THE RETENTION OF VOLATILE MONOCARBONYL COMPOUNDS IN NATURAL MODEL SYSTEMS

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ABSTRACT

THE RETENTION OF VOLATILE MONOCARBONYL COMPOUNDS IN NATURAL MODEL SYSTEMS

By

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The quantitative retention of volatile monocarbonyl compounds during freeze-drying was investigated by incorporating known amounts of 2-alkanones, alkanals and a 2-enal into a natural model system high in lipid content (cream--30.5 per cent milkfat). A substantial loss of added monocarbonyls was observed in the lyophilized cream samples. The retention of each class of volatile carbonyls was enhanced by the selection of moderate temperatures for freeze-drying; at elevated temperature programs the recovery of monocarbonyls from cream samples decreased.

The data suggest that the recovery of higher molecular weight methyl ketones and aldehydes from the freeze-dried cream samples may be determined by factors such as boiling point, solubility in the lipid phase of the cream, molecular size, and properties of absorption and adsorption. Generally, relative volatility seemed to influence the retention of volatile short chain monocarbonyls in the freeze-dried powder. During lyophilization, the low molecular weight aldehydes

appear to be retained by the powder in greater amounts than the short chain methyl ketones. Though the concentration of the 2-alkanones in the cream samples was higher than that of the alkanals and the 2-enal, the per cent retention of both these classes of carbonyls following freeze-drying was superior to that of the methyl ketones. The results of these experiments indicate that the retention of volatiles with an aldehyde group may have been favored.

A technique was pursued for the enrichment of the dried model system by exposure of the system to an atmosphere of volatile flavor compounds. Substantial quantities of methyl ketones were absorbed by the powder. The data show that as the exposure time was lengthened, the amount of each of the flavor compounds absorbed by the freeze-dried cream increased. Short chain methyl ketones were absorbed by the cream powder in larger amounts than were homologs of higher molecular weight. The application of these findings to a commercial absorption process could be of considerable importance in improving the flavor and odor characteristics of a dehydrated food, or in the fabrication of completely new synthetic foods or food analogs. In these experiments the desorption during frozen storage of short chain ketones from cream samples exposed to 2-alkanones was greater than that obtained for the higher molecular weight homologs. To compensate for the loss of highly volatile flavor constituents during prolonged storage, a dried food product could be enriched above the normal level. Following storage residual levels of such volatiles would more closely approximate the initial concentrations in the food.

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INTRODUCTION

During the past few years there has been a growing interest in the freeze-drying or lyophilization of foods. Because they may be rehydrated readily to products closely resembling the fresh material, freeze-dried foods are usually considered superior in quality to the same foods preserved by other dehydration techniques. The retention of volatile flavor compounds is of particular importance to the food industry as it is frequently the major determinant of food quality. Although the flavor of freeze-dried foods is generally accepted to be better than that of air-dehydrated products, there is very little published information regarding the quantitative losses of flavor compounds from foods during lyophilization.

Pure flavor compounds such as esters, aldehydes, monocarboxylic acids and alcohols were found to evaporate almost quantitatively during freeze-drying (Kallistratos and von Sengbusch, 1964). However, the evaporation during lyophilization of flavor constituents in a complex food system may be reduced as a result of various physical and chemical interactions with the food components. A quantitative investigation of the loss of flavor constituents during the lyophilization of a food is difficult because of the large numbers of compounds which are present, some at very minute concentrations. Model systems such as gels and solutions are frequently employed in flavor research. However, selection of a natural model system for the incorporation of known compounds at concentrations typical of foods may be more representative of the conditions present during the freeze-dehydration of food products. Research of this nature may also provide information regarding the extent to which volatile organic constituents may be retained in foods after processing.

The major purpose of this project was to investigate the quantitative retention of selected monocarbonyl compounds during the lyophilization of a natural model system high in lipid content. An attempt was made to evaluate the effect of freeze-drying conditions on the losses of selected methyl ketones, aldehydes and a 2-enal from cream. A technique for restoring the volatile aroma constituents lost during processing and thereby enhancing the flavor quality of freeze-dried foods was also studied. Minor emphasis was placed on determining the retention of added volatiles in fresh and freeze-dried samples during frozen storage.

LITERATURE REVIEW

Flavor is generally accepted as a primary food attribute. The flavor and aroma characteristics of foods are dependent upon the presence of specific organic compounds. Monocarbonyls--namely, alkan-2-ones, alkanals, alk-2-enals, and alk-2,4-dienals--are among the important flavorful volatiles most frequently encountered in food lipids. An investigation into the nature of chocolate flavor revealed that each carbonyl class was a potent reservoir of aroma-emitting constituents (Boyd et al., 1965). According to the authors, methyl ketones were cheese-like; saturated aldehydes, waxy and fruity; 2-enals, oily and painty; 2,4-dienals, spicy and like nutmeg. Furthermore, their quantitative data indicated that each group of carbonyls was a significant contributor to chocolate aroma. Studies of the organic volatiles predominant in coffee have demonstrated the essential role of aldehydes in coffee aroma and flavor (Sivetz and Foote, 1963).

The carbonyl components of dairy products have been examined extensively. The characteristic piquant flavor of Blue cheese has been attributed to alkan-2-ones (Patton, 1950; Schwartz and Parks, 1963; Day and Anderson, 1965; Anderson and Day, 1966). In Blue cheese, methyl ketone formation has occurred as a result of β-oxidation of fatty acids by the spores of *Penicillium roqueforti* during ripening (Gehrig and and Knight, 1963). Several aldehydes--methanal, ethanal, propanal, 3-methyl butanal and methional--have been isolated from Cheddar cheese (Day *et al*., 1960; Day and Libbey, 1964). They result from the transamination and decarboxylation of amino acids (MacLeod and Morgan, 1958) by bacteria. Alkanals that may originate from the Strecker degradation of amino acids (Keeney and Day, 1957) have also been detected. Of these, methional has been recognized as a major volatile component in toasted Cheddar cheese. Cis-4-heptenal, which arises from the oxidation of linoleic acid, has been shown to impart the creamy aroma desirable in cream fudges and cream caramels (Begemann and Koster, 1964).

These few examples illustrate the significance of carbonyl compounds in flavoring foods, particularly foods high in lipid content. In food dehydration, the retention and loss of these organic volatiles are important factors relating to the quality of the dried food. Lyophilized foods are usually considered superior in flavor and quality to the same foods preserved by more conventional methods. Since the primary emphasis of this investigation is concerned with the retention of monocarbonyls during freeze-drying, studies relating to losses of organic volatiles in dehydration as well as methods of improving retention of volatiles are reviewed.

Literature pertinent to the variables employed in the freeze-drying process and the lyophilization of dairy products is also summarized.

Freeze-Drying

Freeze-drying may be described as a dehydration technique in which moisture is removed from a frozen product by sublimation. In their comprehensive review of the early developments in the freeze-drying of foods, Harper and Tappel (1957) discussed the basic principles and methods. Operational parameters involved in freeze-dehydration of foods include preparation of the raw material, freezing, drying and packaging, and they markedly affect the quality of the final product. The process variables of particular importance are the temperature, rate and method of freezing and the temperature, pressure and rate of dehydration.

Variables in the Freezing Phase of Freeze-Drying

In his Traité de Lyophilisation, Rey (1961) stated that for many food processors the freezing phase of lyophilization was of little importance since its only purpose was to solidify the material. During freeze-drying, a product of optimum quality was obtained by freezing to the temperature of complete solidification, or lower, and drying at temperatures below the incipient melting point (Rey, 1960). The point of complete solidification could be determined by resistivity measurements;

the temperature of incipient melting by thermal analysis. Rey (1960) also observed that many substances did not crystallize quantitatively. Such materials reached a metastable condition during which time interstitial fluids became extremely viscous and eventually formed a glass-like solid. During drying, the softening of the glass-like bodies might cause denaturation of sensitive constituents. In studies conducted on both model systems and orange juice, Rey and Bastien (1962) showed that the electrical resistance of a frozen material was a more valid and reproducible index of rigidity of the material than was temperature. Moreover, changes in resistance could be employed to automatically control the process.

Luyet (1962) demonstrated that the rate of initial freezing had a fundamental role in the structure of the frozen material, and on the subsequent processes of dehydration and rehydration. The effects of slow, rapid and intermediate freezing rates on sublimed muscle tissue were observed by light and electron microscopy. Slowly frozen lyophilized samples were shrunken, had a coarse, spongy structure and large interstitial cavities. The cavities were the spaces previously occupied by ice as well as those resulting from the shrinkage of the non-frozen parts of the tissue. In contrast, rapidly frozen freeze-dried tissues were not shrunken and had a relatively soft consistency. Individual fibers were porous as a result of the development of numerous ice spears during the initial freezing. The large cavities typical of slow freezing were absent. Thus, variation in freezing velocity affected the size and number of ice crystals.

Later, Rey (1963) reported that fast freezing resulted in the formation of small crystals and products of high quality. However, for products that develop glassy structures, progressive and careful rewarming to the temperature of devitrification was suggested to rupture the unstable equilibrium of the glass and thereby induce crystal formation. Subsequent recooling of the solution to the desired drying temperature was recommended. This technique was especially promising for delicate products.

Although Luyet (1962) observed differences in rehydration for slow and rapidly frozen muscle tissue, additional information on the binding of water by tissue solids was required. Smithies (1962) noted that relatively rapid freezing (dry ice-acetone mixture) gave lyophilized meat that rehydrated more slowly. In addition, cooked samples which had been frozen rapidly were tougher and drier than comparable slowly frozen samples (-20 to -10^{o}C). Freezing shrimp at moderate rates and temperatures (0^{o}F) resulted in products with better rehydration characteristics than did freezing at a rapid rate in liquid nitrogen (Goldblith _et al_., 1963; Karel, 1963). However, experiments with Swiss chard showed that rapidly frozen lyophilized samples exhibited a greater uptake of water (McIlrath and Dekazos, 1962). In this case, freezing in liquid nitrogen was superior to an ethanol-dry ice mixture. Lusk _et al_. (1965) determined the effect of freezing rate on the rate of dehydration. The drying rate was faster for shrimp frozen at $0^{\circ}F$ than for similar samples frozen in liquid nitrogen. Larger crystals, which developed during slower freezing, created a more porous dried layer, permitting more efficient water vapor transfer across the dried layer.

In studies relative to the structure of frozen solutions, Mackenzie and Luyet (1963) noted that very rapidly frozen 30 per cent gelatin gels freeze-dried faster at $-30^{\circ}C$ when they had not been permitted to recrystallize prior to freezing than when they had recrystallized. Their findings suggested that samples which contain very many ice crystallites freeze-dry somewhat more rapidly than samples containing comparatively large ice particles.

Simple food gels frozen slowly at $-10^{\circ}C$ dehydrated faster than gels frozen at much lower temperatures with dry ice or liquid nitrogen (Saravacos, 1965). A similar effect was noted with milk (Kramers, 1958). Experiments with Romano cheese showed that the rate of freezing affected the rate of drying (Keppeler, 1968). Fast frozen samples exhibited the slowest rate of dehydration. Samples subjected to a moderate freezing rate lost moisture rapidly at the beginning of the drying cycle. However, the rate diminished after a few minutes. Slowly frozen samples showed a slow water loss initially but the desired moisture content was attained more quickly.

Quast and Karel (1968) investigated the effects of composition, of freezing rates and of pressure on dry layer permeability of freeze-dehydrated liquids. They found that drying time, based on mathematical models, was reliable only in the case of slush frozen samples. Predictions were inadequate for samples frozen by other methods due to anistrophic distributions of ice crystals. In general, permeability increased as ice crystal size increased. Slowly frozen and slush frozen samples of coffee and a model system, had permeabilities several times higher than those obtained with similar fast frozen counterparts.

Variables in the Drying Phase of Freeze-Drying

Heat transfer is definitely important in lyophilization (Burke and Decareau, 1964). During freeze-drying studies with radiant energy, drying curves for various operating conditions were established (Zamzow and Marshall, 1952). A comparison of conduction and radiation freeze-dehydration indicated that the latter was more rapid because of the penetration of radiation into the sample. The drying rate was affected by the nature of the contact between the retaining medium and the frozen solid. Poor contact greatly reduced the drying rate in both conduction and radiation freeze-drying. Abelow and Flosdorf (1957) described practical methods for obtaining shorter cycles and more uniform temperature conditions for those engaged in bulk sublimation of sensitive materials.

Experiments with shrimp illustrated the effect of platen temperature on the rate of freeze-drying (Lusk et al., 1965). Four different platen temperature programs were selected: 125°F; 175°F; initially 175°F, lowered to 150 and 125°F to prevent a surface temperature in excess of 125°F; and initially 250°F, successively lowered to 200, 175, 150 and 125°F. The drying rates accelerated with increasing platen temperatures. It was possible to reduce the total drying time substantially by employing high initial platen temperatures. Center and surface product temperature profiles were obtained for each of the temperature programs. The center temperature of the product decreased to a temperature in equilibrium with the chamber pressure immediately after the drying chamber was evacuated. This was followed by a slow temperature rise. Near the completion of the drying cycle, the center temperature rose rapidly to 5 to 15°F below the platen temperature at the end of the drying period. Surface product temperature increased rapidly during the initial stages of drying, and then increased more slowly to a level slightly below the platen temperature at the completion of the drying period. For programs which involved successively lowering the platen temperature, the surface temperature: a) decreased slightly with each decrease in temperature of the radiating surface, and b) increased again after equilibrium between the platen and surface temperatures had been re-established. Similar time-temperature profiles for the freeze-drying cycle have been obtained in other studies (Goldblith *et al.*, 1963; Saravacos, 1965; Radanovics, 1969). Weight loss during lyophilization appeared to be a curvilinear plot until the point at which drying became essentially complete (Goldblith *et al.*, 1963; Radanovics, 1969).

The reduction in total freeze-drying time that occurs at high platen temperatures is well substantiated in the literature (Zamzow and Marshall, 1952; Harper and Tappel, 1957; Goldblith *et al.*, 1963; Radanovics, 1969). However, undesirable changes that occur during dehydration may be accelerated. Thus, the actual temperatures employed in freeze-drying must reflect a compromise between process efficiency and optimum product quality (Karel, 1963).

In their experiments on freeze-drying shrimp, Lusk *et al.* (1965) found that differences in total drying time at a chamber pressure of 1.5 mm Hg, and of 0.08 mm Hg were not significant. A similar observation was made by Saravacos (1965).

A direct relationship between total drying time and sample thickness has been reported (Zamzow and Marshall, 1952; Harper and Tappel, 1957; Saravacos, 1965; Radanovics, 1969). The freeze-drying of gelatin and starch gels (Saravacos, 1965) and of beef slices (Harper and Tappel, 1957) showed that sample thickness and drying time were linearly related. Saravacos (1965) also observed that the drying rate depended on the nature of the product. Under identical conditions, total freeze-dehydration time for simple food gels varied from 4.3 hours (cellulose gum) to 11.5 hours (egg albumen).

A comprehensive study of heat and mass transfer in the freeze-dehydration of several products was made by Lambert *et al*. (1962). The authors described the existence of a surface film or crust resistance to mass transfer. This resistance decreased with solids content of the sample. Quast and Karel (1968) also acknowledged the existence of a surface layer. Samples which developed a surface layer showed a constant rate period during lyophilization (Lambert *et al*., 1962; Burke and Decareau, 1964; Quast and Karel, 1968). This constant drying rate may have been due to the surface resistance, which is the major component of the resistance to mass flow during the initial period of drying (Quast and Karel, 1968). Mechanical removal of the surface at a suitable temperature, reduced the surface resistance (Lambert *et al*., 1962; Quast and Karel, 1968).

Mechanisms of Freeze-Drying

Meryman (1962) described the general mechanism of the lyophilization process as the introduction of heat to supply the energy required for sublimation, the conduction of heat to an ice-dried layer interface and the sublimation of water vapor from the interface, followed by the movement of water vapor through the dried layer, and finally its removal from the dried surface.

More recently, Mackenzie (1966), as a result of extensive study of structure in the frozen state, indicated the existence of four principal alternative freeze-drying mechanisms: 1) Direct sublimation drying. This mechanism was characterized by ice crystals that connect directly with each other and with the free surface of the specimen. During sublimation these channels, previously filled with ice, served as paths for vapor flow. The solute matrix retained its rigidity and original microstructure throughout sublimation. 2) Molecular diffusion through concentrated solute (structure preserved). Separate ice crystals surrounded by solute represented this mechanism. Moisture travelled from the freeze-drying "front" by diffusion through the solute in the absence of channels. 3) Direct sublimation via cracks induced in the solute matrix by shrinkage. The simultaneous cracking of the solute framework during sublimation was indicative of this mechanism. Water vapor flowed from the freeze-drying "front" via cracks induced in the matrix by secondary dehydration. 4) Molecular diffusion through concentrated solute. This phenomenon was observed in almost all frozen solutions. In addition to one of the other mechanisms, recession of the freeze-drying "front" was accompanied by total loss of solute matrix structure. Each system had a particular temperature, usually much lower than the eutectic temperature, above which sublimation was impossible without structure collapse. The disppearance of ice was attended by the viscous flow of solute. Mackenzie (1966) stressed that generally freeze-drying occurred by a combination of two or three of the mechanisms and that their relative contribution to the over-all mechanism might change during the run. Furthermore, an understanding of the molecular processes that took place during lyophilization permitted the application of this technique to a remarkable variety of products.

Freeze-Drying of Dairy Products

Although freeze-drying has been utilized for many foods, its application to dairy products has been somewhat limited. In an early investigation, Nickerson *et al*. (1952) observed that freeze-dehydrated milk was not superior to milk which had been spray-dried. The fat emulsion of the freeze-dried powder was partially destabilized. Moreover, it was difficult to reconstitute the freeze-dried milk powder because of the presence of free fat. Studies on lyophilized milk and cream established that emulsion destabilization occurred during the drying portion of the process (Rutz and Winder, 1953). Attempts to overcome this problem by the addition of sodium citrate, disodium phosphate, or the partial removal of calcium ions were generally unsuccessful. Mickle (1966) found that the emulsion stability of freeze-dried model milk systems was improved by the selection of emulsifiers with hydrophile-lipophile balance (HLB) ranges of 11 to 14. In addition, as emulsifier concentrations were increased, the amount of fat recovered in reconstituted systems also increased.

The application of freeze-drying to liquid milk products was described in a patent (Ogden, 1967). Liquid milk, concentrated to one-third of its original volume, was frozen in thin sheets. The frozen sheets of milk were flaked, pressed into blocks and freeze-dried to a moisture content of less than 5 per cent.

Desai (1966) observed that freeze-dried reconstituted sour cream had a pleasant flavor but that its body was unlike that of the fresh product. However, recently Hamilton (1970) indicated that fortification of the fresh cream, with stabilizers and stabilizing additives, resulted in a freeze-dried reconstituted cultured cream possessing body and texture properties similar to those of a fresh cultured cream.

Studies on the lyophilization of cheese have been more numerous. Evstrat'eva et al. (1959) investigated the application of this procedure to quarg. A process for the successful commercial production of freeze-dried quarg has been described (Anon.,1964). Flosdorf and Hamilton (1957) obtained a patent for freeze-drying soft cheeses such as cottage and Neufchâtel. Several reports (Jokay and Meyer, 1959; Anastos, 1964) have discussed the development of a satisfactory freeze-dehydrated cottage cheese.

Meyer and Jokay (1959) observed the characteristics of lyophilized Cheddar, Brick, Munster, Blue, cream and cottage cheese. Generally, the flavor of all freeze-dried samples was milder than that of the untreated controls. All cheeses rehydrated to a soft but natural consistency. A study of the structure and physical properties of various freeze-dried cheeses was reported by Schultz (1966). Results indicated that the body of most cheeses was unsatisfactory upon rehydration. Only the "high moisture" cheeses such as quarg and double-cream cheese could be readily reconstituted. Keppeler (1968) applied freeze-dehydration techniques to Romano cheese.

Factors Affecting the Retention of Flavor Volatiles during Dehydration

The loss of volatile flavor compounds that accompanies food dehydration is a major problem. Numerous factors affect the retention of organic aroma constituents.

Generally, as compound volatility is increased, flavor retention in a dried product is decreased (Boudreau *et al*., 1966; Saravacos, 1968). The processing of spray-dried butter containing known amounts of volatile fatty acids, showed that more than two-thirds of the butyric and caproic acid content was lost during drying (Boudreau *et al*., 1966). About one-half of the caprylic and capric acids, and approximately three-fourths of the lauric acid, were retained.

During evaporation and drying processes, the loss of a volatile aroma compound in the pure state is directly related to its vapor pressure. In aqueous solutions, however, the volatility of an aroma constituent is dependent upon its vapor pressure and its solubility. The evaporation from aqueous solutions of completely soluble substances such as acetic acid follows the normal pattern of water-liquid equilibria of two miscible compounds (Saravacos, 1968). However, flavor compounds such as esters, which are only partly soluble in water (thus more volatile) form azeotropes. Their volatilization during dehydration is dependent on the relative volatility of the azeotrope. High relative volatility results from high vapor pressure and low solubility in water.

The volatility of a specific flavor component is also dependent upon such factors as the miscibility of the compound with other organic constituents and the presence of salts and sugars. Sivetz and Foote (1963) listed factors which may influence the relative vapor pressure of volatiles in a coffee extract medium. Coffee solubles increased the solubility of aldehydes. This affinity (between the alkanals and solubles) tended to lower the relative vapor pressure of aldehyde to water compared to that found in a pure aldehyde-water system. Other coffee organics mixed with the volatiles influenced the volatile behavior of each. Therefore, the true vapor pressure curve differed from that of the pure volatile. Coffee solubles such as phospholipids and fatty acids exerted adsorption and absorption properties. Thus phospholipids, which were only slightly water soluble, retained organic carbonyls better than water. Although oils were immiscible with water and coffee extract, they had a high solubility coefficient for aliphatics and long chain carbonyls. As a result, the concentration of some coffee volatiles in the oils (petroleum ether solubles) might be 1,000 times that of the contacting soluble coffee extract (Sivetz and Foote, 1963).

The effect of food lipid content on ketone volatility was illustrated by determining the headspace response of 2-heptanone in milk (4 per cent fat), skimmilk and water (Nawar, 1966). At the same ketone concentration, the greater solubility of 2-heptanone in milk, compared to that in skimmilk or water, was reflected by the lowered headspace response. Changes in the headspace response of aqueous 2-heptanone, at two levels of concentration, with the addition of various substances, were also observed. An equal amount of 2-octanone did not affect the headspace concentration of 2-heptanone in the dilute system; however, in the concentrated system, the 2-heptanone level was reduced to about one-half following the addition of 2-octanone. Thus, in some cases differences in volatility (e.g., those of a sample before and after treatment) could result from the appearance or disappearance of another component rather than from a true increase or decrease in the compound examined.

Reineccius and Coulter (1969) observed that individual skimmilk constituents varied in their ability to retain diacetyl during spray-drying. Milk protein extracts retained greater amounts of diacetyl during drying than did lactose solutions of equivalent concentration. They suggested that this may have been caused by the substantial reduction in the vapor pressure of diacetyl following the addition of milk protein to a diacetyl-water solution. The addition of lactose to the diacetyl-water solution resulted in an increase in the vapor pressure of diacetyl.

The loss of volatiles may be a function of the boiling point of a flavor constituent. During the spray-drying of butter, the loss of a homologous series of fatty acids was directly related to the boiling points of these compounds (Boudreau _et al._, 1966). The gas chromatographic analysis of spray-dried cheese slurries showed that substantial losses of low boiling compounds occurred during processing (Bradley and Stine, 1964). Katayama _et al._ (1966) reported that boiling and lyophilization removed considerable amounts of the low-boiling components present in apples. Similar findings have been observed by Saravacos and Moyer (1968b) during the vacuum drying of fruit juices. However, Reineccius (1967) indicated that boiling point exerted only a minor influence upon the retention of volatiles during the spray-drying of skimmilk.

The loss of a flavor component may be related to its concentration in the liquid phase. McBean _et al._ (1965) noted a small but consistent decrease in the retention of sulfur dioxide in fruit sulfured at higher concentrations (3 per cent sulfur dioxide). In the freeze-drying of acidified cream, the relative loss of diacetyl was independent of the initial absolute concentration between the range of 5 to 100 ppm (Radanovics, 1969). Reineccius and Coulter (1969) determined the loss of diacetyl (at concentrations of 500 and 6,000 ppm) from 30 per cent total solids skimmilk during spray-drying. When the lower diacetyl concentration was dried, 75 per cent of the added diacetyl was retained. However, only 60 per cent of the volatile was retained when the higher concentration was dehydrated.

The role of total solids in the retention of volatiles has been investigated. As the concentration of solids is increased to an optimum, loss of volatiles is decreased. Sivetz and Foote (1963) found that aroma losses from freeze-concentrated coffee extracts containing 45 per cent total solids were low. However, a reduction in the concentration of solubles noticeably impaired the flavor of spray-dried coffee.

Menting and Hoogstad (1967) obtained similar results when they determined acetone retention during the drying of a droplet of aqueous carbohydrate solution. Until the carbohydrate droplet attained an average total solids content of 40 per cent, acetone losses were significant. However, further dehydration resulted in a minimal reduction of acetone. During the freeze-drying of glucose solutions, the percentage of residual acetone was directly proportional to the amount of glucose present in the solution (Rey and Bastien, 1962). Acetone retention in glucose solutions of 5 and 25 per cent total solids was 5 and 45 per cent, respectively. The data of Saravacos (1968) show that pectin solutions and grape juice, with high sugar concentrations, retained significantly greater quantities of aroma constituents than did their counterparts with less sugar.

More recently, Reineccius and Coulter (1969) reported total solids concentration to be a major factor in the retention of added flavors during dehydration. When 50 per cent total solids skimmilk and diacetyl were spray-dried, essentially all of the diacetyl was retained. However, similar experiments with 10 per cent total solids skimmilk yielded a diacetyl retention of only 35 per cent. Furthermore, concentration of the 10 per cent skimmilk prior to dehydration resulted in greater losses of added diacetyl than if the skimmilk had not been pre-concentrated.

Boudreau et al. (1966) studied the effect of pH on the recovery of volatile fatty acids from spray-dried butter. The free fatty acids were incorporated into melted butter adjusted to a pH of 9.0. Conversion of the acids to their sodium salts reduced volatility significantly. Losses of sodium butyrate and caproate were less than 20 per cent; higher members were completely recovered. An association between aroma retention and pH has been obtained by Saravacos and Moyer (1968a). Freeze-dried food gels that were acidic retained flavor constituents to a lesser degree. Relatively high amounts of volatiles were present in gels with a higher pH. Such findings concur with those of Saravacos (1968) on the vacuum-drying of aroma compounds.

A relationship between particle size and volatile loss has been observed. McBean _et al_. (1965) determined the loss of absorbed sulfur dioxide from apricot tissue during dehydration. The larger fruit retained greater amounts of sulfur dioxide than did the smaller fruit.

The improved retention of fatty acids in butter extracts spray-dried at reduced pressure was assumed to be due to the larger particle size (Boudreau _et al_., 1966). Bradley and Stine (1963, 1964) indicated that Cheddar cheese powders containing large particles showed a definite flavor superiority over powders having small particle sizes. In the manufacture of instant coffee, the production of large thick-walled particles resulted in an improvement in flavor quality (Sivetz and Foote, 1963). Flavor retention was attributed to a decrease in surface area per unit mass of the powder when large particles are produced. Reineccius and Coulter (1969) observed that diacetyl retention in spray-dried milk powders, from 30 per cent total solids skimmilk, was independent of particle size. A delay in membrane formation in larger particles, due to a reduction in the vapor pressure differential and a decrease in diffusivity with increase in concentration, may have lengthened the volatilization period prior to effective membrane formation. Consequently, the effect on volatile loss of the decreased surface area per unit of mass, for larger particles, may have been offset.

The influence of inlet air temperature on the retention of volatiles has been reported (Sivetz and Foote, 1963;

Reineccius and Coulter, 1969). The loss of added flavors became more pronounced as the inlet temperature was raised (Reineccius and Coulter, 1969). Powders of higher moisture contents, larger mean particle sizes and lower absolute densities were obtained at high inlet air temperatures. According to Sivetz and Foote (1963), inlet temperatures of less than 204°C were required to cause a significant improvement in coffee volatile retention. They suggested that greater losses at high inlet temperatures were due to "blossoming" of the drying particle. Thin-walled particles with a large surface area per unit mass were obtained.

The research of Bradley and Stine (1963) showed that high exit air temperatures were detrimental to the flavor of spray-dried Cheddar cheese. Cheese powders dried at the lowest exit air temperature (160°F) received the highest organoleptic flavor scores. Powders processed at higher exit temperatures (190°F) developed a noticeable stale flavor which was attributed to heat damage of the cheese. Findings in opposition to these (Bradley and Stine, 1964) have been reported by Reineccius and Coulter (1969). High exit air temperatures (194 to 212°F) favored the retention of flavor compounds added to skimmilk. Reineccius and Coulter (1969) theorized that the improved retention of organic flavors at higher exit air temperatures may be caused by the lower relative humidity of the drying air, and therefore, an increased rate of water removal from the atomized particles.

The more rapid formation of a selective membrane around the atomized particles was suggested as the reason for the reduced loss of volatile compounds.

Saravacos and Moyer (1968b) observed that retention of aroma in vacuum-dried fruit juices was improved when high drying temperatures were avoided. These findings have been substantiated in experiments related to the volatility of flavor compounds during lyophilization (Saravacos and Moyer, 1968a). Higher losses of volatile esters were noted when the final product temperature was increased.

The effect of platen temperature on the loss of both diacetyl and acetoin from freeze-dried acidified cream was determined (Radanovics, 1969). Experiments, employing platen temperatures ranging from 75 to 225^{o}F, resulted in improved retention for both volatiles at reduced temperatures.

The dehydration technique has influenced the loss of flavorful constituents. Bradley and Stine (1964) reported that cheese powders obtained by foam spray-drying were superior to cheese powders spray-dried conventionally. Staleness present in conventionally dried powders was absent in samples which had been foam spray-dried. They suggested that the presence of gases in the interstitial areas of the larger particles could serve to reduce the volatilization of cheese flavors and thus contribute to the improved flavor of foam spray-dried powders. In addition, the larger surface area per unit mass of the foam spray-dried particles permitted a more rapid rate of water removal from the particles. There was a more pronounced cooling effect on the particles as a result of the more rapid rate of evaporation. Thus, the particles were not as prone to the deteriorative effects of the heated atmosphere encountered in the dryer.

Sivetz and Foote (1963) found that foam spray-drying techniques resulted in the occurrence of fragmented particles with poor fluidibility and a greater flavor loss. They (Sivetz and Foote, 1963) attributed the reduction of flavor and aroma in foam spray-dried coffee powders to the increase in surface to mass ratio.

Losses of diacetyl, diacetyl and acetoin, and volatile acidity were greater in foam spray-dried sour cream than those obtained from similar freeze-dried counterparts (Desai, 1966). The effect of drying technique on the retention of diacetyl added to skimmilk was determined (Reineccius and Coulter, 1969). The volatile constituent was lost during roller-drying. However, similar and substantially greater proportions (60 per cent) were retained during spray- and freeze-drying. In the double-drum drying process moisture was essentially boiled from the milk. This vigorous agitation during roller-drying interfered with the formation of a selective membrane for the retention of organic volatiles.

Lee _et al_. (1966) studied the effects of several dehydration processes on the flavor components of peaches. Lyophilized peaches retained 90 per cent of their original volatile carbonyl compounds. However, losses of volatile carbonyls from sun-dried and hot-air-dehydrated peaches were greater than 60 per cent.

Freeze-drying experiments (Saravacos and Moyer, 1968a) have shown that during the initial stages of the process, the loss of aroma compounds was substantial and similar to that encountered in vacuum-drying. However, as freeze-drying progressed, retention of volatiles was generally higher than that in comparable vacuum-drying experiments.

Several investigators have acknowledged the existence of a critical moisture level below which volatile desorption ceases (Menting and Hoogstad, 1967; Radanovics, 1969; Flink and Karel, 1970). For a malto-dextrin model system, the critical moisture level was 9 per cent (Menting and Hoogstad, 1967). Volatilization of acetone did not occur at moisture levels lower than 9 per cent, whereas higher moisture levels facilitated desorption of volatile materials. Radanovics (1969) observed the effect of extended lyophilization on the retention of diacetyl. Although additional losses occurred, they were of lesser magnitude than those during the initial stages of freeze-drying. Similar findings for the loss of 1-butanol during freeze-dehydration of maltose solutions have been reported (Flink and Karel, 1970).

Mechanisms for Retention of Flavor Volatiles during Freeze-Drying

The high degree to which organic volatiles are retained by freeze-dried materials has prompted the investigation of mechanisms to control or minimize the loss of volatiles. In experiments with freeze-dehydrated pure volatile compounds, Kallistratos and von Sengbusch (1964) illustrated the importance of solid material in counteracting aroma loss. Lyophilization, at -30^{o}C and 0.2 Torr for 24 hours, resulted in essentially total loss (> 97 per cent) of all the volatiles studied. Similar observations were made in the study of aroma loss from solutions with flavor compounds (Saravacos and Moyer, 1968a). The addition of polymers capable of gel formation (such as pectin or starch) to the initial solution, enhanced the retention of volatile components. Although adsorption was not implied as a factor, little information regarding mechanisms controlling the retention of volatiles was presented.

Studies on the effect of glucose concentration on acetone retention in model systems demonstrated that acetone volatilization was negligible during the secondary stages of freeze-drying (Rey and Bastien, 1962). Adsorption of the volatile on the dry layer was considered to be responsible in promoting acetone retention. However, perhaps the improved retention of acetone was due to a reduction in the rate of diffusion through the dry surface layer at high glucose concentration (Issenberg et al., 1968).

Sorption isotherms, obtained as a result of frontal analysis gas chromatography, have been employed in studying the adsorption of volatile organic compounds (Issenberg et al., 1968; 1969; Boskovic and Issenberg, 1968). These measurements have facilitated the formulation of concepts for mechanisms of the retention of volatiles. Isotherms, initially determined for the vapor-solid adsorption of hexane and acetone on microcrystalline cellulose, revealed the need to examine interactions of volatile food components with non-volatile constituents (Issenberg et al., 1968). Subsequent and similar experiments conducted by Boskovic and Issenberg (1968) with aliphatic straight chain alcohols, and by Issenberg et al., (1969) with alcohols and their acetates, have demonstrated that the affinity of the volatile compound for the system in which it is present determined its ability to adsorb.

The research of Radanovics (1969) with freeze-dried acidified cream has suggested that an adsorption mechanism was responsible for the retention of flavor volatiles.

In their discussion of aroma loss during lyophilization, Thijssen and Rulkens (1968) discounted theories of volatile adsorption. They postulated that the moisture content dependence of the diffusion coefficients of water and volatile in the dry material were responsible for the retention of the volatiles. More recently, these authors (Rulkens and Thijssen, 1969) presented a numerical method, based on their theory, for the calculation of flavor loss in drying food liquids. The drying rate curve and the retention of the volatile component were determined for a slab composed of water and maltodextrin with the addition of acetone as the model organic volatile. The calculated retentions of water and acetone fit the experimental data within the accuracy of the observations.

A somewhat similar concept for the retention of volatiles, emphasizing the localized structural aspects of the dry material, rather than general diffusion parameters, has been proposed (Flink and Karel, 1970). Aroma losses in lyophilized carbohydrate solutions served as a basis for their theory which stressed the dry material microstructure. Development of the dry material microstructure was dependent on many processing parameters and its effectiveness in preventing the loss of volatiles was, in turn, dependent on the local moisture content.

According to their proposal (Flink and Karel, 1970), pools of concentrated carbohydrate and organic volatile solutions (regions enclosing the volatiles) were formed during freezing. Further re-arrangements of the carbohydrate molecules in the frozen and/or interface layers occurred during drying. While the microregion was frozen no loss of volatiles was observed. However, at the passage of the ice interface through the microregion, aroma loss began and continued until the critical moisture level was attained. During dehydration, hydrogen bonds between carbohydrate hydroxyl groups and water were replaced by carbohydrate-carbohydrate hydrogen bonds. At the critical moisture level, the microregion was sealed and the loss of volatiles ceased.

Methods of Improving the Retention of Volatiles

Control of operational variables during processing and an understanding of the mechanisms of the retention of volatiles have enhanced the flavor quality of food products. In addition, however, diverse techniques have been employed to overcome the inevitable loss of volatile flavor constituents during food dehydration.

The retention of greater proportions of many volatile compounds during drying has been facilitated by the addition of flavor carriers such as coffee oils. They possess a unique ability to bind certain volatile constituents and thus prevent volatile loss during processing. The flavor of spray-dried coffee may be substantially improved by increasing the quantity of coffee oils that are carried through the extraction process (Sivetz and Foote, 1963).

Micro-encapsulation of flavor materials has been widely utilized for the addition of aroma (Olsen and Seltzer, 1945; Griffin, 1952; Broderick, 1954; Schultz *et al*., 1956; Sirine, 1967). A number of substances, including gelatin (Olsen and Seltzer, 1945), vegetable gums (Broderick, 1954), sorbitol (Griffin, 1952), and sucrose or dextrose (Schultz *et al*., 1956), have been used or proposed for coating essential oils and flavors. All carriers have been acknowledged to give superior powdered flavors. Sirine (1967) has presented a comprehensive review of the background and the technique associated with micro-encapsulation.

The retention of volatile components by the process of micro-encapsulation has been attributed to the rapid formation of a selective membrane around the drying droplet (Thijssen, 1965; Brooks, 1965). This selective membrane was assumed to be impermeable to organic flavor molecules but permeable to water. Other workers have confirmed the theory of membrane selectivity (Olsen and Seltzer, 1945; de Gruyter, 1965; Menting and Hoogstad, 1967).

Coincidental with the development of encapsulation has been the need for the preparation of natural volatile flavors. Numerous processes, such as vaporization and distillation (Dimick et al., 1957; Roger and Turkot, 1965), vacuum stripping and condensation (Bomben et al., 1969), and freeze concentration (Muller, 1967) have been utilized in the production of aroma-bearing concentrates.

Other methods have been utilized to enhance flavor retention. Recently, Nelson (1970) described the production of a fermented milk-like product containing 7 to 12 times the ketone content of good quality commercial Blue cheese, but with a flavor efficacy of four times that of Blue cheese. It is claimed to be of particular value in formulas where dried Blue cheese is employed since the relatively high ketone content with respect to flavor efficacy effectively counterbalances the Blue cheese volatiles lost in spray-drying. Hedrick (1968) was able to compensate for flavor losses in spray-dried Blue cheese by drying a rapid curing Blue cheese, previously cured to the desired flavor intensity.

Hamilton (1970) observed that the flavor of reconstituted freeze-dried cultured cream was improved by increasing the level of the major flavor components in the fresh cultured cream prior to freeze-drying. However, the addition of extra acetaldehyde prior to the lyophilization of quarg had little effect on acetaldehyde retention (Vitez, 1966). Addition of the organic volatile prior to packaging or during rehydration was suggested.

Flavors, lost during dehydration, have been restored by the addition of organic flavor molecules, or flavor preparations, to the dried product (Czulak _et al_., 1961; Bomben _et al_., 1969). In their experiments with cheese, Czulak _et al_. (1961) observed that most of the volatiles were lost during processing. A Romano-type flavor was obtained by the addition of butyric and caproic acids to the powder. A powder with a flavor resembling that of Blue cheese resulted from the introduction of butyric acid, caproic acid, 2-heptanone and 2-nonanone. Other types of cheese flavor were simulated with varying quantities of fatty acids, ketones and commercial cheese flavors.

Bomben _et al_. (1969) described a simple technique for directly adding aroma to a dehydrated product. A cold trap was employed for the direct condensation of a 1,000-fold orange aroma on foam-mat orange powder. Chromatograms of the orange powder, before and after aroma condensation, indicated that a substantial amount of fresh orange aroma was absorbed. In addition, the powder remained free flowing and showed only a slight increase in moisture content.

In experiments to enhance the flavor of freeze-dried sour cream, Hamilton (1970) observed that diacetyl was readily adsorbed onto the powder. The organic volatile had saturated the powder within 15 minutes of exposure.

The porosity and specific area of a dehydrated food may affect the adsorption and desorption of volatile constituents. Berlin et al. (1966) determined the surface areas and densities of various freeze-dried foods. True and apparent densities showed that a micropore structure curtailing rapid gas diffusion through the dried mass was restricted to those layers which formed the outer surface of some foods. The data also indicated that the physical form of a food prior to dehydration may determine the porosity of the dried material. In related experiments, Berlin et al. (1968 a,b) observed differences in sorption isotherms for milk products due to variations in fat content and methods of drying.

Saravacos (1967) compared the sorption isotherms of air-, puff-, and freeze-dried potatoes and apples. The higher water adsorption capacity of freeze-dried samples was attributed to their highly porous structure.

Organic vapors which have been adsorbed by organic solids such as cellulose and cellulose acetate cannot be readily desorbed (Sheppard and Newsome, 1932; Russell et al., 1937).

Methanol was completely desorbed from wool by evacuation at 80°C (Watt, 1964). However, sorbed alcohols of higher molecular weight could only be removed by exposure to water vapor at the saturation vapor pressure prior to the desorption process.

Bushuk and Winkler (1957) showed that alcohol adsorbed as a vapor on wheat flour could not be removed by evacuation. However, the sorbed alcohol was removed by adding water to the vapor space and then evacuating the wheat flour.

Experiments, on the binding of flavor compounds to food, demonstrated that the water content of the food was the determining factor in the sorption of flavor constituents (Maier, 1968). Sorption enthalpies for glucose and lactose with organic volatiles indicated a physical sorption. With the aid of infra-red absorption spectra, it was possible to observe the binding (by inclusion) of flavor substances to carbohydrates and food. Some experiments with alcohols and carbonyls showed the presence of hydrogen bridges in the inclusions. A strong reversible bond was present between carbohydrates and volatile amines.

A simple sensory method was employed to study the binding of volatile aroma components in foods (Maier, 1969). Menthol or isoamyl acetate were exposed to sixteen foods or food constituents, such as whole milk powder, skimmilk powder, glycine, lactose and ovalbumin. Volatile desorption was determined at room temperature, at 60°C and after the addition of water at room temperature. The odor of menthol was evident in lactose after 14 days, and following heating at 60°C for 30 and 60 minutes. The retention of both volatiles was greater in whole milk powder than in skimmilk powder. The desorption of menthol from whole milk powder was not observed until after 5 days; however, isoamyl acetate losses from milk powder were noted 5 hours after exposure. Skimmilk powder retained a menthol odor for approximately 6 hours. Isoamyl acetate was present in both ovalbumin and lactose 48 hours after contact. Losses of isoamyl acetate from cellulose, starch and pectin were observed 30 minutes after heating. The addition of water resulted in the retention of isoamyl acetate in lactose for 9 hours, glucose for 20 minutes, and lactose for 10 minutes. These observations have shown that, in many cases, volatile substances were to some extent very tightly bound to the solids. Inclusion may have been responsible for this binding.

PROCEDURE

Standardization of the Cream

Fresh whipping cream, standardized with skimmilk to 30.5 per cent milkfat, was selected as the model system for the addition of specific carbonyl compounds. Both dairy products were procured from a local dairy.

Preparation of the Carbonyl Stock Solution

The volatile organic compounds employed in these experiments were chosen for their volatility, their frequency in lipid materials and their suitability to the analyses.

Methyl ketones (acetone, 2-pentanone, 2-heptanone, 2-nonanone, 2-undecanone and 2-tridecanone) of 99.5 per cent purity were obtained from Lachat Chemicals, Inc., Chicago, Illinois.

The Aldrich Chemical Co., Inc., Milwaukee, Wisconsin supplied the butanal. Other aldehydes (pentanal, hexanal, heptanal, octanal, nonanal and decanal) were received from K and K Laboratories, Inc., Plainview, New York. The unsaturated aldehyde, 2-nonen-1-al, was also obtained from K and K Laboratories, Inc. The aldehydes were of the highest purity commercially supplied.

To minimize the loss of volatiles and to facilitate recovery, concentrated carbonyl-oil solutions were prepared. Because it is a solid at room temperature, 2-tridecanone was weighed into a small amount of corn oil in a 100 ml Erlenmeyer flask. The flask, containing the organic volatile and oil, was tightly sealed with a glass stopper, heated carefully in a water bath (15 min at 160°F) and cooled. After the 2-tridecanone-oil solution had been thoroughly chilled (18 hr), to insure that the ketone had been thoroughly dispersed, the solution was warmed to room temperature and the remaining carbonyl compounds (at room temperature) were added. When all the organic volatiles had been weighed, sufficient oil was added to give a stock solution of the desired concentration. The carbonyl-oil solution was placed in an ice-water bath and stirred thoroughly with a magnetic stirrer.

Preparation of the Samples for the Retention of Volatiles

An aliquot of the volatile stock solution was pipetted into approximately 200 g of cream in a 3 l flask. The remaining cream was subsequently added to the flask making a total of 2,300 g of carbonyl-cream mixture. Following the careful introduction of a large magnetic stirrer, the flask was tightly stoppered, placed in an ice-water bath and stirred thoroughly for 40 minutes.

An appropriate quantity of the carbonyl-cream solution, for the control sample, was placed in a small glass jar, tightly sealed and refrigerated at 40°F. Immediately after sample preparation for all of the experiments was completed, analysis of the control commenced.

The typical concentration of monocarbonyls obtained from analysis of the control is given in Table 1.

For the frozen storage studies, 10 g samples of the carbonyl-cream solution were weighed into coded glass vials. Each vial was tightly sealed with an air-tight stopper and promptly frozen at -20°F. The samples were stored at this temperature for analysis after intervals of 3 and 6 months.

Samples, consisting of 500 g of the carbonyl-cream mixture, were weighed into prepared aluminum trays, with dimensions of 11 1/4 x 7 1/2 x 1 1/2 inches, for the freeze-drying experiments. Prior to weighing the samples, an iron constantan thermocouple had been positioned on the bottom, inside each of the coded pans. To insure the removal of any air pockets that may have formed in the cream during weighing, the pans were tapped gently. Each tray was tightly covered with a film of plastic, sealed with freezer tape and frozen immediately at -20°F. The samples were held at this same temperature for a period of time demanded by the nature of the freeze-drying experiments.

A Virtis RePP #FFD 42 WS freeze-dryer, with a capacity of 50 lbs of water removal per drying run, was employed in these studies. The freeze-dryer was equipped with instrumentation for the control of freeze-drying variables.

Table 1. Typical concentration[a] of methyl ketones, saturated aldehydes and 2-enal present in control samples.

n-Methyl Ketones	
Acetone	22.5
2-Pentanone	103.2
2-Heptanone	610.4
2-Nonanone	774.6
2-Undecanone	171.2
2-Tridecanone	130.0
Saturated Alkanals	
Butanal	21.8
Pentanal	25.9
Hexanal	78.7
Heptanal	160.9
Octanal	137.3
Nonanal	111.8
Decanal	100.6
2-Enal	
2-Nonen-1-al	67.1

[a]Calculated as μg/10 g of fresh cream (30.5 per cent milkfat)

The temperature of the heating platens was adjustable for the range between 60 to 250^{o}F. An 8-point recording potentiometer (Honeywell Electronic 15 Multipoint Strip Chart Recorder) and a system of 8 thermocouple connections, for temperature measurements in the product, or at selected points within the dryer, facilitated a record of temperature during lyophilization. The vacuum within the drying chamber was adjustable. The thermocouple vacuum gauge, connected to a strip chart recorder (Speedomax W) monitored changes in pressure during the drying cycle. Additional pressure measurements were obtained with a McLeod gauge. For the determination of the rate of drying, a weight system connected to a recorder (Speedomax W) was available.

In each of the experiments, the randomly selected frozen sample was placed in a similar location on the same platen. After the product had cooled to -50^{o}F, freeze-drying at the required temperature, and at an absolute pressure of 10^{-3} Torr was initiated. Temperatures of the product, the platens and points within the dryer were recorded throughout lyophilization. The freeze-drying cycle was terminated when the sample temperature was within 10 to 15^{o}F of the platen temperature, unless the experimental design advised otherwise. The platen temperature programs employed in these experiments appear in Table 2. The freeze-dried powder was removed from the tray, screened, put into a small brown glass jar, tightly sealed and stored at -10^{o}F. To guard against autoxidation and a

Table 2. The platen temperature programs employed in the freeze-drying experiments.

Sample Number	Platen Temperature[a] (°F)	Sample Temperature[b] (°F)	Drying Time (hours)
1	80	65	19.6
2	80	63	19.6
3	80	80	24.0
4	100	80	14.5
5	120	85	13.0
6	120	85	15.0
7	160	100	9.2
8	160	115	9.0

[a]Temperature of the control platen

[b]Temperature at the end of drying

corresponding increase in the carbonyl content of the samples, the periods of frozen storage before and after freeze-drying were scheduled so that none of the dried powders was held in excess of three weeks.

<u>Preparation of Cream for Absorption Studies</u>

Fresh whipping cream was standardized with skimmilk to a 30 per cent milkfat content. Thermocouples were positioned on the bottom, inside each of three stainless steel trays with dimensions of 18 1/4 x 23 x 1 inches. A 4-lb and two 8-lb portions of cream were weighed into the stainless steel trays. Air bubbles in the cream were removed.

The trays were transferred to the freeze-dryer. The tray containing 4 lbs of cream was positioned so that the rate of drying could be measured. After the cream had reached a temperature of $-50^{o}F$, freeze-drying at a platen temperature of $100^{o}F$ and at an absolute pressure of 10^{-3} Torr was begun. During lyophilization, temperatures of the cream, the platens and locations within the dryer were monitored. Freeze-drying was discontinued when the product temperature was within $10^{o}F$ of the platen temperature and the rate of drying remained constant. The freeze-dried cream was removed from the trays, screened, and placed in brown glass jars, tightly sealed and stored at $-10^{o}F$.

Absorption of Organic Volatiles on Freeze-Dried Cream

A glass vacuum desiccator served as the absorption chamber. During the absorption studies, the equilibrated absorption chamber was maintained at a temperature of $68 \pm 1^{\circ}F$ by placing it in a constant temperature unit.

Prior to each absorption experiment, the freeze-dried cream and the organic volatile(s) were removed from frozen storage and allowed to come to room temperature. Fifty grams of sieved (U.S. Standard #12 sieve) freeze-dried cream were weighed and evenly distributed onto a large watch glass. For the absorption of a single ketone, 1 g was weighed into a small glass container. However, in experiments with a mixture of volatiles, 0.5 g of a single ketone was individually placed in a 5 ml beaker and covered with a glass stopper until all of the carbonyls had been weighed. To further minimize the loss of volatiles during the weighing interval, a mixture of ketones was weighed in order of increasing volatility.

When weighing was completed, the volatile(s) was transferred to the base of the desiccator, and the watch glass of freeze-dried cream was put on the desiccator plate as shown in Figure 1. The sealed absorption chamber was maintained at a constant temperature for 15, 30 or 60 minutes. Following absorption, the sample was removed, spooned into a brown jar, tightly sealed and mixed well. The cream was allowed to equilibrate at $40^{\circ}F$ for 24 hr before analysis.

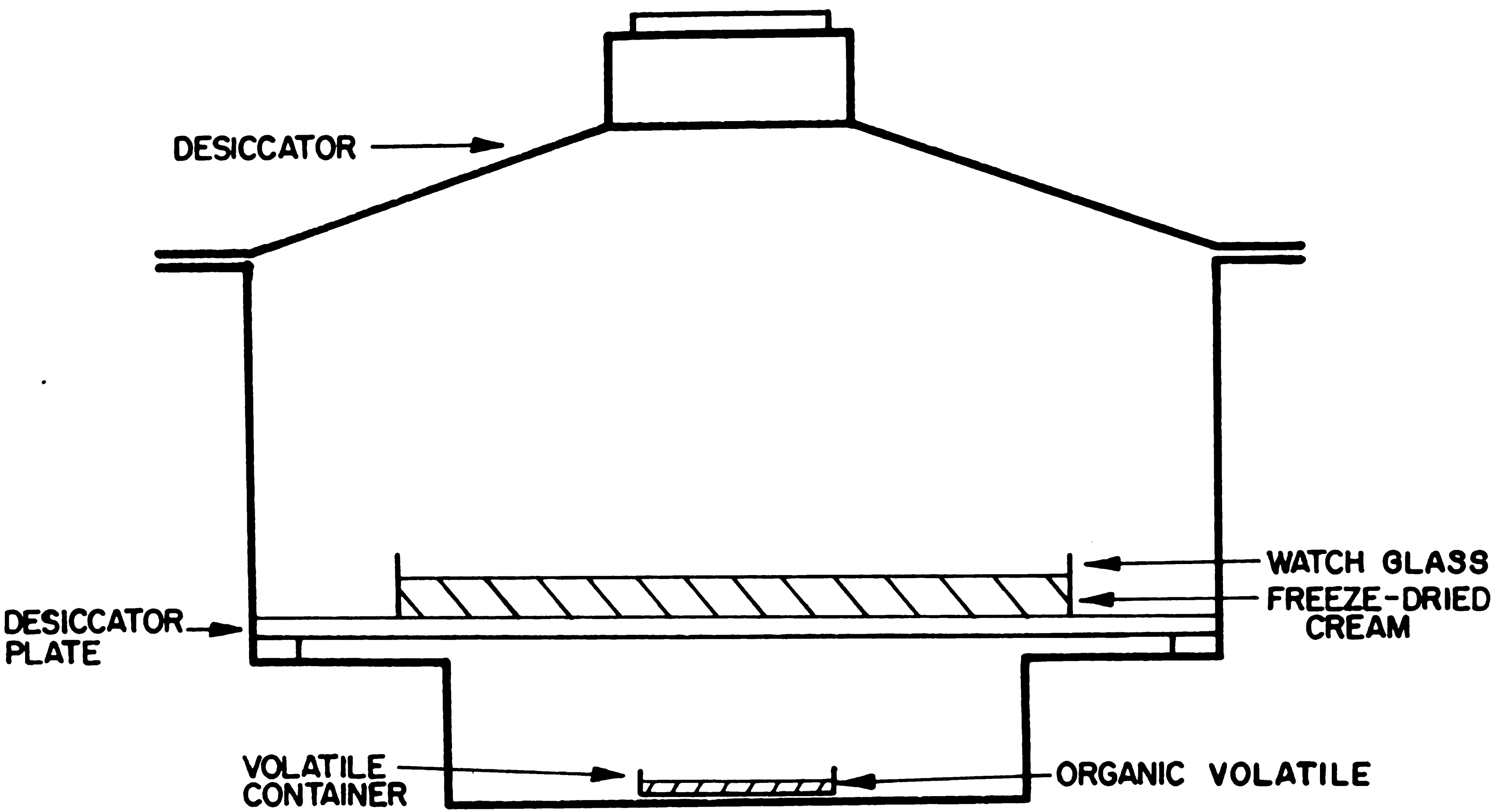

Figure 1. Schematic of absorption chamber.

<u>Retention of Volatiles Absorbed by Freeze-Dried Cream</u>

Samples obtained from the 1 hr absorption of individual ketones on freeze-dried cream were held at -10°F. The retention of each volatile was determined after 1 and 2 months storage.

Chemical Analyses

<u>Fat</u>

The official Babcock procedure (AOAC, 1965) was employed for determination of fat content of the bulk cream and the standardized cream.

<u>Moisture</u>

The percentage of moisture in liquid cream samples was determined by the method of the Association of Official Agricultural Chemists (AOAC, 1965) established for dairy products. The moisture content of freeze-dried cream samples was obtained by employing the Karl Fischer method as recommended by the American Dry Milk Institute, Inc., Bulletin 911 (1954).

<u>Quantitation of Monocarbonyls</u>

<u>Solvent Purification</u>

<u>Carbonyl-free Hexane</u>. High purity n-hexane (Phillips Petroleum Co.) was treated for the removal of carbonyls by the methods of Hornstein and Crowe (1962) and Schwartz and Parks (1961). Re-distilled hexane was passed over a column prepared

from Celite impregnated with concentrated sulfuric acid, and then through a reaction column consisting of Celite impregnated with 2,4-dinitrophenylhydrazine, phosphoric acid and water. To precipitate the colored impurities, the hexane was slurried with Sea Sorb 43 (iodine number 80) and filtered as suggested by Blakely (1970).

Carbonyl-free Benzene. Reagent grade benzene was refluxed with 1 gm of 2,4-dinitrophenylhydrazine per 500 ml of solvent and then re-distilled.

Nitromethane. Nitromethane (Fishers highest purity) was re-distilled over boric acid as recommended by Anderson (1966).

Other Solvents. Other solvents, including high purity hexane, benzene, methanol, chloroform and acetonitrile were re-distilled prior to use.

Preparation of 2,4-Dinitrophenylhydrazone Derivatives

Standards of the 2,4-dinitrophenylhydrazone derivatives of the methyl ketones, aldehydes and 2-enal employed in this research were prepared according to the procedures outlined by Shriner, Fuson and Curtin (1967). The 2,4-dinitrophenylhydrazine (Eastman Organic Chemicals) was purified as recommended by Vogel (1962).

Isolation of Carbonyl Compounds from Fresh, Frozen and Freeze-Dried Cream

Extraction of Fat from the Sample

The quantitative procedure for the isolation of monocarbonyl compounds was similar to that described for fats and oils (Schwartz, Haller and Keeney, 1963) and adapted to the analyses of Blue cheese by Anderson (1966). Fifteen grams of Celite 545 and 10 g of cream were thoroughly ground in a mortar and pestle. The damp mixture was packed into a 2 cm ID chromatographic column fitted with a coarse fritted glass filter plate at the bottom. The fat was extracted from the cream-Celite 545 mixture by allowing 200 ml of carbonyl-free hexane to pass through the column.

Formation of 2,4-Dinitrophenylhydrazones

The hexane-fat extract was passed through a 10 g reaction column (Schwartz and Parks, 1961), consisting of analytical grade Celite homogeneously impregnated with 2,4-dinitrophenylhydrazine, phosphoric acid and water, to convert all the monocarbonyl compounds in the fat quantitatively into their 2,4-DNP-hydrazones. When the last of the extract had entered the column, the column sides were washed down with carbonyl-free hexane. The column was flushed with carbonyl-free hexane until the effluent had the same spectral properties as that of the carbonyl-free hexane which had passed through the column prior to sample addition. Usually this required the collection of a 500 ml sample. The total concentration of monocarbonyl DNP-hydrazone was estimated by determining the absorbancy of the solution compared to carbonyl-free hexane at 340 nanometers (nm) with a Beckman DU Spectrophotometer. When the column had been washed sufficiently, the hexane was evaporated over steam with nitrogen.

Removal of Fat from the Dinitrophenylhydrazones

The lipid material was removed from the hydrazones by employing the procedure of Schwartz, Haller and Keeney (1963) as modified by Anderson (1966). Fourteen grams of Sea Sorb 43 (iodine number 80) and 28 g of Celite 545 (dried 24 hrs at 150°C) were slurried in hexane and poured into a 2.8 cm ID chromatographic column containing a coarse fritted glass disc. After the slurry was packed with moderate nitrogen pressure (3-5 psi), the fat-hydrazone mixture was dissolved in 5 ml of hexane and applied to the column. The fat was flushed from the column by the successive additions of 200 ml of hexane, 100 ml of a hexane-benzene mixture (1:1 v/v) and 200 ml of benzene. Elution of the hydrazones from the column was effected with 175 ml of a chloroform-nitromethane mixture (3:1 v/v). The chloroform-nitromethane solvent was removed from the eluent by evaporation on a steam bath with nitrogen.

Fractionation of Derivatives on Weak Alumina

An alumina column (Schwartz and Parks, 1961) was employed for the removal of decomposition products and 2,4-DNP-hydrazones of ketoglycerides. Five grams of partially deactivated alumina (Chromatographic Alumina F-20) were packed into a chromatographic column (1 cm ID) containing hexane and plugged with glass wool at the bottom. The residue from the preceding step was dissolved in hexane and applied to the column. The monocarbonyl fraction was eluted with a 50 ml mixture of benzene-hexane (1:1 v/v). Evaporation on a steam bath with nitrogen resulted in solvent removal.

Separation of Monocarbonyl Fraction into Classes

The 2,4-DNP-hydrazone derivatives of the aliphatic monocarbonyls were separated into classes using a modification of the method of Boyd, Keeney and Patton (1965). Sea Sorb 43 and Celite 545 were employed in a 1:2 ratio rather than the 1:1 ratio reported. This procedure reduced the destruction of DNP-hydrazones from freeze-dried samples. The solvent system of Boyd, Keeney and Patton (1965) as modified by Blakely (1970) was employed. Using nitrogen pressure (1-3 psi), a hexane slurry of 7.5 g of Sea Sorb 43 (iodine number 80) and 15 g of Celite 545 (dried 24 hrs at 150^{o}C) was packed into a 1.8 cm ID chromatographic column containing a coarse fritted glass disc. Because of the substantial reduction in the quantity of derivatives obtained from freeze-dried samples, the amount of column packing employed in the preparation of columns for those analyses was 5 g Sea Sorb 43 and 10 g Celite 545. The hydrazone derivatives were dissolved in hexane (about 2 ml) and carefully applied to the column. Separation of the three classes of 2,4-DNP-hydrazones was effected by employing the following sequence of solvents: 50-ml quantities of 15, 25, 40, 60 and 80 per cent chloroform in hexane, 150 ml of chloroform, 50-ml quantities of 2, 4, 6, 8 and 10 per cent methanol in chloroform, and finally, 50 ml of 25 per cent nitromethane in chloroform. A slight variation in the retention volume for each of the classes was observed due to the variation in carbonyl content among samples. Ketone elution was usually complete following the addition of 4 per cent methanol in chloroform. The saturated aldehydes and enal were eluted with the higher concentrations of methanol in chloroform.

Class separation was monitored at 254 nm with an ultraviolet liquid flow analyzer (Model UA-2, Instrumentation Specialties Company, Inc., Lincoln, Nebraska). The solution which constituted each peak was collected separately and evaporated to dryness. Classes were identified by determining the absorption maximum of the fractions in chloroform with a spectrophotometer (Ratio Recording Beckman DK-2A). The maxima employed for the three classes in chloroform were: methyl ketones, 363; saturated aldehydes, 358; 2-enal, 374 as given by Day (1965). In a few instances where the absorption maximum of a ketone peak was lowered by the presence of an anomolous hydrazone, the fraction was pooled with the ketone class and separated in the subsequent step of the procedure.

Where they were required, time studies of the deterioration of color formed by carbonyl derivatives in alcoholic base (Jones, Holmes and Seligman, 1956) were employed to classify the parent carbonyl compound. Class authenticity was also verified by studying the infrared absorption spectra of derivatives in chloroform with a Beckman Infrared Spectrophotometer 12.

Separation of Ketone and Aldehyde Derivatives into Individual Chain Lengths

Liquid-liquid partition columns (acetonitrile) of Corbin, Schwartz and Keeney (1960) were utilized to separate both the methyl ketone hydrazone and aldehyde hydrazone classes into their individual members. Twenty-five grams of analytical grade Celite (activated at 160°C for 24 hrs) were employed to pack each chromatographic column (2.8 cm ID). Acetonitrile served as the stationary phase on the Celite column and hexane saturated with acetonitrile was the mobile phase. Elution of the derivatives of individual members of ketones and aldehydes required about 2,000 ml of the mobile phase. The column eluate was monitored at 254 nm with an ultraviolet liquid flow analyzer (ISCO Model UA-2).

Determination of Individual Methyl Ketone, Aldehyde and Alk-2-enal Concentrations

The concentrations of C_3, C_5, C_7, C_9, C_{11} and C_{13} methyl ketones were determined by measuring the absorbance of their 2,4-DNP-hydrazone derivatives in chloroform with a spectrophotometer (Beckman DU) at 363 nm. The chain length of each member was tentatively assigned by its retention volume on the partition column and by determining the absorption maxima of the fractions in chloroform on a Ratio Recording Spectrophotometer (Beckman DK-2A). The thin-layer chromatographic technique of Edwards (1966) with the modifications recommended by Blakely (1970) was employed to confirm the chain length of each ketone.

The concentrations of C_4, C_5, C_6, C_7, C_8, C_9 and C_{10} alkanal derivatives were ascertained by measuring their absorbance in chloroform with a spectrophotometer (Beckman DU) at 358 nm. Each of the aldehydes was tentatively identified by the procedures outlined for methyl ketones, and was verified by the thin-layer chromatographic technique (Method E) of Schwartz et al. (1968).

The concentration of the non-2-enal (obtained from the separation of monocarbonyls into classes) was obtained by measuring the absorbance of its 2,4-DNP-hydrazone in chloroform at 374 nm (Beckman DU Spectrophotometer). The thin-layer chromatographic technique for aldehydes of Schwartz et al. (1968) facilitated enal confirmation.

All analyses were carried out in duplicate. A quantitative value for each of the methyl ketones, saturated aldehydes and the enal was obtained by applying the appropriate molar extinction coefficient (Day, 1965). During each of the analyses, the recovery of fat, from cream samples for monocarbonyl analyses, was obtained by the concomitant extraction of fat from additional similar samples. The hexane, from the hexane-fat extract, was evaporated on a steam bath with nitrogen, and the fat was quantitatively transferred with petroleum ether into a tared aluminum weighing dish. Following careful evaporation of the ether, the quantity of fat recovered was calculated. The per cent removal of fat was employed in the quantitation of the monocarbonyls.

The percentage recovery of the individual ketones, the aldehydes and the enal was determined by adding a standard mixture of each of the monocarbonyls to fresh cream (30 per cent milkfat). Concentrations of methyl ketones employed were approximately those found in Blue cheese; aldehyde concentrations were typical of those in dairy products. The resulting samples were analyzed as described. Average percentage recovery of each ketone, each alkanal and the enal, as determined by triplicate analyses, was used in the calculation of monocarbonyl concentrations of the cream samples. In addition, the percentage recovery of the individual ketones was determined by passing a standard mixture of the C_3, C_5, C_7, C_9, C_{11} and C_{13} methyl ketones in hexane over a reaction column and following the recovery procedure employed by Anderson (1966) and Blakely (1970). Recoveries similar to those of Blakely (1970) were obtained for each ketone.

Isolation and Determination of Absorbed Methyl Ketones in Freeze-Dried Cream

The absorption of a mixture of ketones on freeze-dried cream was determined by the analytical techniques which have been described.

However, for the absorption of a single ketone, the analyses of samples and blanks of freeze-dried cream were terminated following the separation of the monocarbonyl fraction, from decomposition products and 2,4-DNP-hydrazones of ketoglycerides, on the alumina column (Schwartz and Parks, 1961). The concentration of an individual ketone and that of the blank was determined by measuring the absorbance of its 2,4-DNP-hydrazone derivative in chloroform with a spectrophotometer at 363 nm. By the application of the proper molar extinction coefficient (Day, 1965) a quantitative value for each ketone was obtained. The actual amount of ketone absorbed was found by deducting the blank, computed as the absorbed ketone, from the quantity of absorbed ketone.

Each ketone was tentatively identified by its absorption maximum in chloroform with a Ratio Recording Spectrophotometer (Beckman DK-2A), and confirmed by thin-layer chromatography (Edwards, 1966, as modified by Blakely, 1970). Ketone authenticity was also verified through analysis by a combination of mass spectrometry and gas chromatography.

Mass spectrometric analyses were made with a single focusing, rapid magnetic-scanning mass spectrometer (LKB 9000) coupled through molecule separators with a gas chromatographic system.

Operating conditions were:

Filament emission current, μa	60
Electron energy, ev	70
Accelerating voltage, v	3,500
Separator temperature, °C	250
Ion source temperature, °C	290

A PDP 8/1 Digital Computer was on the line with the mass spectrometer. A print-out of spectra recorded on magnetic tape was obtained.

A 4-foot glass GLC column (2 mm inside diameter) packed with 3 per cent SE-30, GC grade 80/100 mesh (Supelco Inc., Bellefonte, Pa.) was employed in this study. Samples of 5 μl were injected into the gas chromatograph.

Chromatographic conditions were:

Flash heater, °C	150
Detector block temperature, °C	275
Helium flow, cc/min	30
Voltage, v	0.2 or 0.5
Attenuation	1
Column temperature, °C	200 or 250

Identification of the mass spectra of the 2,4-DNP-hydrazones of absorbed ketones was accomplished by comparing the principal mass spectral features of 2,4-DNP-hydrazones of ketone standards and by noting the molecular ion peaks suggested by Kleipool and Heins (1964).

RESULTS AND DISCUSSION

Addition of Monocarbonyls to Cream

An investigation of the retention of added volatiles in cream during frozen storage and freeze-drying required that an appropriate technique for the incorporation of the monocarbonyls into the cream be considered. Numerous experiments were conducted to insure that the cream could be reproducibly enriched with monocarbonyls and that the level of organic volatiles was appropriate to the methods of analyses. The average percentage of individual members of each class of carbonyls in the stock solution that was incorporated into the cream of three typical experiments appears in Table 3. The solubility of both the methyl ketones and the aldehydes in the medium appears to affect the concentration of their individual members in the cream. In both classes of compounds, the higher molecular weight, water insoluble members were retained in similar, greater amounts; the recovery of short chain hydrophilic components is lower. These more polar substances may bond to aqueous solvent molecules and thus are not readily extracted.

Allen and Parks (1969) determined the recovery of methyl ketones added to milkfat which was homogenized with skimmilk.

Table 3. Recovery of monocarbonyls added to cream.

Monocarbonyls[a]	Average[b] per cent[c] recovery
Methyl ketones	
2-Tridecanone	77.6
2-Undecanone	86.4
2-Nonanone	64.0
2-Heptanone	50.9
2-Pentanone	18.3
Acetone	10.0
Aldehydes	
Decanal	55.2
Nonanal	69.3
Octanal	56.5
Heptanal	34.8
Hexanal	27.0
Pentanal	15.1
Butanal	14.1
2-Enal	
2-Nonen-1-al	67.3

[a]Calculated as μg/10 g cream (30 per cent milkfat)
[b]Triplicate analyses
[c]Determined as: $\frac{\mu g \text{ recovered}}{\mu g \text{ added}} \times 100$

Their recovery values based on the actual amount of 2-pentadecanone, 2-tridecanone and 2-undecanone added to recombined whole milk were lower than those reported here (2-pentadecanone, 84.5 per cent; 2-tridecanone, 62.5 per cent; and 2-undecanone, 30.3 per cent). However, a lowering in the retention of organic volatiles with decreasing chain length was observed. When the recovery data were based on the micromoles of methyl ketones per gram of fat recovered from the product, higher values were obtained. Though the recovery of 2-pentadecanone exceeded 100 per cent, the values obtained for 2-tridecanone and 2-undecanone were lower than those found for the corresponding volatiles in these experiments. According to Allen and Parks (1969), these higher results suggest either a portion of the methyl ketone exists in the cream plug independent of the fat phase, making them readily available to extraction procedures, or are extracted from the residual fat-protein complex in a manner similar to that demonstrated by Patton (1961). Furthermore, the authors (Allen and Parks, 1969) indicated that recoveries based on the actual amount recovered relative to the quantities added to the product are more accurate when applied to a fluid product.

For their investigation of the methyl ketones in stored sterilized concentrated milk, Arnold and Lindsay (1969) obtained the percentage recovery of the individual ketones by the addition of a standard mixture of C_3, C_5, C_7, C_9 and C_{11} alkanones to steam-stripped samples of fresh concentrate.

Although the recovery of acetone was lower than that of 2-pentanone, a linear relationship with decreasing chain length was not reported. The average percentage recovery of higher molecular weight homologs was 45 per cent.

The average percentage recovery (Table 3) was employed in the calculation of carbonyl concentrations in freeze-dried cream samples where required. The data were reported, in duplicate analyses, as μg of specific carbonyl/10 g of fresh cream (30.5 per cent milkfat). They are usually shown as the average per cent recovered based on the initial carbonyl concentration determined in the control. The average per cent deviation of duplicates from their mean was: 2-tridecanone, 5.9; 2-undecanone, 4.8; 2-nonanone, 6.0; 2-heptanone, 3.6; 2-pentanone, 8.3; acetone, 7.9; decanal, 5.3; nonanal, 8.7; octanal, 6.2; heptanal, 7.3; hexanal, 6.5; pentanal, 8.8; butanal, 9.6; and 2-nonen-1-al, 5.1.

The Retention of Monocarbonyls during Frozen Storage

Since it is not always possible to freeze-dry samples immediately after their preparation, experiments were conducted to determine the effect of frozen storage on the monocarbonyls added to cream. At fat levels of 25 per cent or greater, cream freezes homogeneously (Trelogan and Combs, 1934). Thus, this kind of study seemed feasible. Ten gram samples of carbonyl-enriched cream, placed in tightly sealed glass vials, were held at -20°F for periods of 3 and 6 months.

Table 4 summarizes the average per cent recovery of added methyl ketones following frozen storage. In general, the results appear to indicate that the 2-alkanone content of cream samples after both intervals of freezer storage is similar. The data show that the losses of the higher molecular weight, less volatile compounds are only slight. Apparently the binding or absorption of these ketones with constituents of the cream retards their volatilization. The findings obtained for 2-pentanone do not indicate a definite trend. The concentration of acetone and 2-pentanone added to the cream (Table 1) was lower than that of the higher molecular weight homologs. As a result, small changes in the recovery of acetone and 2-pentanone have a more pronounced effect on percentage recovery.

Hrdlicka, Vit and Janicek (1970) studied the effect of various freezing procedures on the changes of volatile organic constituents, including acetone, in apricots. The fresh fruit was frozen in a tunnel at -30°C, or by immersion in liquid nitrogen to -30 or -60°C, and then stored at -18°C for 6 months. A marked loss of flavor volatiles from stored samples frozen in the freezing tunnel was observed.

The loss of acidic carbonyl compounds during the frozen storage of cheese was observed (Harper and Kristoffersen, 1958). After 3 months of frozen storage (-10°F) analyses of samples of Romano cheese that originally contained the β-keto acids, α-acetolactic acid, oxalacetic acid, and acetoacetic

Table 4. Average per cent recovery[a] of added methyl ketones[b] from cream[c] held in frozen storage.

Methyl ketone chain length	Frozen storage at $-20^{o}F$			
	First trial		Second trial	
	3 months	6 months	3 months	6 months
13	93.5	95.4	99.8	97.1
11	100.8	96.4	99.7	103.5
9	96.2	95.6	96.0	98.9
7	96.1	99.9	101.7	94.0
5	70.1	102.5	108.6	85.4
3	d	76.9	83.6	86.0

[a]Based on concentration of control

[b]Calculated as μg/10 g fresh cream (30.5 per cent milkfat)

[c]Average of duplicate analyses

[d]Not determined

acid, did not reveal the presence of these acidic carbonyl compounds. The nature of the chemical change in the β-keto acids was not determined. However, an increase in the neutral carbonyl compounds in the cheese, coupled with the known instability of the carbonyl group in β-keto acids suggested a decarboxylation reaction. In Cheddar cheese there appeared to be some modification in flavor which may be associated with changes in the β-keto acids. Although no marked alteration in flavors could be observed in Romano cheese, subtle changes may have occurred.

Initial observations on Blue cheeses stored at $-10^{\circ}F$ for 1 year indicated the complete absence of the β-keto acids normally present in these cheeses (Harper and Kristoffersen, 1958). In Blue cheese, methyl ketone formation has occurred (via β-keto acids) as a result of β-oxidation of fatty acids by the spores of P. roqueforti during ripening (Gehrig and Knight, 1963). The storage of spore suspensions with sodium octanoate (as a substrate) at $-5^{\circ}C$ resulted in almost two times the amount of 2-heptanone formed at $4^{\circ}C$ (Gehrig and Knight, 1963). The authors suggested that this phenomenon may have occurred due to a permeability factor or because the spores may be better protected in the frozen state than at normal refrigeration temperatures. Spores are extremely durable in their resistance to autolysis. Furthermore, spores stored at $4^{\circ}C$ for 3 hrs produced 2-heptanone when sodium octanoate was the substrate.

The keto acid components of keto glycerides form about 0.3 to 0.5 per cent of the component acids of milkfat (Webb and Johnson, 1965). The greater part of the keto acids were shown to consist of a series of isomeric keto stearic acids (Keeney, Katz and Schwartz, 1962). Boldingh and Taylor (1962) indicated the presence of glyceride bound β-keto acids in milkfat and postulated that they were the precursors of the 2-alkanones. Ketone concentrations were increased with the application of heat (Patton and Tharp, 1959). Investigating the normal flavor of raw milk and cream, Wong and Patton (1952) isolated a homologous series of methyl ketones from 3 to 7 carbon atoms. They suggested that the spontaneous decarboxylation of β-keto acids, which are intermediates in the β-oxidation of fatty acids may have accounted for the ketone formation and also for the fact that β-keto acids have never been identified in milk. In view of these findings, an increase in the methyl ketone constituents in these experiments (Table 4) would seem to be unlikely.

The average percentage recovery of saturated aldehydes from cream following frozen storage is given in Table 5. Aldehyde concentrations in samples after both intervals of freezer storage appear to be analogous. The data indicate a slight reduction in the initial quantity of both decanal and octanal. However, the concentration of each of the remaining aldehydes is similar to that determined for the control. It is interesting to note that the retention of the higher

Table 5. Average per cent recovery[a] of added saturated aldehydes[b] from cream[c] held in frozen storage.

Aldehyde chain length	Frozen storage at -20°F First trial 3 months	6 months	Second trial 3 months	6 months
10			86.1	82.4
8	90.9	90.7	92.7	84.1
7	103.8	104.3		
6	97.2	101.4	104.1	94.9
5	99.8	102.1		
4	92.6	97.6	101.2	96.8

[a]Based on concentration of control

[b]Calculated as µg/10 g fresh cream (30.5 per cent milkfat)

[c]Average of duplicate analyses

molecular weight alkanals (decanal and octanal) is lower than that obtained for 2-nonanone and 2-undecanone. Because of their very potent odor, the aldehydes were added to the cream at minimal levels which still permitted their analyses. Thus, the initial concentration of both decanal and octanal was low.

Apricots, held at -18°C for 6 months, following tunnel freezing at -30°C, showed a loss of volatile carbonyl compounds (Hrdlicka, Vit and Janicek, 1970). Aldehydes with 1 to 11 atoms of carbon were among these volatile constituents.

The loss of 2-nonen-1-al during freezer storage (Table 6) resembles that found for the higher molecular weight saturated aldehydes of the second trial (Table 5). The reduction in the concentration of the 2-enal between the two intervals of freezer storage is within the limit of experimental error. Since unsaturated aldehydes are known to oxidize readily, one might account for the loss of 2-nonen-1-al in this way. However, if this were so, heptanal should have been observed in the samples of the second trial (Table 5). There should also have been a corresponding increase in the level of heptanal in the samples of the first trial. Although there appears to have been a slight increase in the heptanal concentration of the first study, heptanal was not detected in the samples of the second experiment. Perhaps the relatively low initial concentration of 2-nonen-1-al has accented the loss.

Table 6. Average per cent recovery[a] of added 2-enal[b] from cream[c] held in frozen storage.

Storage	2-Nonen-1-al First trial	2-Nonen-1-al Second trial
After 3 months	84.5	88.3
After 6 months	77.8	84.4

[a]Based on concentration of control
[b]Calculated as μg/10 g fresh cream (30.5 per cent milkfat)
[c]Average of duplicate analyses

The Retention of Monocarbonyls during Freeze-Drying

Time-temperature profiles for three respective temperature programs are illustrated in Figure 2. These time-temperature relationships resemble those obtained by Radanovics (1969) for lyophilized acidified cream. Similar freeze-drying curves were obtained during the dehydration of shrimp and salmon steaks (Goldblith et al., 1963; Lusk et al., 1965).

The temperature of the platen on which the sample was placed was approximately 20°F lower than that of the control platen and did not approach the temperature of the control platen until the final stages of the drying cycle (Figure 2, 80°F platen). In his experiments, Radanovics (1969) found that the sample platen and control platen temperatures were similar during the last half hour of the drying cycle.

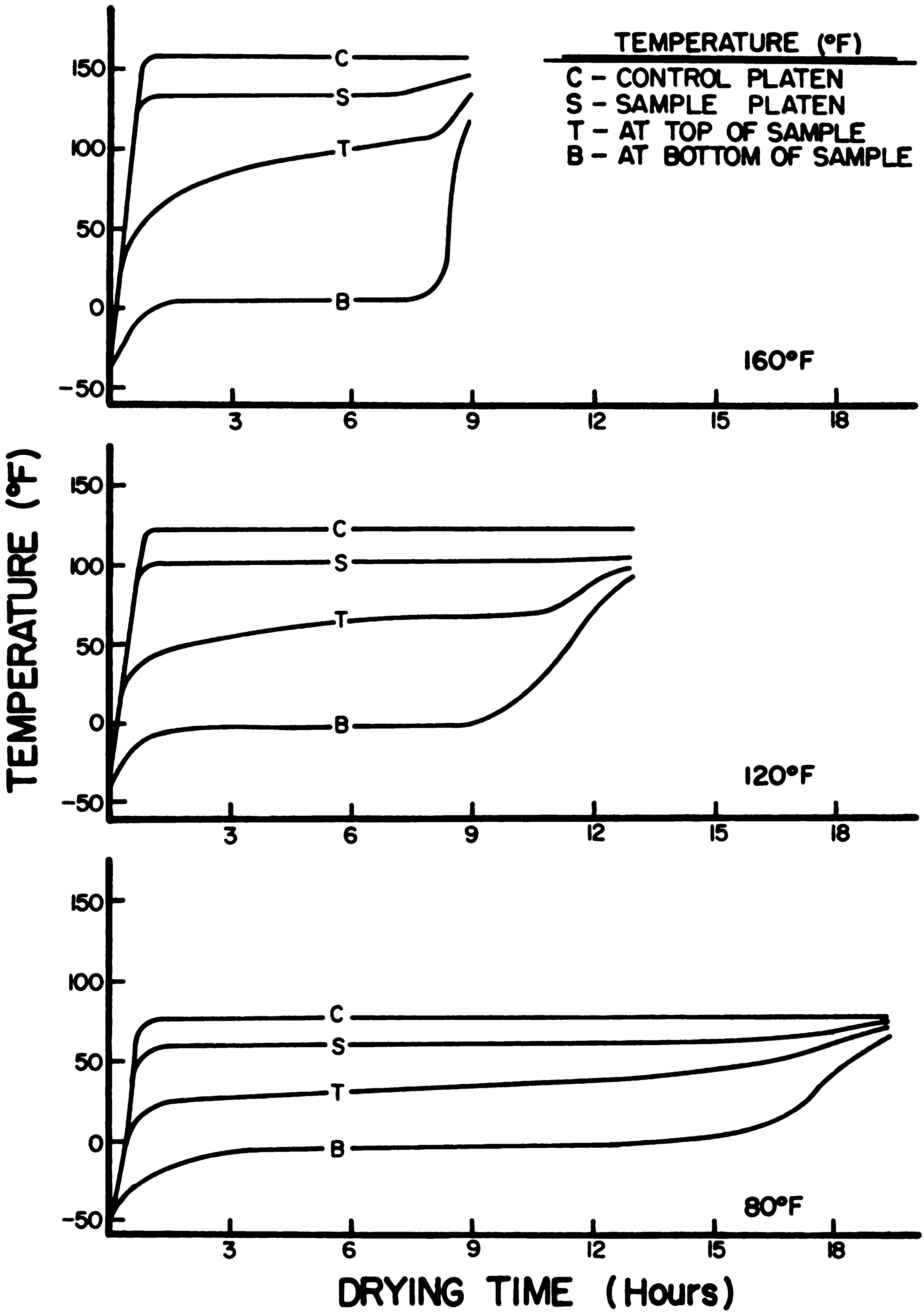

Figure 2. Time-temperature profiles of carbonyl-enriched cream at various platen temperatures.

Radanovics (1969) attributed this lower temperature, measured at the interface of the surface of the platen and sample trays, to several factors:

"1. The constant BTU requirement for the latent heat of sublimation,

2. The cooling effect of the sample, which had a significantly lower temperature averaging about -20^{o}F,

3. The inadequate heat transfer from the heating fluid, circulating at the bottom of the platen, to the surface of the platen."

Temperatures for the product, measured at the bottom and surface during the freeze-drying cycle, appear in Figure 2. Lyophilization at a temperature of 80^{o}F was terminated when the temperature of the sample at the bottom was within 10^{o}F of the sample platen temperature. This "endpoint" was employed on the basis of the findings of Radanovics (1969). At this stage of the drying cycle all the ice in the product was sublimed and the moisture content of the cream had equilibrated at 1-1.5 per cent. Since the loss of monocarbonyls during freeze-drying was substantial, temperature programs at platens of 100, 120 and 160^{o}F were terminated when the temperature of the sample at the bottom was 80, 85 and 100^{o}F, respectively, and on the basis of a similar total drying time (Table 2, p. 41).

During the initial stages of drying the surface temperature of the product was greater than the mean product temperature, and increased rapidly as freeze-drying progressed. Although this difference may be observed at all platen temperatures, it is more significant at elevated platen temperatures. During one of the temperature programs at 160°F (Table 2), the mean surface temperature of the product exceeded 100°F near the end of the drying cycle (Figure 2). Similar findings were obtained for lyophilized acidified cream as platen temperatures were raised (Radanovics, 1969). Although the surface temperature was higher than the mean product temperature, it did not begin to approach the temperature of the sample platen until the later stages of the drying cycle. The subliming water vapors diffusing through the already freeze-dried portion of the food exerted a cooling effect on the dried food.

As the temperature programs were raised, an increase in the mean product temperature was observed. However, the eutectic temperature of the produce was never attained. Near the completion of each of the drying cycles, the mean product temperature began to increase quite rapidly. This rise in temperature became more pronounced as higher platen temperatures were employed. These findings also are in agreement with those of Radanovics (1969).

Total freeze-drying time was reduced as temperature programs were elevated. While 19.6 hours were required for lyophilization at 80°F, the drying time was reduced to about 9 hours at a platen temperature of 160°F. The freeze-drying time for one of the samples dried at 120°F was slightly greater than that which would be expected (Table 2, p. 41).

Perhaps this may be due to poorer contact between the platen and the sample (Zamzow and Marshall, 1952). The reduction in total lyophilization time that occurs as platen temperature is elevated, is well substantiated (Zamzow and Marshall, 1952; Harper and Tappel, 1957; Goldblith _et_ _al_., 1963; Radanovics, 1969). As a result, the rate of freeze-dehydration can be predicted and controlled depending on the platen temperatures employed. Since the undesirable changes that occur during dehydration may be accelerated at elevated platen temperatures, the temperature programs selected for freeze-drying should maximize process efficiency and optimum product quality.

The drying time data (Table 2) for carbonyl-enriched cream samples obtained in these experiments (Table 2, p. 41) closely resemble those reported by Radanovics (1969) for acidified cream samples of a similar size and lyophilized at comparable platen temperatures. Saravacos (1965) observed that the drying rate depended on the nature of the product. Under identical conditions, total freeze-dehydration time for simple food gels varied from 4.3 hours for cellulose gum to 11.5 hours for egg albumen.

The average per cent recovery of added methyl ketones from freeze-dried cream as a function of platen temperature is shown in Table 7. Generally, the retention of ketones decreases as platen temperatures are elevated. This trend is particularly evident for the ketones of the first trial and for the higher molecular weight volatiles with 7, 9, 11 and 13 atoms of carbon in the second study at platen temperatures of 100, 120 and 160°F. Losses of 2-pentanone and acetone are variable in the second trial. Saravacos and Moyer (1968a) noted that the losses of volatile esters during lyophilization were greater when the final product temperature was increased. The loss of diacetyl appeared to be a linear function of platen temperature for lyophilization between 75 to 225°F (Radanovics, 1969). In experiments reported in this thesis dehydration was terminated at an earlier stage of the drying cycle, when elevated temperature programs were employed (Table 2). Thus, a linear relationship cannot be determined readily for these data (Table 7).

Although the retention of both 2-undecanone and 2-tridecanone is greater in the second experiment than in the first, the loss of organic volatiles is reduced when high drying temperatures are avoided. Usually, the retention of 2-tridecanone is greater than that of 2-undecanone. At all temperature programs, the recovery of these high molecular weight ketones is significantly greater than that of the shorter chain homologs. While they are somewhat similar, the concentration of 2-heptanone is lower than that of 2-nonanone in both experiments. The retention of the methyl ketones with 5 carbon atoms exceeds that of 2-heptanone and resembles that of 2-nonanone in the first trial; 2-pentanone increases beyond the levels of 2-heptanone and 2-nonanone in the second

Table 7. Average per cent recovery[a] of added methyl ketones[b] from cream[c] freeze-dried at various platen temperatures.

Methyl ketone chain length	First trial Platen temperature (°F) 80	120	160	Second trial Platen temperature (°F) 100	120	160
13	31.5	24.0	24.0	54.2	52.0	28.5
11	36.4	27.6	23.0	41.6	37.6	18.4
9	5.4	6.2	1.8	5.8	4.2	3.4
7	4.4	1.9	2.0	3.7	3.5	2.7
5	4.6	3.5	1.6	6.2	5.9	6.5
3	5.3	4.3	2.9	3.9	3.2	4.0

[a]Based on concentration of control

[b]Calculated as μg/10 g fresh cream (30.5 per cent milkfat)

[c]Average of duplicate analyses

study. The results obtained for acetone are variable. Acetone losses are generally greater than those of 2-pentanone but they are of lesser magnitude than those of 2-heptanone.

Numerous factors may affect the retention of volatile compounds. Table 8 presents the methyl ketones and their physical characteristics. Generally, as compound volatility is increased, flavor retention in a dried product is decreased (Boudreau et al., 1966; Saravacos, 1968). The loss of flavorful fatty acids, during the spray-drying of butter, was reduced as the volatility of the organic compounds decreased (Boudreau et al., 1966).

During dehydration processes, the loss of a volatile flavor compound in the pure state is directly related to its vapor pressure. However, in an aqueous solution, the volatility of an aroma constituent is dependent upon its vapor pressure and its solubility. Therefore, acetone which is infinitely soluble in water and has a low vapor pressure is least volatile in an aqueous media. 2-Heptanone (very slightly water soluble, high vapor pressure) is extremely volatile; while 2-pentanone is slightly less volatile. The high volatilities of these ketones result from a combination of high vapor pressures and low solubilities in water. 2-Nonanone which is insoluble in water is more volatile. The data (Table 7) for the first trial show that the retention of acetone was superior to that of 2-pentanone. 2-Pentanone was retained in greater amounts

Table 8. Physical characteristics[a] of the methyl ketones.

Methyl ketone	Structure	Molecular Wt.(g/mole)	Solubility	Vapor Pressure[b] (1 Atm.)	Boiling Point (°C)
Acetone	CH_3COCH_3	58.8	infinite	56.5	56.2
2-Pentanone	$CH_3(CH_2)_2COCH_3$	86.1	sl. soluble	103.3	102.0
2-Heptanone	$CH_3(CH_2)_4COCH_3$	114.2	very sl. soluble	150.2	151.5
2-Nonanone	$CH_3(CH_2)_6COCH_3$	142.2	insoluble	195.0	193.0
2-Undecanone	$CH_3(CH_2)_8COCH_3$	170.3	insoluble	224.0	228.0
2-Tridecanone	$CH_3(CH_2)_{10}COCH_3$	198.4	insoluble	262.5	263.0

[a]Compiled from Handbook of Chemistry and Physics, 1968-1969
[b]Taken from Chemical Engineers' Handbook, 1963

than 2-heptanone. In the second experiment, however, the high concentrations of 2-pentanone offset the retention of acetone. The data of both studies show that 2-nonanone (with a high relative volatility) was recovered in larger amounts as were the ketones of higher molecular weight. Perhaps in this system, the strong affinity of the water insoluble ketones for the lipid phase of the cream is more dominant in their retention than their relative volatility in an aqueous solution.

Nawar and Fagerson (1962) employed gas chromatography to determine the headspace concentration of ketones in an aqueous solution. Their results substantiate the recovery data for acetone, 2-pentanone and 2-heptanone (Table 7) reported in this thesis. Although the headspace response (HR) of 2-heptanone (24) was greater than that of 2-pentanone (22), a maximum was attained at 2-hexanone (29). Apparently effective vapor pressure is increased as water solubility decreases. However, at a certain point, such as that of 2-heptanone, the effect of lowered solubility on vapor pressure is offset by the high boiling point of a volatile. 2-Nonanone was not included in their study (Nawar and Fagerson, 1962). Possibly in the samples of this research the high boiling point of 2-nonanone reduced its high relative volatility (Table 7).

Studies on the evaporation of aroma components during the sublimation of ice showed that methyl anthranilate, a highly volatile ester of low water solubility, was retained in greater amounts than a substance of low volatility and high water solubility like acetic acid (Saravacos, 1968). The behavior of the higher molecular weight ketones is analogous to that of methyl anthranilate. Acetone may behave like acetic acid. Upon freezing, acetone, with a low relative volatility, may become more volatile than 2-nonanone. Thus, following sublimation the retention of these methyl ketones based on their relative volatility may be: the 2-alkanones of high molecular weight, 2-nonanone, acetone, 2-pentanone and finally 2-heptanone. The data (Table 7) indicate that 2-nonanone and the ketones of longer chain length were retained in greater amounts than acetone. Retention of 2-pentanone was superior to that of 2-heptanone. The assumptions for the evaporation of aroma constituents and their relative volatility during the sublimation of ice provide an explanation for the recovery of these methyl ketones. However, the concentrations of 2-pentanone in the second experiment cannot be accounted for on this basis.

The volatility of a specific flavor component is also dependent upon the concentration of the compound in the liquid phase. Although the level of methyl ketones added to the cream was similar in both experiments, fewer aldehydes were included in the samples of the second study (Table 9). Thus, the total alkanal concentration was reduced by approximately 40 per cent. Perhaps this alteration in the aldehyde content may have contributed to the improved recovery of 2-pentanone. Nawar (1966) observed changes in the headspace response of aqueous 2-heptanone, at two concentrations with the addition of various substances. An equivalent amount of 2-octanone did not affect the headspace concentration of 2-heptanone in the dilute system; however, in the concentrated system, the 2-heptanone level was reduced to about one-half following the addition of 2-octanone. In some cases, differences in volatility, such as those of the sample before and after treatment, could result from the appearance or disappearance of another component rather than from a true increase or decrease in the compound examined (Nawar, 1966).

In determining the partition coefficients of food volatiles, Nelson and Hoff (1968) observed that the methyl ketones exhibited a greater preference for the aqueous phase than the aldehydes. Possibly the elimination of the slightly water soluble alkanals (pentanal and heptanal) in the second trial of this research (Table 9) facilitated an increase in the solubility of 2-pentanone and thereby enhanced its retention (Table 7).

In general, the results (Table 7) show that for a homologous series of methyl ketones with 7 or more carbon atoms, molecular size may have influenced the retention of volatiles. At a platen temperature of 100°F the retention of 2-tridecanone was approximately 14 times greater for 2-heptanone. However, molecular weight appears to be unrelated to the recovery of the short chain ketones. During spray-drying, the retention of 2-butanone was about 4 times that of acetone (Reineccius, 1967).

The loss of a homologous series of fatty acids (C_4 to C_{12}) from butter emulsions during spray-drying has been shown to be a function of the boiling point (Boudreau et al., 1966). The data in this thesis indicate that the boiling point may be significant for a homologous series of ketones with seven or more atoms of carbon but of lesser importance for acetone (B pt. 56.2^{o}C) and 2-pentanone (B pt. 102^{o}C). Reineccius (1967) indicated that boiling point was not influential when comparing the retention of organic compounds, with boiling points ranging from 56.5^{o}C (acetone) to 148^{o}C (3 hydroxy-2-butanone) during the spray-drying of skimmilk. Similar findings for the losses of acetoin and diacetyl from lyophilized acidified cream have been noted (Radanovics, 1969).

During freeze-drying substantial amounts of the methyl ketones with low boiling points were removed. Katayama et al. (1966) observed that the low boiling compounds present in apples were lost during freeze-drying. Findings in agreement with these have been reported for the vacuum-drying of fruit juices (Saravacos and Moyer, 1968b) and the spray-drying of cheese slurries (Bradley and Stine, 1964).

The average per cent recovery of added aldehydes from lyophilized cream as a function of platen temperature appears in Table 9. As platen temperatures are elevated, the retention

Table 9. Average per cent recovery[a] of added aldehydes[b] from cream[c] freeze-dried at various platen temperatures.

Aldehyde chain length	First trial Platen temperature (°F)			Second trial Platen temperature (°F)			
	80	120	160	80	100	120	160
10				33.4	29.8	24.1	14.5
9	40.9	28.5	9.4				
8	7.9	11.2	4.5	14.5	15.2	12.4	7.1
7	16.2	15.6	9.5				
6	47.6	36.0	24.8	52.6	d	35.3	32.7
5	19.1	16.9	9.3				
4	60.4	39.2	33.9	65.6	d	58.2	52.6

[a]Based on concentration of control

[b]Calculated as μg/10 g fresh cream (30.5 per cent milkfat)

[c]Average of duplicate analyses

[d]Not determined

of aldehydes is lowered. These trends resemble those obtained for the methyl ketones and are in agreement with the observations of other researchers (Saravacos and Moyer, 1968a; Radanovics, 1969). With the exception of octanal, the retention of alkanals in both studies is similar.

Although the recovery of nonanal may appear to be somewhat greater than that of decanal, both aldehydes are retained in similar amounts. Losses of both decanal and nonanal are not as great as those of the aldehyde with 8 carbon atoms. In spite of the differences in the magnitude of the per cent retention of octanal in both experiments, heptanal concentrations are slightly greater than those of octanal at all temperature programs. The retention of hexanal is superior to that of pentanal, while the levels of butyraldehyde are greater than those of caproaldehyde.

Since the physical characteristics of the aldehydes may be influential in their losses, they are compiled in Table 10. Precise vapor pressure data were not obtainable for the lower molecular weight alkanals. However, with the information available estimates may be made which, when combined with solubility characteristics permit a determination of the relative volatility of the aldehydes in aqueous media. On this basis, the relative volatility of the aldehydes should increase with increasing carbon chain length. Usually, as compound volatility increases, the retention of volatiles decreases. The data (Table 9) generally indicate this kind of relationship

Table 10. Physical characteristics[a] of the saturated aldehydes.

Saturated Aldehyde	Structure	Molecular Wt. (g/mole)	Solubility	Vapor Pressure[b] (1 Atm.)	Boiling Point (^oC)
Butanal	$CH_3(CH_2)_2CHO$	72.1	soluble	--	75.7
Pentanal	$CH_3(CH_2)_3CHO$	86.1	slightly	--	103.4
Hexanal	$CH_3(CH_2)_4CHO$	100.2	slightly	--	131.0
Heptanal	$CH_3(CH_2)_5CHO$	114.2	slightly	155.0	152.8
Octanal	$CH_3(CH_2)_6CHO$	128.2	--	168.5	163.4
Nonanal	$CH_3(CH_2)_7CHO$	142.2	--	185.0	185.0
Decanal	$CH_3(CH_2)_8CHO$	156.3	insoluble	208.5	208.9

[a]Compiled from Handbook of Chemistry and Physics, 1968-1969
[b]Taken from Chemical Engineers' Handbook, 1963

for the relatively volatile alkanals of 4 to 8 carbon atoms. However, the low concentrations of pentanal in the first study contradict its relative volatility. The substantial reduction in the amount of heptanal and octanal compared to that of hexanal may be due primarily to solubility. Davis (1968) determined the solubility of these aldehydes. His (Davis, 1968) results expressed in grams per liter were: 5.64, 1.55 and 0.37 for the alkanals with 6 to 8 carbon atoms, respectively. Possibly differences in the levels of octanal between the two studies (Table 9) may be attributed to the total alkanal concentration in each of the trials.

Decanal and nonanal were both retained in larger quantities than octanal. This finding resembles that observed for the ketones with 9 or more atoms of carbon. The higher boiling points of these aldehydes (decanal and nonanal) appear to have offset the effect of lowered water solubility on vapor pressure (Nawar and Fagerson, 1962). In their studies on the volatiles in coffee, Sivetz and Foote (1963) noted that coffee solubles (such as phospholipids and fatty acids) increased the solubility of aldehydes. Thus, the relative vapor pressure of the aldehydes was lowered. Coffee solubles promoted adsorption and absorption. In addition, a high solubility coefficient for long chain carbonyls and coffee oils was observed. Possibly, similar factors in the lipid phase of the cream enhanced the recovery of decanal and nonanal from the samples in these experiments.

Pentanal was retained in lesser amounts than would be expected when its recovery is compared with that obtained for butanal and hexanal. Predictions, based on relative volatility, indicate that the loss of valeraldehyde would have been of lesser magnitude than that obtained (Table 9). It is possible that monocarbonyl concentration may have affected the recovery of pentanal. Since the elimination of this aldehyde from the samples of the second trial may be related to the improved recovery of 2-pentanone in those samples, perhaps mutual solubility effects between pentanal and 2-pentanone may influence the level at which they are retained.

The data (Table 9) obtained from these experiments do not show an increase in the retention of volatile aldehydes with increasing molecular weight. Although greater losses were observed for octanal, there is no difference in the recovery of decanal and nonanal. A similar conclusion may be drawn for the loss of alkanals as a function of boiling point.

In the studies conducted in this research the recovery of an added unsaturated aldehyde from cream freeze-dried at various platen temperatures was determined. The results obtained are found in Table 11.

The data for both trials are similar. Samples freeze-dried at a platen temperature of 80^{o}F retain greater amounts of 2-nonen-1-al than their counterparts lyophilized at higher temperature programs (160^{o}F). At the 160^{o}F platen temperature, the slightly greater loss of 2-nonen-1-al in samples of the

Table 11. Average per cent recovery[a] of added 2-enal[b] from cream[c] freeze-dried at various platen temperatures.

Platen temperature (^{o}F)	2-Nonen-1-al First trial	2-Nonen-1-al Second trial
80	34.4	27.3
120	28.2	29.6
160	11.8	14.0

[a]Based on concentration of control
[b]Calculated as μg/10 g fresh cream (30.5 per cent milkfat)
[c]Average of duplicate analyses

first study may be the result of a higher final sample temperature (Table 2, p. 41).

Since the retention of volatile monocarbonyls is enhanced by the selection of moderate platen temperatures for freeze-drying, a determination of the loss of these volatiles during prolonged lyophilization at a temperature of 80^{o}F was of interest. The effect of extended freeze-drying on the losses of methyl ketones and aldehydes is shown in Figures 3 and 4. Freeze-drying at a temperature program of 80^{o}F was complete after 19.6 hours. A substantial loss of added monocarbonyls occurs during lyophilization. Although additional losses in both classes of carbonyls may be observed during extended dehydration, they are of lesser magnitude than those found initially. During further lyophilization the losses of alkanals

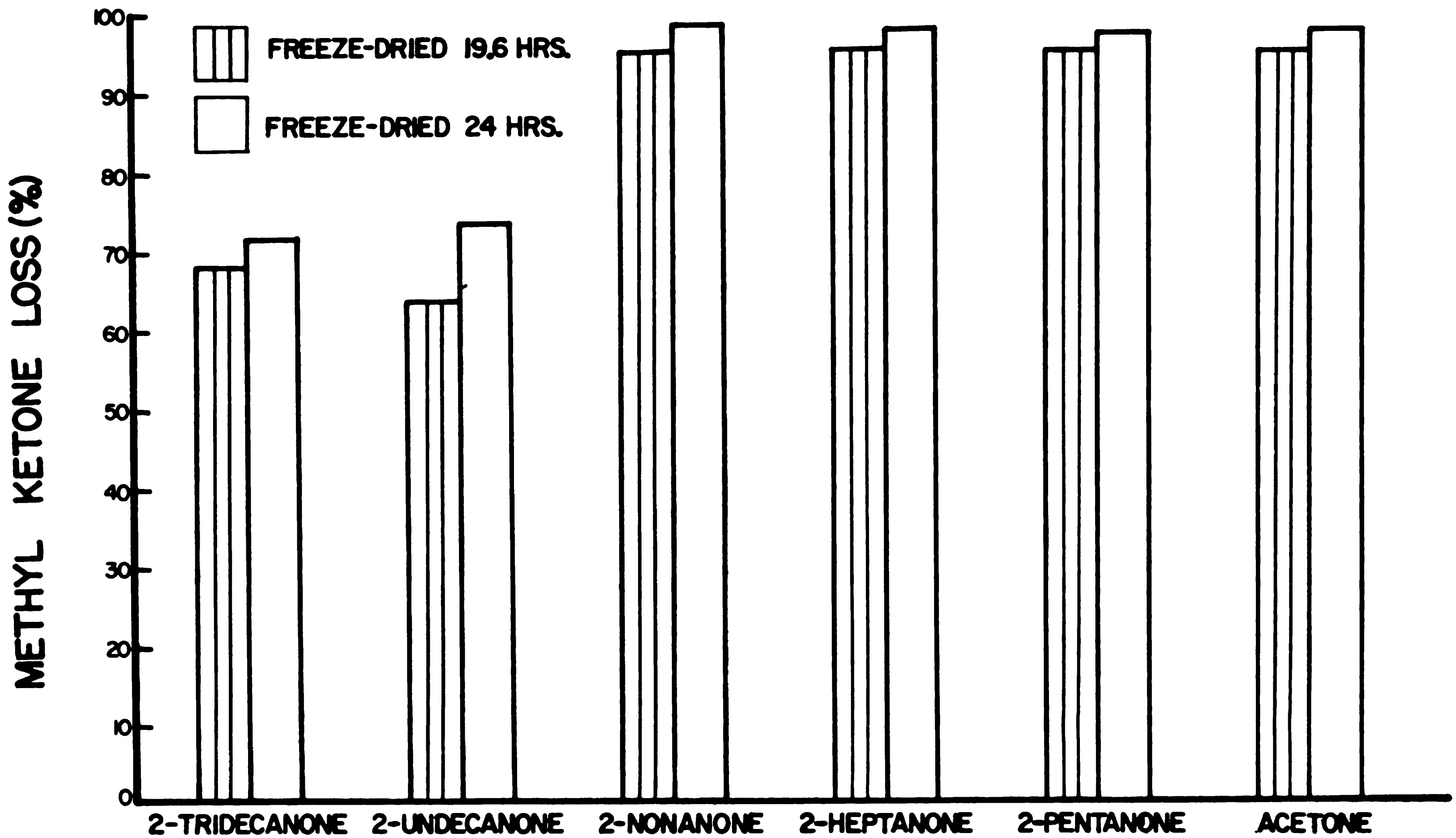

Figure 3. The effect of extended freeze-drying at 80°F on the loss of methyl ketones added to cream.

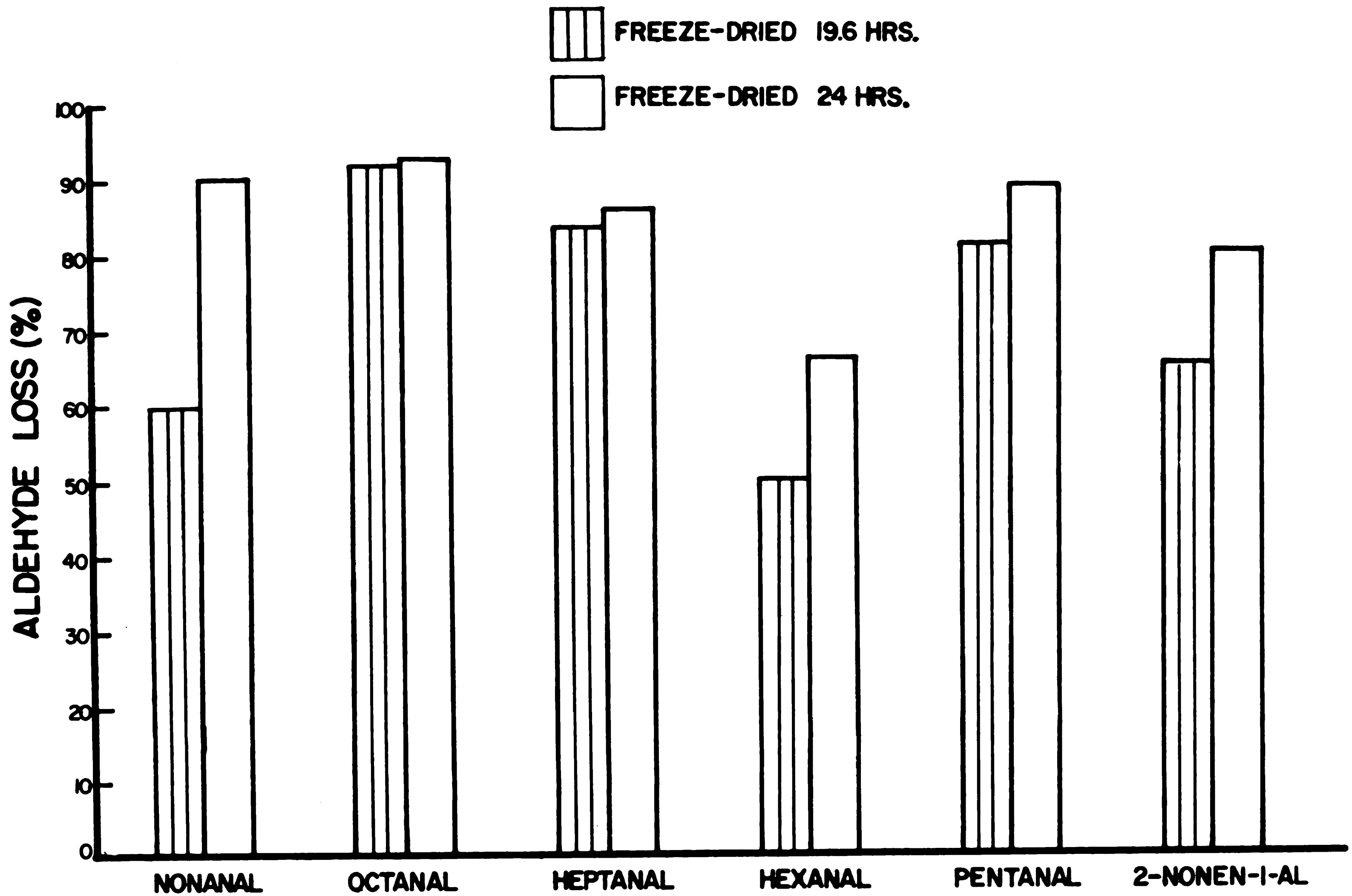

Figure 4. The effect of extended freeze-drying at 80°F on the loss of aldehydes added to cream.

(Figure 4) are greater than those of the ketones (Figure 3). Perhaps the aldehydes are not volatilized as readily as the 2-alkanones. Although extended lyophilization was not quite as detrimental, the retention of volatiles from this temperature program resembles that obtained for volatiles from samples freeze-dried at a platen temperature of $160^{\circ}F$.

Findings similar to these for the loss of diacetyl (Radanovics, 1969) and 1-butanol (Flink and Karel, 1970) following extended freeze-dehydration have been reported.

To facilitate a comparison of the retention of each class of monocarbonyls in these studies, the monocarbonyl content of fresh, frozen and freeze-dried samples of the second trial is illustrated in Figure 5. A substantial amount (92 per cent) of the total carbonyl concentration is retained in the samples held in frozen storage at $-20^{\circ}F$ for 6 months. However, a significant reduction in the total carbonyl content may be observed after freeze-drying. Approximately 14 per cent of the carbonyls found in the fresh samples is recovered from the samples freeze-dried at $80^{\circ}F$; samples lyophilized at $160^{\circ}F$ retain about 9 per cent of their added monocarbonyls.

The comparative proportion of each class of carbonyls in relation to the total monocarbonyls content is also illustrated in Figure 5. It is apparent that the quantity of methyl ketones (82 per cent) present in the fresh samples is substantially greater than that of the aldehydes (15 per cent) and 2-enal (3 per cent). The losses of each class of carbonyls

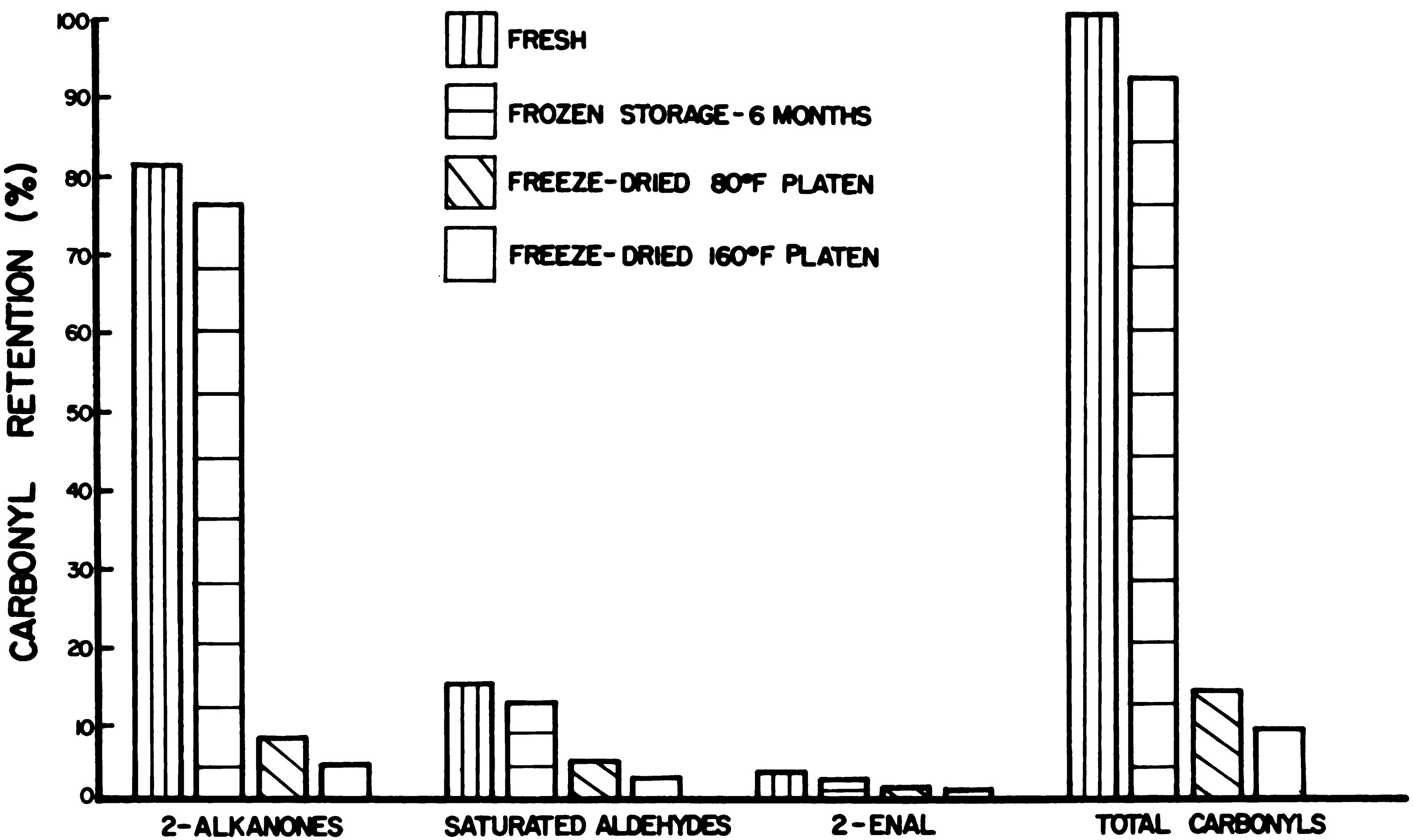

Figure 5. Comparison of added monocarbonyls in fresh, frozen and freeze-dried cream.

from cream during frozen storage and freeze-drying may be observed. In addition, the increased loss of organic volatiles which occurs at elevated platen temperatures is also shown.

Data for the methyl ketones, aldehydes and 2-enal are graphed by treating the initial total concentration of each class of added monocarbonyls in the fresh cream as 100 per cent (Figure 6). The per cent retention of 2-nonen-1-al in samples held in frozen storage compared with that found in the fresh control is lower than that obtained in similar comparisons of the aldehydes and ketones. During frozen storage, the loss of aldehydes appears to be greater than that of the ketones. There is only a slight reduction in the recovery of methyl ketones from cream samples held at -20°F for 6 months. Perhaps the initial low concentration of both the saturated and unsaturated aldehydes has accented the loss of these carbonyls (see earlier discussion).

Freeze-drying at a platen temperature of 80°F results in a substantial loss of each class of added monocarbonyls from the cream samples. However, the retention of the aldehydes (34 per cent) is superior to that of the ketones (10 per cent). The 2-enal is retained in an amount similar to that of the saturated aldehydes (30 per cent). A reduction in the concentration of each class of carbonyls in cream samples lyophilized at a platen temperature of 160°F may be observed. When the loss of flavor volatiles from samples freeze-dried at platen temperatures of 80 and 160°F is compared, the per cent

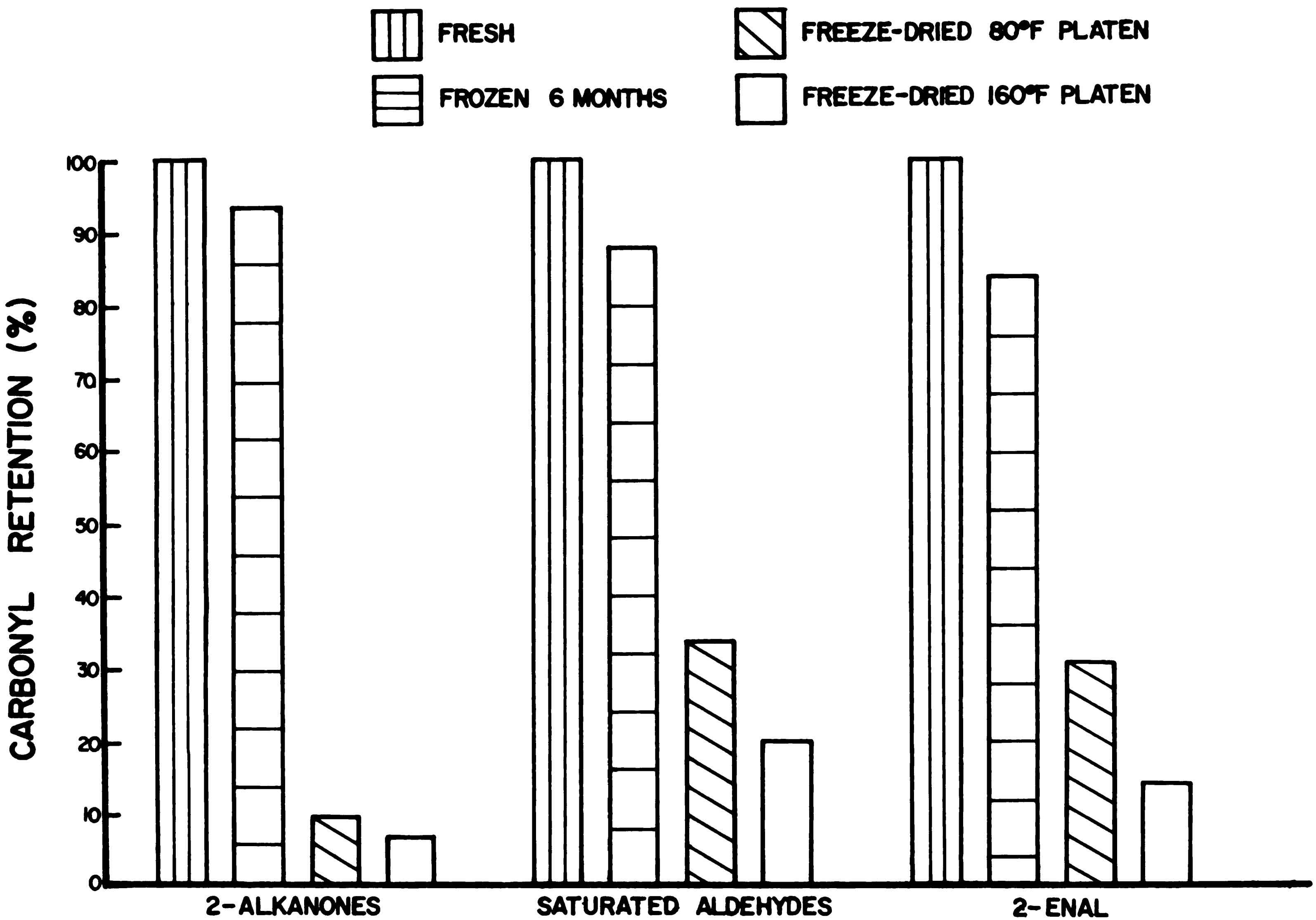

Figure 6. Retention of each class of added monocarbonyl compounds in cream after frozen storage and freeze-drying.

loss of both the aldehydes (14 per cent) and the 2-enal (16 per cent) is of greater magnitude than that found for the methyl ketones (3 per cent). Although the microgram concentration of the 2-alkanones in cream freeze-dried at 160°F is higher than that of the aldehydes and the 2-enal, the per cent retention of both of these classes of carbonyls is superior to the methyl ketones. During his experiments with spray-dried skimmilk, Reineccius (1967) found that the retention of butyraldehyde, acetone and 2-butanone was 67, 22, and 80 per cent, respectively. In these studies, the retention of volatiles with an aldehyde group appears to be favored.

Since the concentration of each class of monocarbonyls and that of individual members within a class varied significantly in these experiments, perhaps a generalization of this kind is not justified. For example, a comparison of the retention of volatiles of equivalent molecular size, at a temperature program of 80°F, shows that nonanal and 2-nonen-1-al were retained in greater proportions (40.9 and 34.4 per cent) than 2-nonanone (5.4 per cent). In this research the experimental design was organized to approximate carbonyl concentrations that may be present in foods, such as Blue cheese and oxidized dairy products. This resulted in a substantial variation in the initial concentration of each of the volatiles with 9 atoms of carbon (Table 1, p. 39). Therefore, while the initial concentration of 2-nonanone was 774.6 μg, there were only 111.8 and 67.1 μg, respectively, of nonanal and 2-nonen-1-al present in the control. The relative loss of diacetyl from lyophilized acidified cream has been shown to be independent of the initial absolute concentration between the range of 5 to 100 ppm (Radanovics, 1969). However, during the spray-drying of skimmilk, Reineccius and Coulter (1969) found that the loss of diacetyl (at levels of 500 and 6,000 ppm) was affected by initial concentration. The retention of diacetyl was reduced by 15 per cent when the higher concentration was dehydrated. McBean _et al_. (1965) noted a small but consistent decrease in the amount of sulfur dioxide retained in fruits sulfured at higher concentrations. Furthermore, the experiments of Nawar (1966) have shown that in some cases differences in the volatility (e.g., those of a sample before and after treatment) could result from alterations in the levels of components rather than from a true increase or decrease in the volatile examined. Compounds present at subthreshold concentrations exhibit a synergistic effect and thus contribute to aroma (Nawar and Fagerson, 1962). This, too, may subsequently affect the volatilization of aroma constituents.

The aldehydes used in this research may have had a greater affinity for the lipid phase of the cream than the ketones. Since the methods of analyses employed in these experiments are based on the removal of monocarbonyls from the fat, the improved retention of the aldehydes may be the result of this factor.

On the basis of relative volatility in an aqueous media, it is difficult to determine whether a greater retention of methyl ketones might be expected. Methyl ketones are generally more soluble than aldehydes of similar molecular weight (e.g., 2-butanone--35 g/100 g water; butanal--3.5 g/100 g water; Reineccius, 1967). However, in this respect, the determination of Henry's constants for similar weight aldehydes and ketones in water indicated that aldehydes were more soluble than methyl ketones (Nelson and Hoff, 1968). According to Henry's Law, "the solubility of a gas in a liquid is directly proportional to the pressure of the gas above the liquid." (Maron and Prutton, 1965). Thus, aldehydes may be more soluble in water. In addition, the experiments of Nawar (1966) have shown that dilution with water affects the vapor pressure of various compounds differently. A relationship between the flavor intensity of certain volatile compounds and the solvent in which they may exist has been observed (Lea and Swoboda, 1958; Jellinek, 1959; Patton, 1964). This behavior is attributed to the effect of the medium on the rate of evaporation of solute molecules (Jellinek, 1961; Nawar and Fagerson, 1962).

Furthermore, the volatility of a flavor constituent is also dependent upon the miscibility of the compound with other organic constituents. Coffee solubles increased the solubility of aldehydes (Sivetz and Foote, 1963). Similarly, the solubility of 2-heptanone was greater in milk (4 per cent fat) than in skimmilk (Nawar, 1966). The affinity of the solubles for the alkanals tended to lower the relative vapor pressure of the aldehydes to water compared to that found in a pure aldehyde-water system (Sivetz and Foote, 1963). However, when volatiles of similar chain length are compared, findings for Henry's constants of aldehydes and ketones in oil (Nelson and Hoff, 1968) indicate that the solubility of particular ketones and aldehydes varies.

Thus, many factors may have influenced the volatility of the monocarbonyls in these experiments. Because of differences in concentration, variety, and possible interaction of the organic volatiles incorporated into the cream, and because of the complex nature of the cream, a determination of these factors cannot be made. However, the direct headspace gas chromatographic analyses of food volatiles in model aqueous and lipid systems by Nawar (1966) have demonstrated "the relationship between the concentration of a specific compound in the vapor phase at a given temperature and the interplay of the following variables: vapor pressure of the compound; type of medium in which it is distributed; degree of its solubility in the medium; concentration of the compound in the liquid phase; its miscibility with other organic compounds in the mixture; and the presence of salts or sugars." Therefore, the results of these experiments possibly indicate that the retention of aldehydes may have been favored.

Absorption of Methyl Ketones by Freeze-Dried Cream

Because the reduction of volatile compounds from monocarbonyl-enriched cream during freeze-drying was substantial, further research directed toward overcoming some of these losses was initiated. The extremely volatile lower molecular weight ketones were selected for the absorption experiments because they were retained in particularly small amounts in lyophilization.

Preliminary studies were conducted to determine the quantities of cream and ketone to employ and to determine the exposure time. Thus, 50 g of the carbonyl-enriched cream freeze-dried at a platen temperature of 160^{o}F and 2 g of 2-pentanone were placed in the absorption chamber for 140 hours at 68^{o}F and equilibrated prior to analysis. 2-Pentanone was absorbed in such large quantities that it could not be determined accurately, because of an inability to class separate the monocarbonyls originally present in the cream. In a subsequent experiment similar amounts of 2-pentanone (2 g) and freeze-dried cream (50 g) were exposed for 1 hour under conditions identical to those just described. Although there was a significant increase in the 2-pentanone content of the cream, the sample was class separated. The initial 2-pentanone concentration of the carbonyl-enriched cream freeze-dried at 160^{o}F was 8.8 μg; however, 4494 μg of this ketone were determined in the exposed cream sample. As a result of these marked increases in ketone content during absorption, 50 g of freeze-dried cream and 1 g of each volatile compound were chosen for studies of the absorption of a single 2-alkanone.

The temperature profile and drying curve of the freeze-dried cream employed in the absorption studies are shown in Figure 7. Since the time-temperature profiles of freeze-dried cream were presented and discussed previously (Figure 2), this discussion is limited to other conditions of these experiments (Figures 2 and 7). The total freeze-drying time of the cream for these studies is considerably longer than that observed for the previous samples. This longer time may be attributed to the substantial increase in the size of the load in the freeze-dryer. This (Figure 7) time-temperature profile was obtained for the 4 lbs of cream lyophilized during the sublimation of a total load of 20 lbs of cream.

During freeze-drying the loss of weight appears to be a curvilinear plot until the point at which drying becomes essentially completed. The rate of moisture loss appears to be constant and no sudden changes in the curve are apparent. The falling rate of the drying cycle, typical of spray-dried and air-dried products (van Arsdel and Copley, 1963) is completely absent in freeze-dehydration. Other workers (Goldblith et al., 1963; Radanovics, 1969) have obtained similar drying curves during the lyophilization of shrimp, salmon and sour cream.

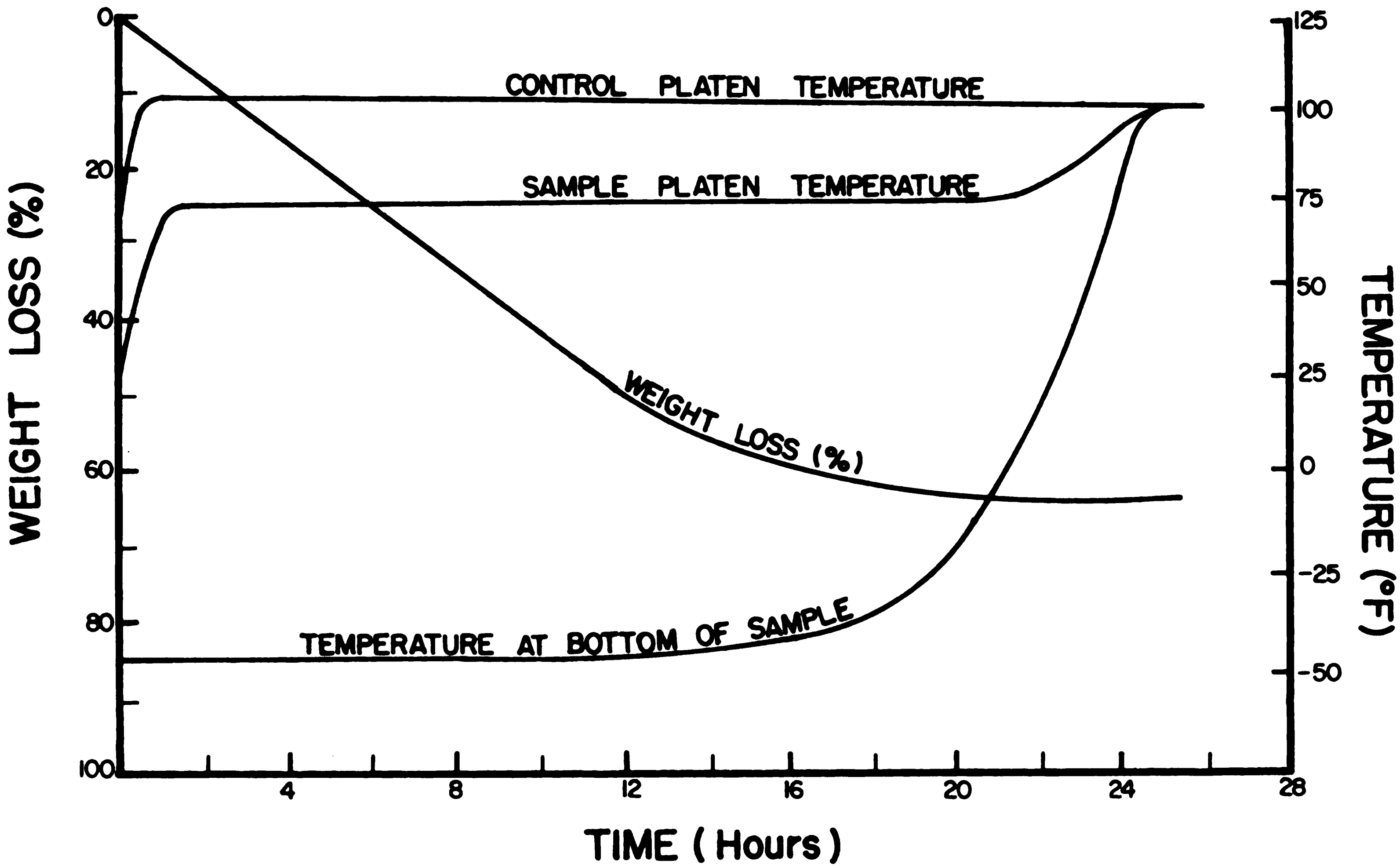

Figure 7. Drying curve and time-temperature profile of cream freeze-dried at a platen temperature of $100^{\circ}F$.

The short chain 2-alkanones absorbed by the freeze-dried cream were converted to their 2,4-dinitrophenylhydrazone derivatives. In addition to confirmation by thin-layer chromatography, ketone authenticity was verified by a combination of mass spectrometric and gas chromatographic analyses. Since 2,4-DNP-hydrazone derivatives are among the most widely used derivatives for the characterization of carbonyl compounds in microquantities, this technique was of particular interest because it permitted an accurate confirmation of absorbed volatiles.

Partial mass spectra for the 2,4-DNP-hydrazone derivative obtained from the 60 minute exposure of 2-heptanone to freeze-dried cream and for the standard (2-heptanone) 2,4-DNP-hydrazone derivative are shown in Figure 8. Fragment ions of m/e above 300 were of very low intensity. The spectrum of the sample resembles that obtained for the standard. In both spectra the 100 per cent intensity peak m/e 97 confirms the base peak of the 2-heptanone-2,4-dinitrophenylhydrazone derivative (Kleipool and Heins, 1964). The appearance of ion fragments at m/e 178 and 238 are indicative of a methyl ketone 2,4-DNP-hydrazone derivative. The ion peak at 294 corresponds to a molecular weight of 2-heptanone-2,4-DNP-hydrazone. Finally, both spectra show a minute but distinct peak at M-35. These findings are similar to those reported by Kleipool and Heins (1964). Retention time data further confirms the identification of the 2-heptanone derivative.

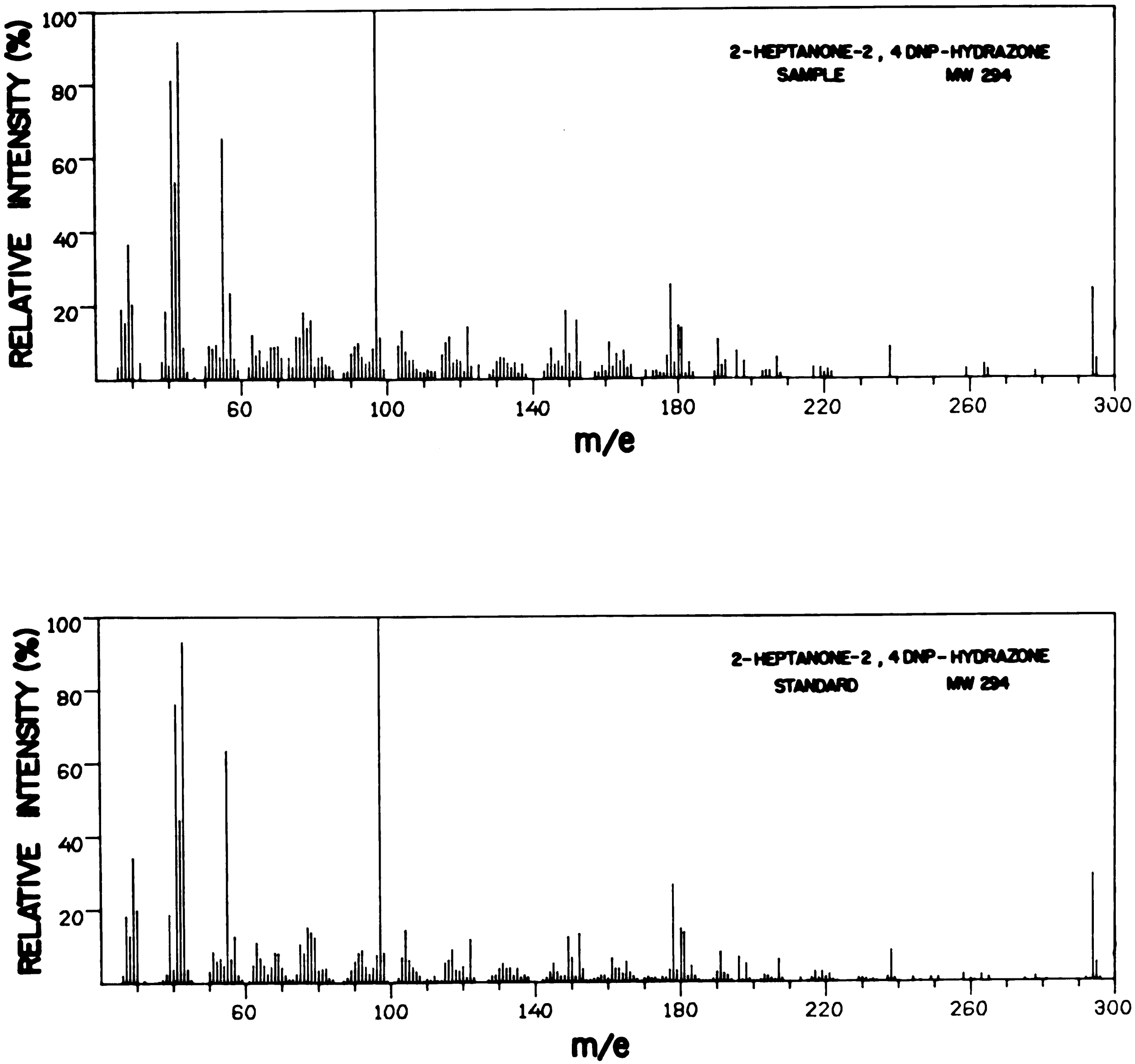

Figure 8. Partial mass spectra of 2,4-dinitrophenylhydrazone derivatives of the sample and the standard.

This method of analysis was employed to identify each of the methyl ketones exposed to freeze-dried cream for 60 minutes.

The recovery of ketones, after the exposure of individual 2-alkanones to freeze-dried cream, is presented as a function of time in Table 12. To facilitate a comparison of these findings with those reported for foods containing monocarbonyls, these data are given in micrograms and micromoles. As would be expected, the quantity of each of the flavor components absorbed by the cream increases as the exposure time is lengthened. Approximately 12.4 μM of acetone were determined in cream samples exposed to the volatile for 15 minutes. However, after 60 minutes in the absorption chamber, the acetone concentration in freeze-dried cream was about 5 times greater than that of the 15 minute samples. Similar increases in the ketone concentration of creams may be observed for the exposure of each homolog as a function of time. Low molecular weight methyl ketones are absorbed more quickly by the powder than are the longer chain 2-alkanones. For each absorption period, the concentration of the more volatile, shorter chain ketones (C_3 and C_5) exceeds that of 2-heptanone and 2-nonanone. 2-Nonanone was absorbed by the cream in the smallest amounts. Although 36.8 μM of acetone were determined in freeze-dried cream exposed for 30 minutes, only 0.7 μM of 2-nonanone were present in comparable samples. At the 60 minute exposure time, the microgram concentration of 2-pentanone exceeded that of

Table 12. Retention of methyl ketones by freeze-dried cream after the absorption of individual 2-alkanones.

Exposure Time (min)[a]	Methyl Ketones[b] Acetone		2-Pentanone		2-Heptanone		2-Nonanone	
	μg	μM	μg	μM	μg	μM	μg	μM
15	722	12.4	534	6.2	291	2.5	43.0	0.3
30[c]	2,140	36.8	1,720	20.0	477	4.2	105	0.7
60	3,770	64.9	4,720	54.8	1,520	13.3	300	2.1

[a]Temperature controlled at 68 ± 1°F

[b]Calculated as μg/10 g fresh cream (30 per cent milkfat)

[c]Average of duplicate analyses; other data: average of two experiments, each determined in duplicate

acetone. The affinity of 2-pentanone for the lipid phase of the cream may account for this finding. For both the 15 and 30 minute time intervals, the acetone which had been placed in the absorption chamber was more rapidly absorbed by the dried cream than the ketone with 5 carbon atoms. However, during the longer absorption period of 60 minutes, the affinity of 2-pentanone for the lipid phase of the cream exceeded that characteristic of acetone.

Table 13 presents the data obtained for the absorption of a mixture of 2-alkanones by freeze-dried cream as a function of time. The results show trends similar to those found for the exposure of individual methyl ketones (Table 12). Highly volatile ketones are absorbed by the freeze-dried powder in larger quantities than those obtained for the higher molecular weight homologs. The concentration of each ketone in freeze-dried cream after 1 hour in the absorption chamber is substantially greater than that determined for similar samples exposed for the shortest period of time.

At each absorption time, the volatile low molecular weight ketones are present in concentrations greater than those of 2-heptanone and 2-nonanone. However, for each exposure time, the microgram concentration of 2-pentanone exceeds that of acetone. Micromole concentrations of both acetone and 2-pentanone are similar for each of the time intervals determined. Several factors may be responsible for this finding. One-half a gram of each ketone was employed in these studies. Relative

Table 13. Retention of methyl ketones by freeze-dried cream[a] after the absorption of a mixture of 2-alkanones.

Exposure[b] Time (min)	Methyl Ketones[c] Acetone μg	Acetone μM	2-Pentanone μg	2-Pentanone μM	2-Heptanone μg	2-Heptanone μM	2-Nonanone μg	2-Nonanone μM
15	75	1.3	155	1.8	51	0.4	9.1	0.1
30	140	2.4	250	2.9	68	0.6	8.4	0.1
60	820	14.1	1180	13.7	210	1.8	36	0.2

[a]Average of duplicate analyses

[b]Temperature controlled at 68 ± 1°F

[c]Calculated as μg/10 g fresh cream (30 per cent milkfat)

to that of 2-pentanone, the concentration of acetone available for absorption by the lipid portion of the powder may have been lower than that of 2-pentanone. Possibly in the presence of a mixture of ketones, the active sites in the cream available for acetone absorption may have been reduced due to a stronger attraction of the other 2-alkanones to these sites.

Although these data (Tables 12 and 13) show that substantial quantities of the methyl ketones were absorbed by the freeze-dried cream, perhaps these findings may be made more meaningful by an examination of the methyl ketones in Blue cheese. Table 14 compares the concentration of low molecular weight methyl ketones in Blue-vein type cheese with those found in freeze-dried cream samples that had been exposed to 2-alkanones. After exposure for 15 minutes, the concentrations of acetone and 2-pentanone in the cream are significantly greater than those determined in Blue cheese. An absorption time of 15 to 30 minutes for the cream and ketone results in a similar level of 2-heptanone in both the cream and the cheese. 2-Nonanone concentrations in Blue cheese are obtained by exposing freeze-dried cream to the methyl ketone for 60 minutes.

The application of these findings to a commercial process could be of considerable importance in improving the flavor and odor characteristics of a dehydrated food. For example, a continuous process could be designed whereby spray-dried or freeze-dried Blue cheese would be conveyed through an air lock into an absorption chamber. The powder would be exposed to

Table 14. Concentration of low molecular weight methyl ketones in Blue-vein type cheese and in freeze-dried cream which had been exposed to 2-alkanones.

	Micromoles/10 g of Extracted Fat						
	Cheese[a]				Freeze-Dried Cream[b] Time (min)		
Methyl ketone chain length	A	B	C	D	15	30	60
3	1.7	1.5	2.5	1.0	41.3	121.4	214.2
5	6.3	2.6	9.0	2.6	20.4	66.0	180.8
7	10.5	5.2	23.3	5.4	8.3	13.9	43.9
9	5.8	4.9	23.0	4.8	1.0	2.3	6.9

[a]Taken from data compiled by Anderson (1966)

[b]Calculated from data presented in Table 12

an atmosphere of volatiles which were lost during dehydration. By controlling the concentration of the volatiles in the atmosphere and residence time in the chamber, complete replacement or enrichment of desired flavor volatiles could be achieved.

Such absorption processes could also be applied in the fabrication of completely new, synthetic foods or food analogs.

Although substantial amounts of the volatile ketones are absorbed by the freeze-dried cream, information regarding the extent to which these flavor compounds are retained by the cream during storage is of importance. Samples obtained from the 1 hour exposure of individual ketones to freeze-dried cream (Table 12) were held at -10°F. The retention of each volatile was determined after 1 and 2 months storage. These data are shown in Figure 9. After 1 month of freezer storage 78 per cent of the initial acetone concentration is retained by the ketone-absorbed cream samples. The per cent retention of 2-pentanone is similar to that determined for acetone. The initial 2-pentanone content of the sample is reduced by 24 per cent. Approximately 93 per cent of the 2-heptanone concentration initially found in the ketone-absorbed cream is retained following storage at -10°F for 1 month.

As the storage time is prolonged, a substantial increase in the desorption of acetone is observed. After 2 months at

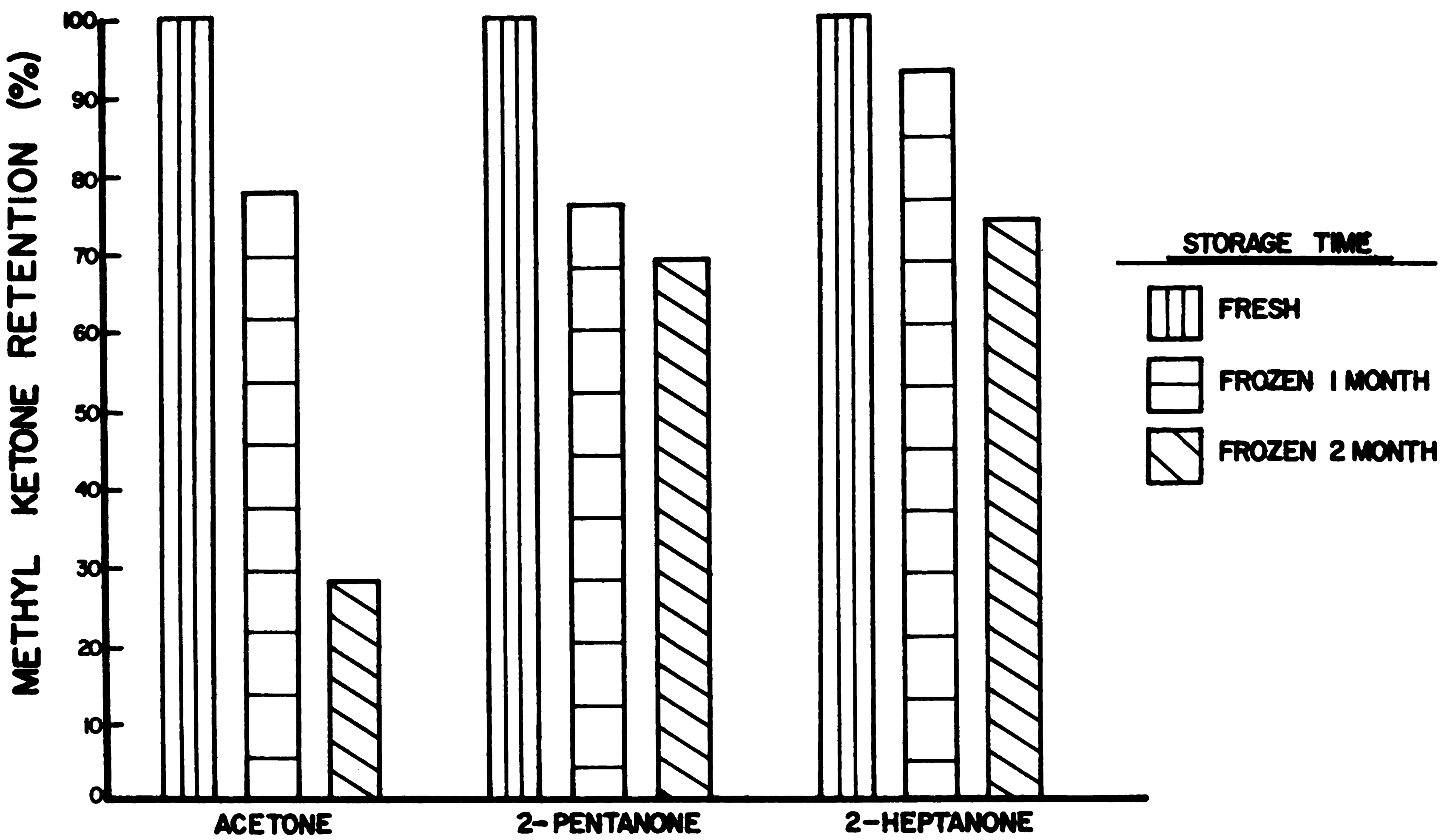

Figure 9. Retention of methyl ketones in freeze-dried cream which had been exposed to 2-alkanones.

$-10^{o}F$, the ketone-absorbed cream samples contain approximately 28 per cent of their initial acetone content. However, greater amounts of both 2-pentanone (69 per cent) and 2-heptanone (74 per cent) are present in the ketone-exposed cream samples after 2 months of frozen storage. These findings suggest that a highly volatile ketone such as acetone desorbs more quickly and steadily than the higher molecular weight homologs. While its initial rate of desorption is similar to that found for acetone, 2-pentanone is volatilized more slowly than acetone as the storage interval is increased. 2-Heptanone desorbs slowly and its per cent retention is greater than that of the more volatile 2-alkanones.

Although the loss of both acetone and 2-pentanone from ketone-absorbed cream samples was greater than that obtained for 2-heptanone, both of these volatiles are absorbed in substantially greater concentrations than the ketone with 7 atoms of carbon (Table 12). The micromole concentration of samples stored at $-10^{o}F$ for 2 months is 18.1, 37.8 and 9.8 for acetone, 2-pentanone and 2-heptanone, respectively (based on data of Table 12 and Figure 9). Thus, after 2 months of frozen storage both acetone and 2-pentanone are present in large concentrations in the freeze-dried powder.

Such findings suggest that a dried food product which slowly loses some of the highly volatile flavor constituents during prolonged storage could be enriched above the normal level. After storage, residual levels of such volatiles would more closely approximate the initial concentrations in the food.

SUMMARY AND CONCLUSIONS

The quantitative retention of volatile monocarbonyl compounds in fresh, frozen and freeze-dried samples was determined by incorporating known amounts of methyl ketones, saturated aldehydes and a 2-enal, that may be present in foods such as Blue cheese and oxidized dairy products, into a natural model system high in lipid content (cream--30.5 per cent milkfat). In these experiments the concentration of individual methyl ketones and aldehydes incorporated into fresh cream (30.5 per cent milkfat) appeared to be affected by the solubility of these organic volatiles in the medium. In both classes of compounds, the higher molecular weight, water insoluble members were retained in similar, greater amounts; the recovery of short chain hydrophilic components was lower. The data for samples of carbonyl-enriched cream held at -20°F for periods of 3 and 6 months show only a slight reduction in the concentration of 2-alkanones; the loss of saturated and unsaturated aldehydes may be slightly greater than that of the methyl ketones. Apparently the binding or absorption of the monocarbonyls with constituents of the cream retards their volatilization during frozen storage.

A substantial loss of added monocarbonyls was observed in the lyophilized cream samples studied in this research. The retention of each class of volatile carbonyls was enhanced by the selection of moderate temperatures for freeze-drying. As temperature programs were elevated, the total freeze-drying time and the recovery of monocarbonyls from cream samples decreased. Although prolonged lyophilization at a temperature of 80^{O}F resulted in additional losses of flavor compounds from the cream, they were of lesser magnitude than those determined initially.

The findings obtained from the freeze-drying experiments suggest that the recovery of higher molecular weight methyl ketones and aldehydes from the samples may be determined by factors such as boiling point, solubility in the lipid phase of the cream, molecular size and properties of absorption and adsorption. Generally, relative volatility seemed to influence the retention of volatile short chain monocarbonyls in the freeze-dried powder. During lyophilization, the low molecular weight aldehydes appear to be retained by the powder in greater amounts than the short chain methyl ketones. Though the concentration of the 2-alkanones in the cream samples was higher than that of the alkanals and the 2-enal, the per cent retention of both these classes of carbonyls following freeze-drying was superior to that of the methyl ketones. The results of these experiments indicate that during lyophilization the retention of volatiles with an aldehyde group may have been favored. Although these studies were occasionally of an exploratory nature the information obtained may help provide an indication of the quantitative retention of volatile monocarbonyl compounds in freeze-dried foods high in lipid content.

A technique for aroma addition in which extremely volatile, low molecular weight methyl ketones were exposed to freeze-dried cream was investigated. Substantial quantities of the 2-alkanones were absorbed by the powder. The data of this research show that as the exposure time was lengthened, the quantity of each of the flavor compounds absorbed by the freeze-dried cream increased. Short chain methyl ketones were absorbed by the cream powder in larger amounts than those determined for the higher molecular weight homologs. The application of these findings to a commercial absorption process could be of considerable importance in improving the flavor and odor characteristics of a dehydrated food, or in the fabrication of completely new synthetic foods or food analogs. In these experiments the desorption, during frozen storage, of highly volatile ketones from freeze-dried cream exposed to 2-alkanones was greater than that obtained for the higher molecular weight homologs. To compensate for the loss of highly volatile flavor constituents during prolonged storage, a dried food product could be enriched above the normal level. Following storage, residual levels of such volatiles would more closely approximate the initial concentrations in the food.

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