ester Concentration in Headspace Gas of Ester Aqueous Solution with CDs

ester	k (mM^{-1})		
	α -CD	β -CD	γ -CD
ethyl acetate	0.03	0.02	0.01
propyl acetate	0.05	0.03	0.01
butyl acetate	0.10	0.05	0.01
pentyl acetate	0.15	0.09	0.01
hexyl acetate	0.20	0.14	0.02
heptyl acetate	0.32	0.19	0.03

per unit amount of CD; therefore, the use of α -CD is most likely to be of importance in the preparation of a powdered flavoring retaining La France pear's flavor components in the CD.

Vacuum Freeze-Dried Juice with CD. Ester Retention of Vacuum Freeze-Dried Juice with CD. The fact that CDs form complexes with various aliphatic acetate esters is apparent from the results shown in **Figure 1**, although this study was conducted to examine whether the addition of a CD is actually effective in retaining esters during the vacuum freeze-drying of La France pear juice. Aliphatic acetate esters are volatile (e.g., boiling point

Table 3. Ester Contents of Freeze-Dried La France Pear Juice with 20% (w/w) Malto-oligosaccharides^a

ester	without	α -CD	β -CD	maltohexaose	maltoheptaose
ethyl acetate	1.00	1.77	1.24	1.10	1.32
butyl acetate	1.00	3.46	2.46	1.58	1.67
hexyl acetate	1.00	3.40	2.07	1.24	1.27

^a Relative ratio of GC peak area.

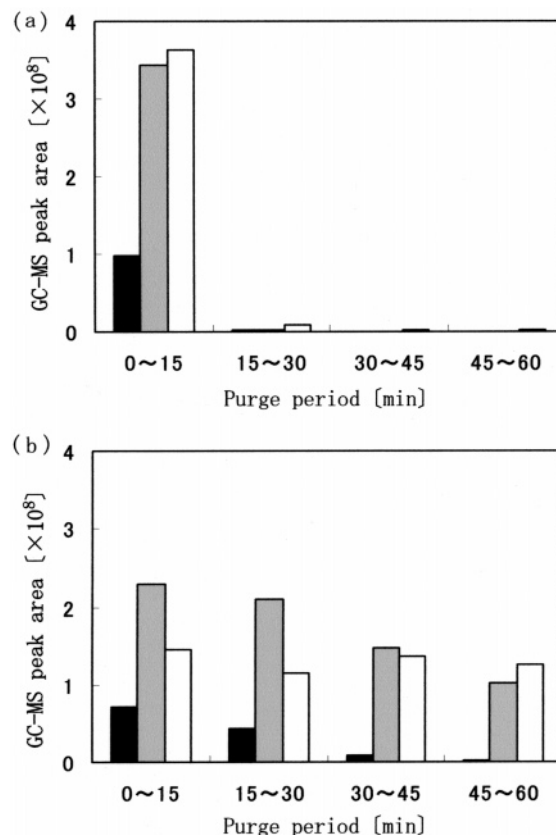


Figure 2. Relationship between the quantity of esters in the headspace gas and the purge period: (a) maltohexaose; (b) α -CD; black bar, ethyl acetate; gray bar, butyl acetate; white bar, hexyl acetate.

of hexyl acetate is 169.2 °C at 101.3 kPa), so aliphatic acetate esters that did not interact with other molecules by inclusion or physical adsorption in pear juice may be lost by evaporation in the process of vacuum freeze-drying. On the basis of the results shown in **Figure 1**, we determined that the volatility of the aliphatic acetate esters decreased due to their formation of complexes with CDs. Thus, if CDs and aliphatic acetate esters form complexes in pear juice, the amount of these esters evaporated in the process of vacuum freeze-drying falls, and more of them remain in the pear juice after vacuum freeze-drying.

Maltohexaose and maltoheptaose, which are straight-chain malto-oligosaccharides with almost the same molecular mass as CDs, were used as reference saccharide compounds that cannot include esters in the manner of CDs. Preliminary testing showed that vacuum freeze-dried juice made with below 25% (w/v) of malto-oligosaccharides or CDs agglomerated, deteriorated during handling, and had a higher hygroscopicity. Therefore, the concentration of them was fixed at 25% (w/v).

The ester content (relative amount) of samples of vacuum freeze-dried La France pear juice prepared with CDs is compared with those using malto-oligosaccharides in **Table 3**. Only three components, ethyl acetate, butyl acetate, and hexyl

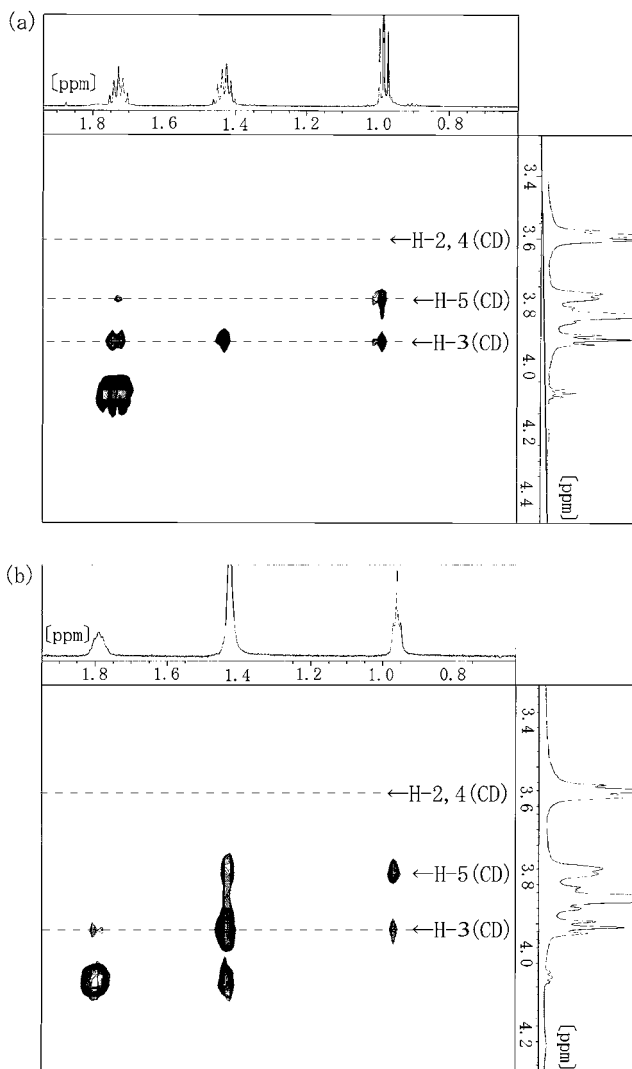


Figure 3. ROESY spectrum of α -CD–ester complexes: (a) α -CD–butyl acetate complex; (b) α -CD–hexyl acetate complex.

acetate, the primary esters in La France pear, were quantified. The freeze-dried juice with the malto-oligosaccharides retained from 1.1 to 1.7 times the amount of esters compared with the

freeze-dried juice without them. In contrast, the freeze-dried juice with the CD retained from 1.2 to 3.5 times the amount of esters. Considering the fact that the molecular structures of α -CD and β -CD are cyclic, whereas the molecular structures of maltohexaose and maltoheptaose are linear, the results described above suggest that the retention of esters is closely related to the cyclic structure of CD. Compared with the freeze-dried juice without malto-oligosaccharides, the juice with α -CD retained the greatest amount of aliphatic acetate, ~ 1.8 times more for ethyl acetate, ~ 3.5 times more for butyl acetate, and ~ 3.4 times more for hexyl acetate. These results are in good agreement with the aqueous solution data shown in **Figure 1**, indicating that α -CD forms the highest numbers of complexes with aliphatic acetate esters and forms more complexes with higher hydrophobic aliphatic acetate esters.

Sustained Release of Esters from Vacuum Freeze-Dried Juice with CD. This study was carried out to elucidate the ester release behavior of vacuum freeze-dried juice containing CD in aqueous solution. Vacuum freeze-dried juice with α -CD was used as the sample, because it retained the most amount of aliphatic acetate esters (**Table 3**). The results of our purge and trap GC-MS measurements, using as a sample the vacuum freeze-dried juice, are plotted in **Figure 2**, which shows the amount of esters released every 15 min (for up to 60 min) from the juice. For the juice with added maltohexaose, most of the aliphatic acetate esters were detected in the headspace gas in the first 15 min, although a very small amount of them was found after another 15 min. In contrast, aliphatic acetate esters were detected in all of the time periods occurring between 0 and 60 min for the juice mixed with α -CD. These findings suggested that aliphatic acetate esters formed CD–ester complexes in juice and that they were gradually liberated in the purging. For the juice with α -CD, ethyl acetate was detected in the headspace gas in the first 30 min, but after that, almost none was present. In contrast, the amount of butyl acetate detected in the headspace gas gradually fell over 0–60 min, and the levels of hexyl acetate decreased slightly. Evidently, esters with a low affinity for α -CD are quickly released from the complex, and esters with a high affinity are gradually released, a scenario that agrees well with the results shown in **Figure 1**.

The results described above indicate that freeze-dried juice with maltohexaose releases many esters in a short time, so the top aroma is strong but does not persist for a long time. In

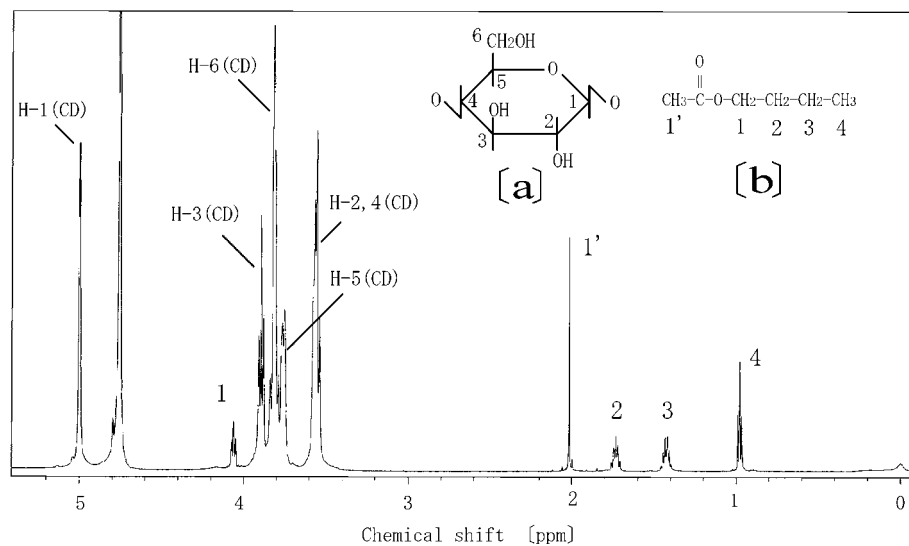


Figure 4. ^1H NMR spectrum of α -CD–butyl acetate complex. The designations H1–H6 (CD) indicate the proton numbers of the glucose unit of α -CD (a). Numbers (1–4) or primed numbers indicate the proton numbers of butyl acetate (b).

contrast, freeze-dried juice with α -CD releases the esters for a long time, so the complex offers a superior retention of aroma. In most cases, the flavor components of processed foods with fruits, including pears, diminish during processing or storage, so an aroma ester's holding effect by a CD is useful in the food industry. However, our research did not include a study of the release rate of the esters and the strength of the aroma actually perceived; these points are topics for the future.

Structural Analysis of α -CD-Aliphatic Acetate Ester Complexes by ^1H NMR Spectroscopy. The above results demonstrate that CD forms complexes with aliphatic acetate esters. To clarify whether this complex formation is due to the inclusion of aliphatic acetate esters in the CD cavity, we carried out a structural analysis of CD-aliphatic acetate ester complexes by ^1H NMR spectroscopy.

CD is known to have the α -D-glucopyranose protons at the 3- and 5-positions positioned inside the cavity (2–4). Thus, if a guest molecule is included in the CD cavity, these protons and the guest molecule's protons are extremely close spatially, so a NOE can be observed. There are papers that have analyzed the structure of various CD-guest molecule complexes via a NOE measurement [vanillin (12), ethyl hexanoate (27), aspartame, neotame (28), benzoic acid, benzoate anions (29), linoleic acid, arachidonic acid (30), and neohesperidin dihydrochalcone (31)]. Following the procedures in these papers, we obtained the spectra shown in **Figure 3** for the α -CD complexes with butyl acetate and hexyl acetate, which are the components most related to La France pear's flavor (18). For reference, we show in **Figure 4** the ^1H NMR spectrum of the α -CD-butyl acetate complex.

Cross-peaks between the α -D-glucopyranose protons at the 3- and 5-positions (positioned inside the cavity of the CD molecule) and the protons of the alkyl chain of esters were present for both the α -CD-butyl acetate complexes and the α -CD-hexyl acetate complexes. However, we did not find a cross-peak between the protons of α -D-glucopyranose at the 2- and 4-positions (positioned outside the cavity of the CD molecule) and the ester alkyl chain protons. These results indicate that the esters were included in the cavity of the α -CD molecule.

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