Cyclodextrin-Complexed Acetal as an Acetaldehyde Generator

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Among the minor but essential constituents of fresh fruit flavors, acetaldehyde appears to be one of the key flavoring agents responsible for the freshness of the flavors of fruits, fruit juices and similar compositions. Acetaldehyde itself possesses, however, high reactivity and volatility under normal conditions. Thus the fixation and stabilization of acetaldehyde both in the physical and chemical sense have long been the subject of many attempts, described in papers and patents,[1,2] to provide fresh fruit flavors for the food industry.

It has been shown in the literature that acetaldehyde can be stabilized by β-cyclodextrin complex formation.[3] Since the reproducibility of this process was difficult, we chose to prepare highly water-soluble cyclodextrin complexes of acetaldehyde diethylacetal, which is the simplest acetaldehyde-generating substance, using 2-hydroxypropyl-β-cyclodextrin (HPBCD).

The other possible technological approach to provide acetaldehyde for food products is the application of acetaldehyde-generating systems, in which the "pro-flavors" like acetals, ketals and dioxolanes are supposed to ensure the release of acetaldehyde under specific—in most cases pH-dependent—conditions. Such acetaldehyde-generator systems are described in numerous patent applications.[4-8]

All these higher molecular weight acetaldehyde-generating systems may have disadvantages, due to the simultaneous formation of corresponding long-chain alcohols, which possess their own flavor. This can further alter the sensory profile of the given flavor composition containing "pro-acetaldehydes." Moreover the release of acetaldehyde is known to depend on the rate of acetal hydrolysis, which is influenced by the pH, the temperature, the presence of phenols, metal ions and other normally occurring constituents of food products. Of course the hydrolysis of the complexed acetal results in ethanol formation which, however, is innocuous in the flavor system.

Materials and Methods

HPBCDs can be prepared with a range of degrees of substitution (DS), where DS is the average number of hydroxypropyl groups attached to the primary and secondary hydroxyls of the β-cyclodextrin ring. At Cyclolab, we prepared three samples of HPBCDs with average degrees of substitution of 2.7, 4.6 and 6.0, respectively. Acetal (acetaldehyde-diethylacetal) was purchased from Fluka AG (Switzerland) and used without further purification. All other reagents used were of analytical purity.

Preparation of the solid acetal/HPBCD complexes: The acetal/HPBCD complexes were prepared by dissolving 50 g of the HPBCD in 100 ml of 0.2% aqueous $NaHCO_3$ solution at ambient temperature under vigorous stirring. Then 10 ml of acetaldehyde-diethylacetal was dropwise added to the clear, continuously stirred cyclodextrin solution. The reaction mixture was stirred for two hours, and water was removed by spray-drying on a laboratory spray-dryer (Buchi, Switzerland). The resulting white powder was found to have an acetal content in the range of 5-8% by weight, depending on the degree of substitution of the HPBCD applied.

Characterization of the formulations: The thermal stability of the acetal in the inclusion complex was investigated by Evolved Gas Analysis (EGA) on a Du Pont Thermal Evolution Analyzer (TEA) in nitrogen atmosphere. The EGA technique is a kind of thermal analysis in which the solid samples on a mg scale are heated in an inert gas atmosphere at a rate of 8°C per minute in the temperature range of 30-300°C. The thermally released volatiles are "washed" continuously from the closed atmosphere to the detector. As this technique uses a flame ionization detector (FID), and the FID does not respond to the loss of *water* from the heated solid samples, this EGA technique detects only the loss of *organic volatiles*.[9] (Normally, the thermoanalytical methods record the *total mass losses* during sample heatings and do not differentiate between organic and inorganic losses.)

The quantitative determination of the acetal content in the solid acetal/HPBCD complexes was carried out by gas chromatography on a Hewlett-Packard 5840A type gas chromatograph according to a previously published

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method. This process involves the dissolution of the solid flavor complex in water and the extraction of the acetal with suitable solvent. The extracted flavor is directly injected into the chromatograph.[10]

The clumping tendency (hygroscopicity) of solid formulations was determined by a screening test through a screen of 400 μm aperture following the storage of samples under relative humidity of 90% at room temperature for 48 hours. The weight percentages of the passed fraction were used to describe the moisture sensitivity of samples tested.[11]

Results and Discussion

Solid state characteristics of the acetal/HPBCD complexes: The acetal/HPBCD inclusion complexes appear as white almost odorless powders, easily soluble in water. We observed that acetal/HPBCD complexes made with HPBCD of DS=2.7 and DS=4.6 showed no considerable hygroscopicity when stored in open vials for two weeks at normal relative humidity (R.H.). However, the storage of the acetal/HPBCD formulations under conditions of higher R.H. (for example, at 90% R.H.) resulted in a noticeable moisture sorption as illustrated by the data (Table I) of a clumping tendency test. Acetal complexes that were prepared using HPBCD of higher DS are more hygroscopic than the corresponding acetal complexes with HPBCD of DS=2.7.

Accelerated stability tests: The solid acetal/HPBCD complexes were stored at 60°C in open test tubes for a week and their actual acetal content was followed by gas chromatography (Table II). It was found that the mechanical mixture of acetal with lactose showed negligible resistance to heat, while all the HPBCD-complexed acetals exhibited acceptable heat stability. Furthermore the effect of the degree of substitution on the stabilization of acetal against heat was less pronounced than the effect of DS on hygroscopicity.

The more significant loss of acetal content of a complex with HPBCD of DS=6.0 may be in part a consequence of the higher moisture sorption of this complex. Higher actual water content results in more rapid dissociation of the inclusion complexes, leading to the generation of free acetal that then evaporates quickly at 60°C.

Table I. Clumping tendency of the acetal/HPBCD complexes measured by a screening test after storage at 90% R.H. for 48 hours

Degree of substitution (DS) of acetal/HPBCD sample	Weight percentages of passed sample fractions at various elapsed times			
	Time zero	12 hours	24 hours	48 hours
2.7	72%	60%	55%	34%
4.6	68	44	27	9
6.0	64	39	11	0

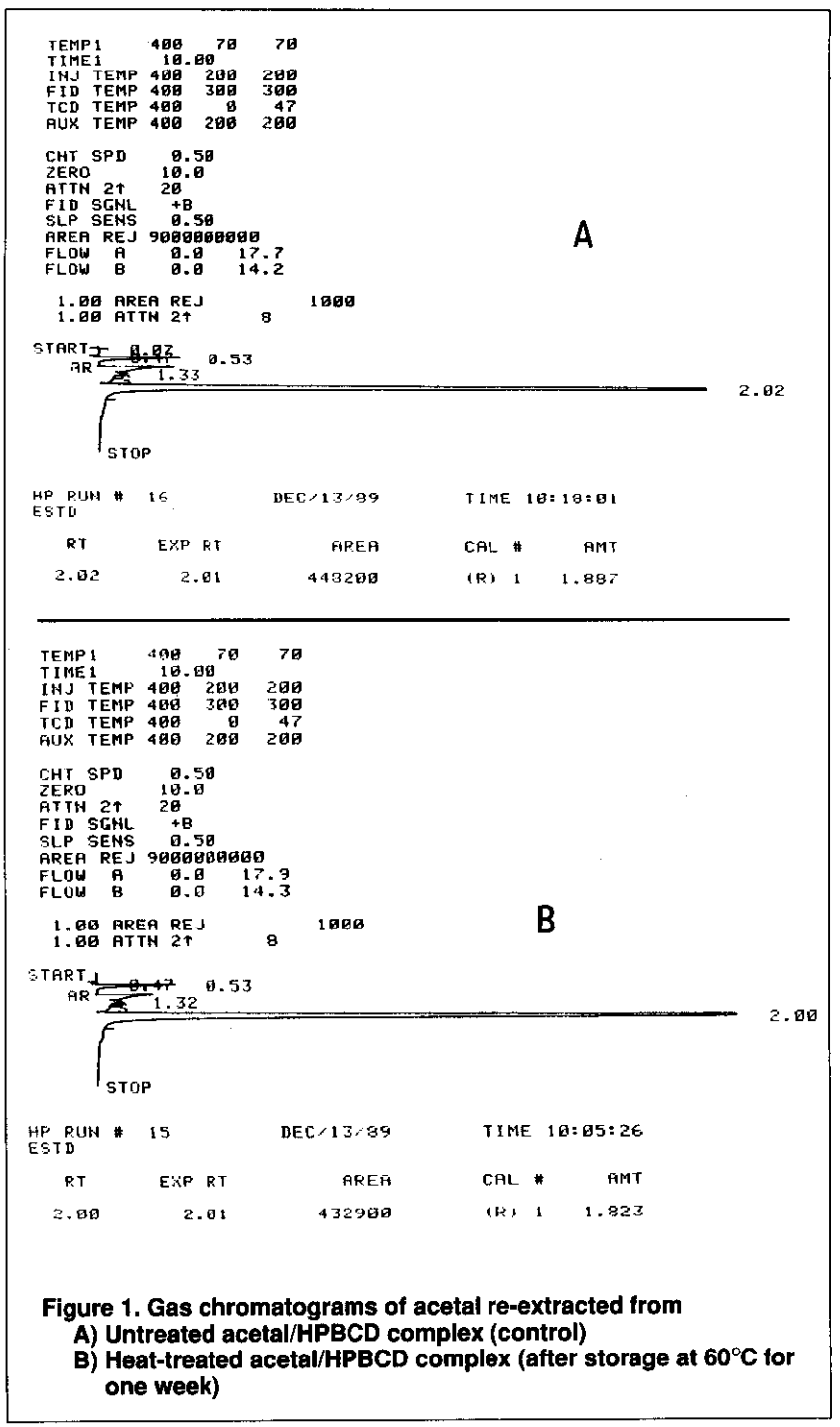

Figure 1. Gas chromatograms of acetal re-extracted from
A) Untreated acetal/HPBCD complex (control)
B) Heat-treated acetal/HPBCD complex (after storage at 60°C for one week)

Table II. Thermal stability of acetal/HPBCD complexes stored at 60°C in open test tubes and measured by GC

	Acetal content (remnant weight percentage) at various elapsed times			
Sample	**Time zero**	**2 days**	**4 days**	**7 days**
acetal/HPBCD (DS=2.7)	7.6%	7.2%	6.6%	5.2%
acetal/HPBCD (DS=4.6)	6.2	4.7	4.4	3.9
acetal/HPBCD (DS=6.0)	5.8	3.9	3.3	3.0
lactose+acetal mixture	6.0	1.7	0.0	0.0

Table III. Change of acetal content of lactose+acetal mixture and of acetal/HPBCD complex during long-term storage (at 22°C under conditions of normal light and humidity) measured by GLC

	Acetal content (remnant weight percentage) at various elapsed times				
Sample	**0 day**	**30 days**	**60 days**	**90 days**	**120 days**
lactose+acetal mixture	6.00%	0.60%	0.10%	0.00%	0.00%
acetal/HPBCD (DS=4.6)	5.78	4.20	4.30	3.97	3.28

The chemical identity of the entrapped acetal stored at elevated temperatures was checked by comparing the GC profiles of the acetal in the complexes at time zero and after a week of storage. As Figure 1 illustrates, the gas chromatogram of the acetal in HPBCD complex after storage at 60°C for a week appears identical with that of the control, non-heated acetal/HPBCD complex. This points again to the remarkable protecting effect of the molecular encapsulation of the volatile flavor.

Similar improved stability of the HPBCD-entrapped acetal was observed during the normal storage tests carried out at 22°C for longer time (Table III).

Evolved gas analysis: The thermal release of the entrapped acetal from the HPBCD complexes as well as from the corresponding lactose+acetal mechanical mixtures was studied using EGA, which showed (Table IV) that the free, non-complexed and lactose-adsorbed acetal almost completely evaporated in the temperature range of 26-60°C upon heating in nitrogen atmosphere with a heating rate of 8°C/min. However, the volatile substance from the acetal / HPBCD complexes was released continuously in the temperature range of 20-140°C.

This heat stress data further supports the results of accelerated storage tests indicating that the molecularly encapsulated acetal is effectively protected against heat-induced losses until it is in a solid, dry state.

Release of entrapped acetal: Since the inclusion complex formulation is a reversible, equilibrium process, the entrapped acetal is immediately released on contact with water. The acetal /HPBCD complexes are all fairly well soluble in water. The dissolution of these inclusion complexes results in the dissociation of the complex into free acetal and "empty" HPBCD. The free acetal then—particularly under acidic conditions such as in fruit juices—decomposes into the desired acetaldehyde and ethanol, thereby providing the fresh note for the instant beverages.

Table IV. Loss of acetal upon heating at 8°C/min in a nitrogen atmosphere, measured by EGA

	Loss of acetal content (released weight percentage) at various temperatures			
Sample	**35°C**	**60°C**	**100°C**	**140°C**
lactose+acetal mixture	78%	93%	100%	100%
acetal/HPBCD (DS=2.7)	16	30	44	87
acetal/HPBCD (DS=4.6)	22	46	68	90
acetal/HPBCD (DS=6.0)	20	38	73	94

Conclusion

Acetaldehyde-diethylacetal, the simplest acetaldehyde-generating substance, can be stabilized and formulated by using HPBCD. The molecular encapsulation of acetal by HPBCD was found to result in a highly water soluble, amorphous solid product. This molecularly encapsulated acetal formulation in solid state was found to be stable in accelerated stability studies at 60°C for several days, and under normal shelf-storage conditions for one year. The inclusion complex releases the entrapped acetal upon contact with water under normal conditions, or upon heating in the presence of moisture.

References

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1. B Byrne and G Sherman, *Food Technology* **38** 57-61 (1984)
2. US Pat 3,767,430, E Earle (1973)
3. US Pat 3,140,184, F Robbins (Jul 7, 1964)
4. US Pat 4,043,752, M Withycombe (1978)
5. US Pat 4,191,785, M Withycombe (1980)
6. US Pat 4,280,011, P De Simone (1981)
7. US Pat 3,857,964, Y Seymour (1979)
8. R DeSimone and B Byrne, *Perf & Flav* **11**(6) 15-26 (1986)
9. L Szente and J Szejtli, *ACS Symp on Flavor Encapsulation*, SJ Risch and GA Reineccius, eds, Washington DC: American Chemical Society (1988) pp 148-157
10. J Harangi and P Nánási, *Anal Chim Acta* **156** 103-109 (1984)
11. L Szente, M Gál-Fuzy and J Szejtli, *Acta Alimentaria* **17** 193-197 (1988)