Retention of Organic Volatiles in Freeze-Dried Carbohydrate Solutions: Microscopic Observations

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Microscopic observations of samples of maltodextrin which had been freeze-dried from solutions containing organic volatile revealed that, in many cases, the dry maltodextrin contained entrapped droplets of the liquid volatile. The amount of volatile retained as measured by gas chromatographic analysis compared favorably with that calculated from microscopic observations. The structure of the freeze-dried maltodextrin cake was observed by optical sectioning in the light microscope and with the scanning electron microscope. The structure consisted of numerous intersecting plates which had the retained volatile within the plate. Droplets of similar appearance were observed within the solids of freeze-dried coffee samples.

The retention of organic volatiles in freeze-dried food systems is very important in insuring the high quality flavor associated with these products. In the past few years much effort has been devoted to measuring the extent of volatile retention in freeze-dried foods, and developing theories by which this retention may be explained. Three basic theories have been presented in the literature (King, 1970). Sorption of the organic volatile on particular sites during its passage through the dry layer has been postulated by Rey and Bastien (1962). Diffusion-based mechanisms have been presented by Thijssen and Rulkens (1969) and King and Chandrasekaran (1971). While these two presentations differ in depth of mathematical analysis, they both are based on the finding that the diffusion coefficients of water and volatile depend on the moisture content in such a way that the diffusion of volatile is much smaller than that of water when the moisture content is low. In King and Chandrasekaran's (1971) latest analysis it is possible (by accounting for the different fluxes of the dissolved solids and the water) for the volatile to undergo a reversal diffusion relative to the water. Flink and Karel (1970) have attributed the retention of the volatile to an entrapment within localized structures ("microregions") in the freeze-dried solute matrix.

Optical microscopic techniques are widely used in chemical and mineralogical laboratories for the study of solid systems (Schaeffer, 1966; Chamot, 1958). Luyet (1960) and his associates have extended the microscopic technique to the evaluation of crystallization and freeze-drying of biological systems. These investigations have generally been related to the state of ice and water in the system during these processes.

The scanning electron microscope (SEM) is a relatively new instrument which is only starting to demonstrate its potential for evaluating the surface structure of materials (Oatley *et al.*, 1965; Johari, 1968). An example of this potential is the SEM's new application to studies of biological material, for instance, to investigations of the changed appearance of the wheat starch granule's surface after enzymatic attack (Evers *et al.*, 1971).

METHODS

Solution Preparation. Aqueous solutions (20% w/v) of maltodextrin (Snowflake maltodextrin 01913, Corn Products Co., Holte, Denmark) were prepared. To 100 ml of the solution was added 1 ml of one of the following reagent grade volatiles: hexanal, isovaleraldehyde, acetone, methyl–ethyl ketone, ethanol, 1-propanol, 1-butanol, and diacetyl. The solutions were shaken by hand until the liquid appeared homogeneous (clear). This was usually accomplished in a matter of seconds (10–20 sec). The solutions were poured into sealable plastic sample holders to a depth of about 4 mm and were frozen at −40°C. The freeze-drying was usually conducted with a minimum of heat and at a chamber pressure of approximately 200 mTorr. This treatment corresponded to a frozen layer temperature of approximately −20°C.

Retained Volatile Analysis. The volatile content after freeze-drying was measured gas chromatographically by reconstituting the dry material to its initial solids concentration with water and injecting a 2-μl sample. Samples of the initial solutions which had been stored at −40°C were used as controls. The ratio of the peak areas for the reconstituted freeze-dried and the initial solutions was taken as the fraction of the volatile retained. A Beckman GC-M dual flame ionization chromatograph was used with 6-ft × $^1/_8$-in. columns of Porapak Q (Waters Assoc., Framingham, Mass.) The temperature and gas flow rates were chosen to give good peaks in a reasonable amount of time.

Optical Microscopic Techniques. Two types of microscopes, a mineralogical polarization microscope (Leitz, SM-POL) and a light field-phase contrast microscope (Olympus Ec4Tr-E2) were used for visual and photographic analyses of the dried samples. The refractive indices of the various materials were measured with a series of 24 solutions composed of either water and glycerol ($1.333 \leq n \leq 1.475$) or paraffin oil and *n*-bromonaphthalene ($1.479 \leq n \leq 1.656$).

The freeze-dried cake was carefully cracked and a fractured surface was gently rubbed with the tip of a fine needle to flake off dry material onto a microscope slide. If it appeared necessary the flakes were crushed further. A drop of the desired refractive index solution was placed on the slide and mixed with the dry solid; finally, a cover slip was placed on the slide.

The refractive index of the solid was measured by the movement of Becke's line when the focus of the microscope was shifted from the optimum focal point. When the distance between the microscope objective and the sample is increased beyond the optimum focus, Becke's line will move to the material possessing the higher refractive index. Thus, if the solid has a refractive index higher than that of the refractive index solution, this difference may be observed when the

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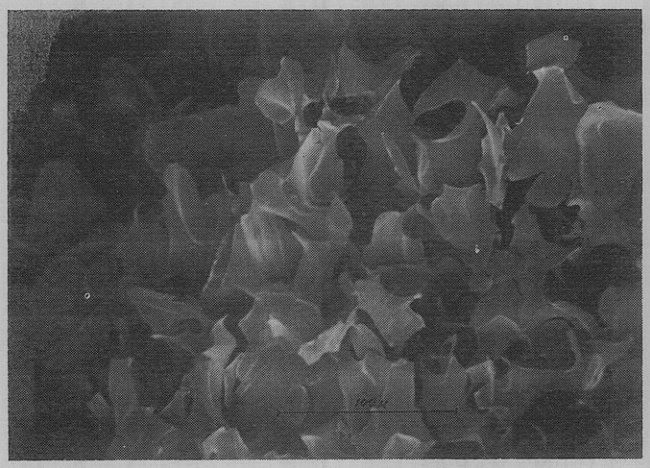

Figure 1. Scanning electron micrograph of freeze-dried 20% maltodextrin solution (208×)

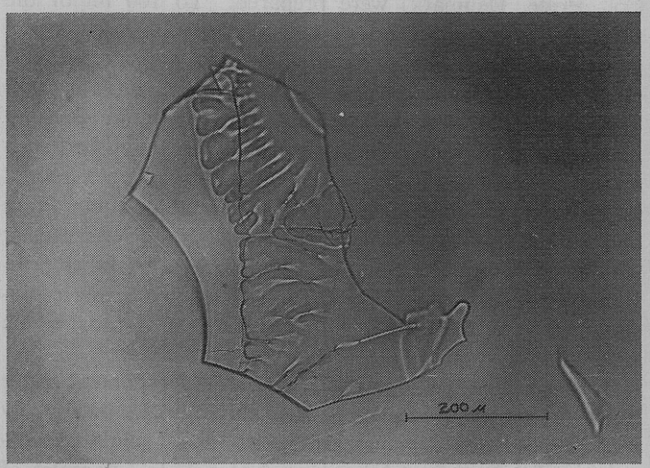

Figure 2. Platelet of freeze-dried maltodextrin showing phase separation of maltodextrin components during solidification (100×)

microscope focus is adjusted so that the distance between the microscope objective and the sample is larger than optimum; Becke's line will then move into the sample. The strength of Becke's line is related to the difference in refractive indices between the sample and the medium. By this method, the actual solid refractive index can be pinpointed more precisely between the values of the available refractive index solutions. The same technique is applicable to liquid samples immiscible in the refractive index solution; the movement of Becke's line is observed at the liquids' interface.

Scanning Electron Microscopic Techniques. Samples of the freeze-dried material were stuck to the sample holders with double-sided adhesive tape. The dry samples were handled in desiccators as much as possible. The samples were coated with a thin layer of gold and observed in a Cambridge Stereo Scan Electron Microscope.

RESULTS AND DISCUSSION

Maltodextrin Structure. Spray-dried maltodextrin, as received from the manufacturer, consisted of a single phase solid material (refractive index = $n = 1.535$) which contained air bubbles. After the spray-dried material was dissolved in water and freeze-dried, it exhibited a very porous structure of intersecting plates, as seen in the scanning electron micrograph for a freeze-dried 20% maltodextrin solution (Figure 1). Closer examination of these plates with the optical microscope revealed that the solid material consisted of a two-phase

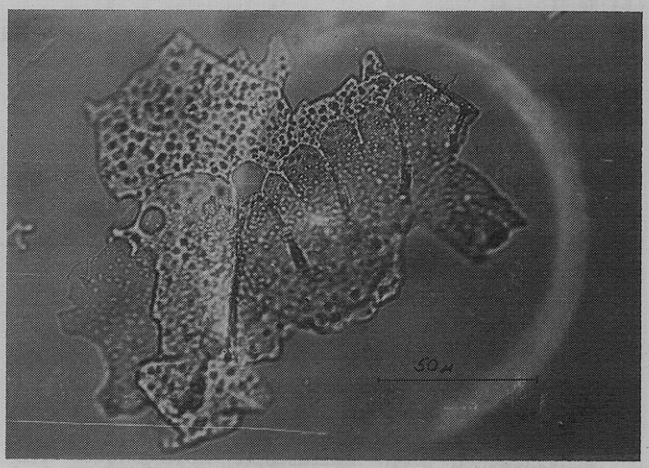

Figure 3. Hexanal droplets in a freeze-dried maltodextrin and hexanal system (400×)

isotropic system, the first solidifying phase having a refractive index $n_1 = 1.535$, and the second solidifying (interstitial) phase having $n_2 = 1.532$. Figure 2 shows a particularly good example of this separation of the two phases. Separation proceeded from the upper right to the lower left. The SEM has also produced pictures in which phase separation can be observed at the sample surface. No air bubbles were observed within the solid platelet structure.

Hexanal–Maltodextrin. The model system of hexanal–maltodextrin–water was chosen for a more detailed microscopic investigation of volatile retention. Figures 3 and 4 show particles of freeze-dried maltodextrin containing many small circular droplets with diameters of about 2–6 μm. As all the droplets are not in the same plane of the sample, some, which are out of focus, appear dark. The droplets are isotropic and have a refractive index lower than the maltodextrin. In a few cases these droplets contained a dark spot which moved around within the circular space. This indicated that the droplet was liquid, and that the dark spot was probably a vapor bubble undergoing brownian motion. The refractive index of the droplets was measured by the careful dissolution of the maltodextrin matrix in the water–glycerol refractive index solutions. The droplet liquid was essentially insoluble in the refractive index solution, so by careful manipulation these small droplets were combined into a larger one whose refractive index could be measured in the usual manner. By interpolation techniques, the droplet's refractive index, which was measured to be between 1.407 and 1.424, was estimated to be 1.412. The measured refractive index for pure hexanal was 1.410. Thus it can be firmly stated, based on the microscopic observations and knowledge of the initial material, that the droplets consisted of liquid hexanal. The hexanal droplets are distributed evenly within the two phases and do not affect the refractive indices of either solid phase, which remain at $n_1 = 1.535$ and $n_2 = 1.532$.

A few particles of freeze-dried maltodextrin provided supporting evidence in scanning electron microscopic observations. The broken platelets of the particle in Figure 5 exhibit circular depressions which have diameters the same order of magnitude as those seen in the optical micrographs. Since the SEM shows only surface features, the fact that the circular depressions are observable only at the broken platelets indicates that, if these depressions are indeed the location of hexanal droplets, the droplets are located within the solute platelets.

Table I. Data on Volatiles Retained by Freeze-Dried Maltodextrin

Volatile	Retention (g volatile/ 100 g solids)[a]	Volatile refractive index	Solubility in water[b]	Number of droplets[c]	Largest refractive index in maltodextrin
Ethanol	2.76	1.361	∞	None	1.531
1-Propanol	3.40	1.385	∞	None	1.530
1-Butanol	2.92	1.399	S (9%)	Many	1.533
Acetone	3.24	1.359	∞	None	1.530
Methyl-ethyl ketone	3.80	1.384	V (35%)	None	1.530
Diacetyl	2.04	1.393	S (25%)	Few	1.530
Isovaleraldehyde		1.390	Sl	Many	1.534
Hexanal	1.28	1.410	Sl	Many	1.535

[a] Initial solution contained 4.25 g of volatile/100 g of maltodextrin. [b] ∞ infinitely soluble; V very soluble; S soluble; Sl slightly soluble. [c] As observed at 400×.

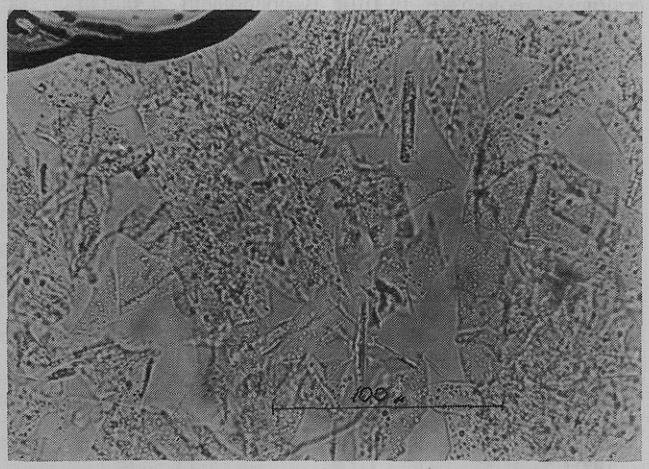

Figure 4. Hexanal droplets in a freeze-dried maltodextrin and hexanal system; this sample was frozen more rapidly and crushed finer than that in Figure 3 (400×)

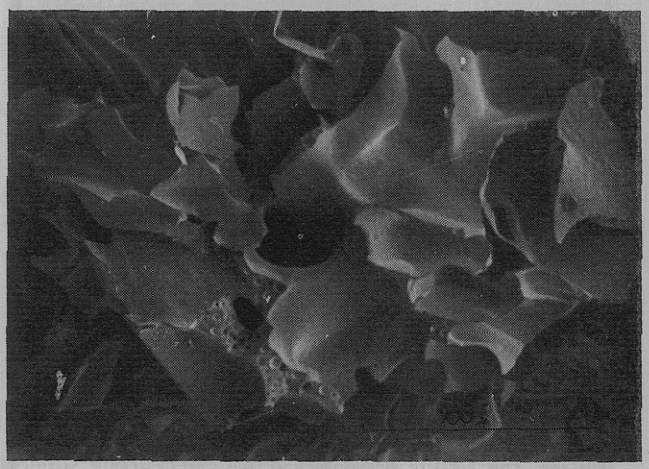

Figure 5. Scanning electron micrograph of freeze-dried maltodextrin and hexanal system (note holes on broken platelet surfaces) (266×)

The hexanal retained after freeze-drying was determined by gas chromatography to be about 1.3 g of hexanal per 100 g of maltodextrin. The hexanal content was also calculated by using an average droplet size, known hexanal density, and by counting the number of droplets on an easily measurable platelet located on the center of the left edge of Figure 4. By assuming the platelet thickness to be three times the average droplet diameter, which seems reasonable based on scanning electron microscopic and optical sectioning observations, we calculated the hexanal retention to be 1.56 g of hexanal per 100 g of maltodextrin, in good agreement with the measured value.

Other Volatiles in Maltodextrin. The other volatiles investigated are listed in Table I, together with pertinent information and results. Sizable amounts of each volatile were retained by the maltodextrin. It should be noted that the droplets observed microscopically have not been proven to be the particular volatile added, but are assumed to be so on the basis of their similarity in appearance with the hexanal samples. Of interest is the relationship between the number of observable droplets, the volatile solubility, and the largest maltodextrin refractive index. Simply stated, the less soluble volatiles show many droplets and none or only small decreases in the maltodextrin refractive index. As the volatile solubility increases, fewer droplets are seen and the maltodextrin's refractive index falls to about 1.530. This behavior can be explained by the fact that refractive indices of mixtures are additive and therefore depend on the fraction of each component present in the mixture. When the freeze-dried material acts as a homogeneous mixture (for example, if the droplets are below the resolving power of the microscope or if the volatile is actually dissolved in the maltodextrin), the measured refractive index of the mixture will decrease due to the presence of the volatile with its much lower refractive index. With acetone, for example, no droplets were observed; the weight-fraction average refractive index was calculated to be 1.529, compared to the measured value of 1.530. Because all the very soluble volatiles have similar refractive indices and retentions, the largest maltodextrin refractive index for all these volatiles was 1.530. In further observations with 1-propanol at 1000×, small droplets were barely discernible in the freeze-dried material, indicating that perhaps the apparent absence of droplets noted for 1-propanol in Table I is due to limitations of the resolving power of the microscope (at 400×). From the above discussion it seems obvious that as the volatiles are less soluble, there is more droplet formation and these droplets tend to be somewhat larger, meaning that there is a higher percentage of them resolvable in the microscope, and the maltodextrin refractive index is higher.

These observations regarding solubility and droplet size can be related to factors affecting the formation of the droplets. The highly insoluble volatile compounds are present in the initial solution as small drops, and remain so as the samples freeze. The size of these droplets can be expected to depend somewhat on mixing conditions used in the solution preparation. Volatiles of intermediate solubilities will reach their solubility limits at different stages of the freezing process and can develop as droplets to an extent dictated by the sample temperature and freezing history. It is uncertain

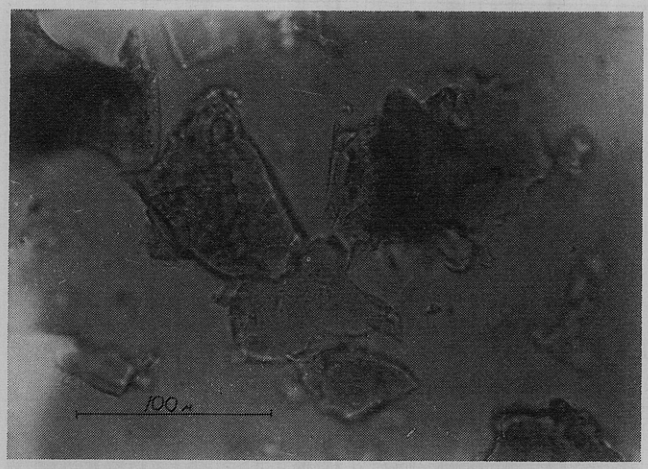

Figure 6. Freeze-dried coffee particles containing small droplets (400×)

what happens to volatiles normally considered to be highly soluble when the low temperatures and high solute concentrations (*i.e.*, low water concentrations) are achieved in the frozen materials prior to freeze-drying; the volatiles may exist as either a fine dispersion or molecular solution.

Similar droplets have been observed in samples of freeze-dried coffee (Figure 6). No attempt has yet been made to identify these droplets as retained liquid volatile. However, based on the observations of the model systems, it does seem likely that these droplets are coffee oil and coffee aroma substances.

We feel the results presented above offer strong support to the "microregion" concept of aroma retention, especially for the case of volatiles of limited solubility or materials having higher concentrations than are present in natural foods (*i.e.*, aroma concentrates). For these cases the presence of minute liquid droplets within the dry solute matrix would not appear to be compatible with concepts based solely on sorption in the dry layer, or diffusion of molecular species. As the volatile solubility increases, however, the droplet diameter apparently decreases; diffusion regulation may therefore become of increasing importance in such cases. However, the presence of droplets in freeze-dried coffee solids may indicate that the most important mechanism for flavor retention is based on the "microregion" concept.

It is obvious from this work that the formation of droplets and the structure of the solute matrix is very important to volatile retention in freeze-drying. The freezing procedure and frozen-layer temperature during drying are particularly important. We are currently investigating the effects of freezing on phase separation and the influences of processing parameters on the conditions influencing droplet formation and freeze-dried solute structure.

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