Retention of Organic Volatiles in Freeze-Dried Solutions of Carbohydrates

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A mechanism by which organic volatiles are retained by soluble carbohydrates after freeze-drying from solution is presented. The dry material is proposed to be organized on a microlevel, and this microstructure is responsible for the retention of volatile. The development of the microstructure depends on many processing parameters, and its effectiveness in preventing volatile loss depends on the local moisture content.

The tenacity with which organic volatiles are retained in dry organic solids is well documented in the literature. Experiments with carbohydrate polymers (Russell *et al.*, 1937; Sheppard and Newsome, 1932; Staudinger *et al.*, 1953) and proteins (Watt, 1964) show that removal of a residual amount of organic volatile from solids by evacuation, even at elevated temperatures, is often impossible.

The flavor characteristics of foods depend critically on the pattern of organic volatiles present. Retention and loss of these volatiles are therefore important factors in food processing. Substantial retention has been observed in dehydration processes despite the fact that in the pure state many retained volatiles have higher vapor pressures than water. High degrees of retention of alcohols, ketones, and/or esters were observed in air-drying (Menting and Hoogstad, 1967; Thijssen and Rulkens, 1968) as well as in freeze-drying (Thijssen and Rulkens, 1968; Rey and Bastien, 1962; Saravacos and Moyer, 1968). Hypotheses for the high retention in dry materials have included adsorption on sites in the dry matrix (Rey and Bastien, 1962), formation of an impermeable surface membrane (Menting and Hoogstad, 1967), increased resistance to diffusion at low water contents (Thijssen and Rulkens, 1968), and formation of inclusion complexes (Russell *et al.*, 1937; Sheppard and Newsome, 1932).

The authors have studied the retention of selected organic volatiles in freeze-dried systems containing soluble carbohydrates. The objective has been to elucidate the mechanisms controlling volatile retention during freeze-drying.

EXPERIMENTAL TECHNIQUES

Sample Preparation. Model systems were prepared from soluble carbohydrates, organic volatiles, and water. All materials were reagent grade. The carbohydrates and volatiles and their sample concentrations are given in Table I. Five-milliliter aliquots of the model solution were pipetted into 25-ml. Erlenmeyer flasks, frozen immediately by immersion in liquid nitrogen, and freeze-dried in the flasks for 48 hours at an ambient platen temperature and a chamber pressure below 100 microns. Other concentrations and processing parameters were investigated but will not be reported in this paper (Flink, 1969).

The individual experiments in this study utilized variations of this sample preparation scheme; the variations are discussed in connection with presentation of results of a particular experiment.

Analysis. The volatile and water analyses were made by dissolving the samples in anhydrous methanol. Aliquots of this methanol solution were analyzed by gas chromatography, using a flame ionization instrument (F & M, Model 1609) for the volatile analysis and a thermal conductivity instrument (Perkin-Elmer, Model 154) for the water analysis. In both instruments Porapak Q columns (Hollis, 1966) were used, with the operating conditions chosen to give quantitative results in the shortest possible time. Calibration samples were used for each individual analysis. Peak areas were used to obtain the sample concentrations. The areas were calculated from measurements of peak height, and peak widths at half height.

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RESULTS AND DISCUSSION

Table II gives typical retentions for model systems freeze-dried as described above. The retentions are similar for volatile compounds differing greatly in volatility, as measured by the vapor pressure of the pure compounds.

In several experiments we obtained evidence that retention was not due to adsorption on the freeze-dried material. Maltose freeze-dried from a solution containing no alcohol was exposed to saturated vapor of isopropyl alcohol for 48 hours. Saturation in the sample flask was confirmed by gas chromatographic analysis. A small amount of the alcohol (approximately 0.4 gram/100 grams of maltose) was found in the maltose but could be removed readily by 5 minutes of evacuation at room temperature. When the alcohol in the vapor space was below saturation levels, none was retained in the maltose, even before evacuation. This behavior contrasts with the tenacity of volatile retention when the alcohol was present in the subsequently freeze-dried solution. The freeze-dried model system was evacuated for 6 and 12 hours at 20°, 37°, and 52° C. The amount of 2-propanol retained by the freeze-dried maltose could not be decreased significantly by these treatments. The lack of an effective vapor pressure for this retained volatile was confirmed by gas chromatographic analysis of the headspace.

Under certain conditions, a surface layer of decreased permeability to water forms on the freeze-dried material (Quast and Karel, 1968). We tested our systems for the presence of similar effects on volatile transport. Samples were frozen

Table I. Model Systems Studied

Carbohydrate, 18.8%, Wt./Wt.	Volatile,[a] 0.75%, Wt./Wt.	Water,[b] 80.4%, Wt./Wt.
Glucose	*n*-Alcohols (C_1–C_5)	
Maltose	Acetone	
Lactose	Methyl Acetate	
Sucrose	2-Propanol	
Dextran-10 (mol. wt. = 10^4)	*tert*-Butanol	

[a] Initial volatile content = 4 g./100 g. [b] Initial water content = 430 g./100 g.

Table II. Retention of Volatiles in Freeze-Dried Model Systems[a]

Organic Volatile	Vapor Pressure of Volatile at −22° F. (mm. Hg)	Volatile Retention in Systems Containing Specified Carbohydrate (G. Volatile/100 G. Solid) Glucose	Maltose	Sucrose	Lactose	Dextran-10
Acetone	11.0	0.99	2.01	2.30	1.83	0.03
Methyl Acetate	9.4	0.67	2.29	2.51	2.20	0.04
Methanol	3.5	1.34	...	...	...	0.03
Ethanol	1.1	2.12	1.76	...	...	...
n-Propanol	0.26	1.91	2.41	...	...	0.17
2-Propanol	0.70	2.11	2.71	3.02	2.71	0.30
n-Butanol	0.10	1.26	2.27	2.83	2.50	0.70
tert-Butanol	0.44	1.93	3.10	3.27	3.15	2.96
1-Pentanol	<0.10	0.63	1.37	...	...	1.37

[a] All systems had the initial composition (% by weight): Carbohydrate 18.8, Organic volatile 0.75, Water 80.45

in beakers; in some of the samples the free surface was scraped prior to freeze-drying to remove any concentrated solute layer which might have formed during freezing. After drying, analysis showed no difference in volatile retention between the scraped and unscraped samples.

In other experiments, we have further confirmed that freeze-dried cakes of soluble carbohydrates have no surface "skin," and that retention of volatiles is determined by properties of microregions in the cakes. A freeze-dried maltose cake which retained 2.47 grams of 2-propanol per 100 grams of solids was ground into a fine powder by mortar and pestle. The pulverized material was evacuated for 13 hours at room temperature and then analyzed for 2-propanol. No loss of volatile was found in either grinding or subsequent evacuation. The experiment not only shows the lack of a "skin" but demonstrates that the regions in which the volatile is sealed must be small compared to the average size of particles produced in grinding.

Confirmation of the localized nature of volatile retention was obtained from experiments using layered systems. Samples were prepared by freezing alternate layers of a volatile-containing solution and layers of a solution containing no volatile. Each layer represented one third of the total height of a 15-ml. sample frozen in a 50-ml. beaker, and each was completely frozen before the next layer was added. After freeze-drying, the layers were separated for individual analysis. Table III shows that, after freeze-drying, retention occurred only in those layers which initially contained the volatile. Results obtained with sample A show that the volatile lost from the bottom layer is not retained on the upper two layers, while results for sample B show that volatile in the top layer is not influenced by the drying which occurs in the lower two layers. The gross structure of the cake must therefore be relatively permeable to the flow of volatile and water.

Further indication of the relative openness of the gross structure is shown by experiments in which samples were sectioned perpendicularly to the direction of drying. The maltose and 2-propanol model system was freeze-dried in glass tubes (I.D. = 25 mm.). The maltose cakes were then sectioned perpendicularly to the direction of drying and each slice was analyzed individually. Figure 1 shows that the retention of 2-propanol is essentially uniform throughout the entire cake. This observation shows that the dry cake is permeable to the flow of 2-propanol, since during freeze-drying the propanol from the still-frozen lower layer of the sample had to be transported through the upper dry layer to the free surface.

Water content plays a key role in retention of volatiles.

Table III. Retention of 2-Propanol in Specified Layers of Freeze-Dried Maltose Solutions

	2-Propanol Content (G./100 G. Solid) Sample A Before freeze-drying	Sample A After freeze-drying	Sample B Before freeze-drying	Sample B After freeze-drying
Top layer	0	0	4	2.52
Middle layer	0	0.05	0	0.05
Bottom layer	4	2.73	0	0.02

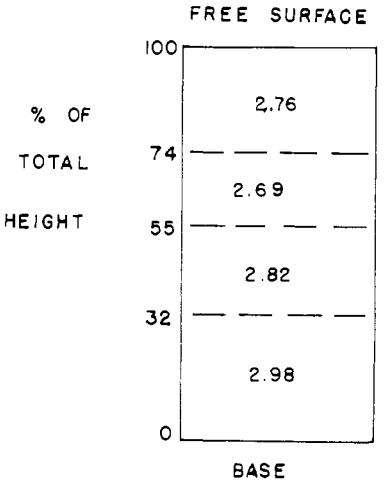

Figure 1. 2-Propanol retention in sectioned, freeze-dried maltose samples (g./100 g. solid)

This fact has been widely recognized (Russell *et al.*, 1937; Saravacos and Moyer, 1968; Thijssen and Rulkens, 1968; Watt, 1964) and has been observed in our experiments as well. Table IV presents the mean volatile and moisture contents during the course of freeze-drying of a solution containing maltose and *n*-butanol. Volatile and water were lost concurrently to a point where volatile loss stopped. The apparent minimum in retention after 6 hours of drying is probably due to experimental error. In particular, it is likely that some additional loss occurred when the vacuum chamber was opened while the sample was in a fairly labile state with respect to the moisture gradient in the dry layer. It can be assumed that at the moisture content where volatile loss stopped, no ice was present in the sample and the moisture gradient was small. This mean moisture content is much higher than the monomolecular layer for water calculated from sorption data

by the method of Brunauer, Emmett, and Teller (Brunauer *et al.*, 1938).

The water content at which no further volatile is lost during drying may be compared with the effects of humidifying the cake after freeze-drying (Table V). Under these conditions, volatile is lost only when moisture contents well in excess of the B.E.T. monolayer value are reached. Humidification to levels initiating volatile loss also led to a change in the cake, which assumed an appearance resembling fused glass. This aspect, which is under further investigation, appears particularly interesting in view of MacKenzie's work concerning structural collapse in freeze-drying (MacKenzie, 1965).

We interpret our results as being consistent with a scheme of volatile retention in which volatiles are entrapped in amorphous microregions of hydrogen-bonded carbohydrate molecules. Freeze-dried carbohydrate cakes are known to exist in an amorphous state (White and Cakebread, 1966; Gane, 1951; Wadehra and St. John Manley, 1966). Our studies as well as those of others indicate that hydrogen bond-breaking solvents, in particular water, cause profound changes in this amorphous structure. Furthermore, we have studied water adsorption on freeze-dried carbohydrate; the results support the conclusion that a large proportion of the hydroxyl groups in our freeze-dried carbohydrate solutions are involved in carbohydrate–carbohydrate hydrogen bonds (Flink, 1969).

The results presented, and results of additional studies (Flink, 1969), show that disruption of the microregion structure by water results in volatile losses. We postulate that regions enclosing the volatiles form during freezing when separation of some water as ice crystals causes the formation of pools of concentrated carbohydrate and organic volatile solutions. During drying, further rearrangements of the carbohydrate molecules occur in the frozen and/or interface layers. The bulk cake outside the microregions is relatively permeable to the flow of volatile and water. No loss of volatile occurs from a microregion as long as it is in the frozen layer. At the passage of the ice interface through the microregion, volatile loss begins. The loss continues as long as the moisture content remains above some critical level. As the water content decreases, the degree of association between carbohydrate molecules is likely to increase sharply as hydrogen bonds between carbohydrate hydroxyls and water are replaced by carbohydrate–carbohydrate hydrogen bonds. When moisture content reaches a critical level, the microregion is sealed and volatile loss ceases. Water loss may still occur, probably because of the plasticizing action of water and the small size of the water molecule. In addition, flow of volatile and water vapor from areas deeper within the sample has no effect on retention in the sealed microregions.

After the studies reported here were completed, the work of Thijssen and Rulkens (1968) came to our attention. These authors postulate a water-content-dependent diffusion coefficient for the volatile. Major features of predicted behavior are similar for our theory and theirs, but our concept emphasizes the localized structural aspects of the dry material, rather than general diffusion parameters.

Table IV. Loss of 1-Butanol During Freeze-Drying of Maltose Solutions

Time, Hr.	Mean Moisture Content, G./100 G. Solid	Mean Volatile Content, G./100 G. Solid
0	430	4
3	178	3.30
6	36	2.20
12	11	2.45
24	0.7	2.50

B.E.T. monolayer, 4.8 grams water/100 grams solid.

Table V. Loss of *tert*-Butanol from Freeze-Dried Maltose after 24 Hours Humidification

Relative Humidity	Mean Moisture Content, G./100 G. Solid	Mean Volatile Content, G./100 G. Solid
0	0.52	3.18
11	2.33	3.08
20	3.52	3.11
32	5.14	3.07
52	9.19	2.80
75	24.61	0.84

B.E.T. Monolayer, 5.24 grams water/100 grams solid.

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