grade lauric acid completely to CO_2 and apparently utilize glucose as an energy source. However, in the absence of glucose, the lauric acid was oxidized by the spores. Earlier Gehrig and Knight (1958) showed that the spores of *P. roqueforti* oxidize fatty acids *via* the classical β oxidation pathway.

The generation of carbonyls from lauric acid by the spores was very sensitive to pH and temperature of incubation, as reported by Lawrence (1966) and Dartey and Kinsella (1973). Gehrig and Knight (1963) found that ketone production from octanoic acid was suppressed at 37°. The stimulation of ketone production from laurate by metabolic CO_2 contrasted with its effects on palmitate, where it depressed carbonyl production (Dartey and Kinsella, 1973). Lawrence (1966) stated that CO_2 accentuated ketone formation and this would be consistent with the conditions prevailing in cheesemaking, where carbon dioxide concentration builds up during maturation.

Methyl ketones were the principal products of lauric acid oxidation by the spores incubated at 30°, pH 6.5. While 2-undecanone was the predominant ketone formed, shorter chain ketones were also produced from lauric acid. Lawrence and Hawke (1968) reported that only heptanone was formed from octanoic acid. The use of uniformly labeled radioactive fatty acids has enabled us to demonstrate that several methyl ketones can be formed *via* β oxidation of lauric and myristic acid (Dartey and Kinsella, 1973). Thus, the long-chain fatty acids of milk fat may be a significant source of the methyl ketones occurring in mold ripened cheese, as reported by Anderson and Day (1966), Dartey and Kinsella (1971), and Patton (1950).

LITERATURE CITED

Anderson, D. F., Day, E. A., *J. Agr. Food Chem.* **14**, 241 (1966).
Dartey, C. K., Kinsella, J. E., *J. Agr. Food Chem.* **19**, 774 (1971).
Dartey, C. K., Kinsella, J. E., *J. Agr. Food Chem.* **21**, 721 (1973).
Franke, W., Platzeck, A., Eichhorn, G., *Arch. Mikrobiol.* **41**, 154 (1962).
Gehrig, R. F., Knight, S. G., *Nature (London)* **182**, 1237 (1958).
Gehrig, R. F., Knight, S. G., *Appl. Microbiol.* **11**, 166 (1963).
Hammer, B. W., Bryant, E. W., *Iowa State Coll. J. Sci.* **11**, 281 (1937).
Lawrence, R. C., *Nature (London)* **205**, 1313 (1965).
Lawrence, R. C., *J. Gen. Microbiol.* **44**, 393 (1966).
Lawrence, R. C., Hawke, J. C., *J. Gen. Microbiol.* **51**, 289 (1968).
Niki, T., Yoshioka, Y., Ahiko, K., *Int. Dairy Congr. Proc. 17th Sec. D:2* 531 (1966).
Patton, S., *J. Dairy Sci.* **33**, 680 (1950).
Rolinson, G. N., *J. Appl. Bacteriol.* **17**, 190 (1954).
Schwartz, D. P., Shamey, J., Brewington, C. R., Parks, O. W., *Microchem. J.* **13**, 407 (1968).
Starkle, M., *Biochem. Z.* **151**, 371 (1924).
Vinze, V. L., Ghosh, D., *Hindustan Antibiot. Bull.* **4**, 119 (1962).

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Volatile Retention during Freeze Drying of Aqueous Suspensions of Cellulose and Starch

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This paper studies the retention of ^{14}C-labeled 2-propanol in freeze-dried starch or cellulose suspensions. Among the variables affecting the retention level are concentration of solids and initial concentration of the alcohol in the suspension. The observed retentions can be explained by inclusion within the polymer chains, the predominant mechanism of retention, and adsorption. Cellulose gave a much lower retention than starch, probably because the low mobility of the chains in the highly crystalline cellulose reduces the capacity for retention of the alcohol through inclusion.

In the past few years, significant progress has been made in studies on the mechanism of volatile retention in freeze-dried foods. Most of these studies have been based on model systems, mainly carbohydrate solutions (Flink and Karel, 1970a,b; King, 1970; Rulkens and Thijssen, 1972; Thijssen and Rulkens, 1968) and water-soluble polymers (Chirife and Karel, 1973b; Chirife *et al.*, 1973). It is to be expected that studies on model systems, based on individual food components, could eventually lead to a better understanding of volatile retention in more complex food systems.

In this study we present results which characterize the retention of 2-propanol in model systems based on cellulose and starch, polysaccharides widely found in fruits and vegetables. The observed retentions are analyzed in terms of possible interactions between the polymeric substrates with volatile.

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EXPERIMENTAL SECTION

Model Systems Preparation. The model systems consisted of either cellulose powder (Whatman CC 41, mean particle size passing 200 B.S.S.) or starch (Merck, Soluble Starch), ^{14}C-labeled 2-propanol, and water. They were prepared by suspending the desired amount of cellulose or starch in water and adding 2-propanol; 0.1% (w/w) of carboxymethylcellulose (CMC) was added to facilitate the handling of the suspensions.

Five-milliliter aliquots of the suspensions were pipetted into 50-ml Erlenmeyer flasks and frozen immediately in liquid nitrogen to maintain the solids in the suspended state. The resultant sample thickness was about 4 mm. The samples were then freeze-dried for 48 hr at ambient temperature and at a chamber pressure of less than 100 μm in a Virtis freeze drier (model 10-MRTR).

Reagent grade 2-propanol was mixed with ^{14}C-labeled 2-propanol to give the desired specific radioactivity. The

radioactive propanol was obtained from International Chemical and Nuclear Corporation, Irving, Calif.

Humidification Experiments. In several experiments, freeze-dried samples were humidified by placing tared and weighed flasks in vacuum desiccators containing saturated salt solutions, which maintained the desired constant relative humidities.

2-Propanol Analysis. The 2-propanol content was determined by measuring the radioactivity of the samples with a liquid scintillation counter.

Reproducible measurements with a constant counting efficiency were obtained by dispersing the dried samples of cellulose or starch in water (to 10% w/w) and adding 1 ml of this suspension to 10 ml of the scintillator solution [2,5-diphenyloxazole (1 g), naphthalene (100 g), dioxane to 1000-ml volume]. The resulting dispersion was counted with a liquid scintillator counter (Beckman LSD series).

RESULTS

Figure 1 shows the effect of initial solids concentration on 2-propanol retention by freeze-dried cellulose and starch suspensions. In both cases the alcohol retention increases linearly with solids content in the range examined. For these experiments the initial concentration of 2-propanol was fixed at 0.1% (w/w) for the cellulose system and 0.05% (w/w) for the starch-based model.

For both cellulose and starch, when solids content is kept constant, relative retention increases as initial alcohol concentration decreases. The use of ^{14}C-labeled 2-propanol of relatively high specific radioactivity allowed for a wide range of concentration from 2–3 ppm to 5000 ppm for the starch system.

Results obtained during freeze drying of 20% (w/w) suspensions are shown in Figure 2. The curve which characterizes the behavior of starch suspensions is particularly interesting because the low volatile concentrations investigated are similar to those encountered in natural food systems. The 2-propanol retention in freeze-dried cellulose suspensions appears to level off at 7.3% retention as the initial volatile concentration is decreased.

Table I summarizes the alcohol retention (1-propanol or 2-propanol) observed during freeze drying of several model systems (carbohydrates and polymers). All the experiments were performed in very similar processing conditions (plate temperature, drying time, chamber pressure, frozen layer thickness) and system composition (solids concentration, volatile content), so the observed retentions give a direct indication of the particular ability of each solid substrate to retain the volatile. For high initial alcohol concentrations (0.5 to 1.0% w/w), the low molecular weight carbohydrates are much more effective than the polymeric systems (PVP, Dextran, Starch, Cellulose). However, at low volatile concentrations, the polymers are also able to produce significant alcohol retentions. The exception is cellulose, which even at low initial volatile concentration gives low retention values.

Readsorption of 2-propanol in the dry layer of cellulose during freeze drying was investigated in the following experiment. Samples were prepared by freezing alternate layers of a cellulose suspension containing no volatile and layers of a solution containing the volatile. Each layer was completely frozen before the next layer was added. The composition of the systems for these experiments was fixed: cellulose 20% (w/w) and 2-propanol 0.5% (w/w). During freezing and drying, the layers were separated by thin brass mesh to avoid any "contamination" between them. After the standard cycle of freeze drying (48 hr), the layers were separated for individual analysis. Excellent agreement was found among all the samples. It was observed that the amount of 2-propanol adsorbed in the layers originally containing no volatile was 31.5% of the retention found in the layers originally containing the volatile, the absolute amount being between 0.0244–0.0269 g of 2-propanol/100 g of cellulose.

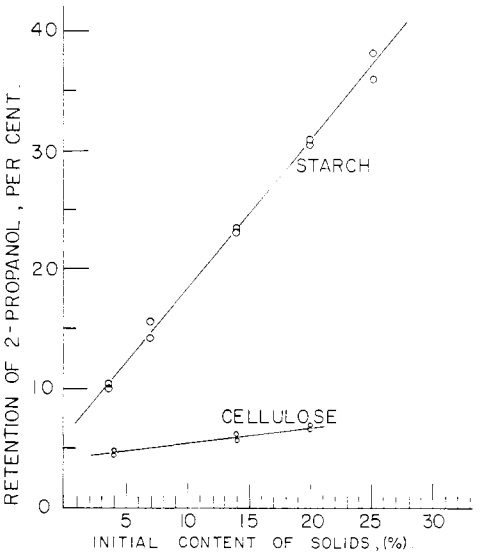

Figure 1. Effects of initial solids content on retention of 2-propanol in freeze-dried aqueous suspensions containing starch or cellulose. Initial 2-propanol content: 0.05% (w/w) in starch suspensions, and 0.1% (w/w) in cellulose suspension.

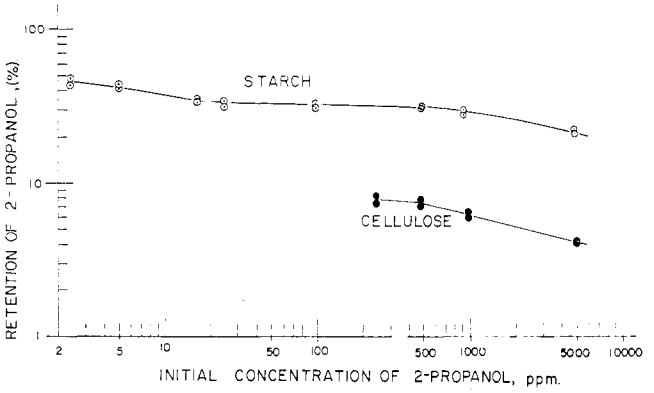

Figure 2. Effects of initial concentration of 2-propanol on retention in freeze-dried suspensions containing 20% of starch or of cellulose.

In another experiment, we studied alcohol release due to adsorption of water. Twenty percent suspensions of starch containing 0.05% (w/w) were freeze dried under standard conditions. These conditions resulted in the retention of 0.0775 g of 2-propanol/100 g of starch. The freeze-dried systems were then equilibrated to different relative humidities and the loss of alcohol was determined. Figure 3 shows the 2-propanol retention in freeze-dried starch humidified to 11, 52, and 75% relative humidity.

DISCUSSION

Two types of interactions between the solid substrates and the volatile will be discussed in connection with the mechanism of 2-propanol retention during freeze drying of cellulose and starch suspensions; they are inclusion and adsorption in the dry layer. The first one refers to an entrapment of the volatile between the polymeric units of cellulose and starch, and the second to the binding of the alcohol to specific sites of these polymers. Russell *et al.* (1937) studied the sorption isotherm of alcohols in cellulose. They found that after sorption of alcohol, evacuation at room temperature did not completely remove the alcohol. They postulated that as a result of the process of removal of alcohol, cellulose chains interact, forming internally stressed structures that hold the residual "solvent."

Table I. Alcohol Retention during Freeze Drying of Carbohydrate and Polymer Solutions

Solid	Concentration of initial solids, % (w/w)	Volatile	Initial concentration of volatile, % (w/w)	Retention, % Rapidly frozen samples	Retention, % Slowly frozen samples	Reference
			Initial alcohol concentration between 0.5–1.0%			
Maltose	20	1-Propanol	1.0	69.5		Chirife and Karel (1973b)
Maltose	18.8	2-Propanol	0.75	67.6		Flink and Karel (1970a)
Malto-dextrin	20	1-Propanol	0.75		80	Flink and Gejl-Hansen (1972)
Glucose	18.8	1-Propanol	0.75	47.8		Flink and Karel (1970a)
Glucose	18.8	2-Propanol	0.75	52.8		Flink and Karel (1970a)
Starch	20	2-Propanol	0.5	21.0		Present work
PVP	20	1-Propanol	0.5		25.5	Chirife *et al.* (1973)
PVP	20	1-Propanol	1.0	9.8	24	Chirife *et al.* (1973)
Dextran 10	20	2-Propanol	0.75	7.5		Flink and Karel (1970a)
Dextran 10	20	1-Propanol	0.75	4.2		Flink and Karel (1970a)
Cellulose	20	2-Propanol	0.5	4.0		Present work
			Initial alcohol concentration between 100–250 ppm			
Dextran 10	20	2-Propanol	100	56	97	Flink and Labuza (1972)
Maltose	20	2-Propanol	100	16	88	Flink and Labuza (1972)
PVP	20	1-Propanol	100		58	Chirife *et al.* (1973)
Starch	20	2-Propanol	100	38		Present work
Cellulose	20	2-Propanol	250	8.4		Present work

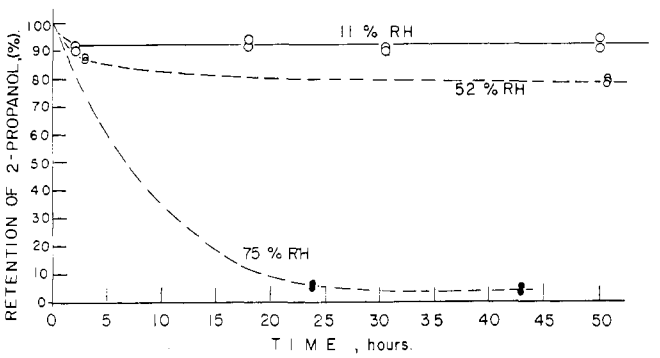

Figure 3. Retention of 2-propanol during exposure of freeze-dried starch suspensions to specified levels of relative humidity at 25°.

Staudinger *et al.* (1953) also showed that nonpolar liquids which do not react with cellulose cannot be completely removed by drying in vacuum. They also postulated an inclusion of the organic liquids produced when hydroxyl groups of the cellulose chains hydrogen-bond together.

We have demonstrated in previous studies that most of the retention of volatiles in water-soluble polymers, in particular dextran and polyvinylpyrrolidone, is due primarily to entrapment in microregions similar in their retention properties to those formed by low molecular weight carbohydrates (Chirife *et al.*, 1973; Flink and Karel, 1970a). However, in polyvinylpyrrolidone, adsorption also plays a role and, in fact, a small but significant readsorption of propanol occurs in dry layers formed during freeze-drying (Chirife and Karel, 1973). The "layered system" experiments reported in this paper show that readsorption of 2-propanol in the dry layer can contribute to retention in freeze-dried cellulose suspensions.

Sorption of alcohols in dry cellulose has been measured by several workers, including Lauer and Ayer (1957), Colombo and Immergut (1970), and Le Maguer (1972). Through measurements of heats of sorption, Colombo and Immergut (1970) found that the interaction cellulose–methanol involves hydrogen-bond formation which extended over a wide range of vapor coverage. However, sorption isotherms measured at high volatile partial pressures may not be valid at the low levels expected in freeze drying of model systems. A small amount of very active sites may be available for strong adsorption of small quantities of the volatile, leading to retention. Sorption of volatile compounds in starch was studied by a number of authors; Maier and Bauer (1972) suggest that most aroma compounds may be bound by hydrogen bonds or inclusion. Starch has the capacity to complex many ligand molecules such as aliphatic alcohols, fatty acids, and aliphatic hydrocarbons (Bear, 1944; Kuge and Takeo, 1967; Osman-Ismail and Solms, 1972; Takashi and Takeo, 1968). It was considered that complexing occurs mainly within the helical regions of the amylose fraction. Several possibilities were taken into account for the forces that stabilize the inclusion complex, namely hydrophobic bonding, hydrogen bonding, dipolar interactions, etc. (Kuge and Takeo, 1967). Based on this property of amylose, Solms *et al.* (1973) suggest that the formation of starch-inclusion complexes is an important mechanism of volatile retention during food processing. However, some characteristics which regulate the formation of such complexes strongly indicate that this is not the case for the 2-propanol retention during freeze drying of starch suspensions. To begin with, a certain minimum concentration of ligand was necessary to initiate the formation of insoluble complexes (Solms *et al.*, 1973). Also, reaction mixtures did not give inclusion compounds under constant temperature conditions but only if a temperature gradient was applied ranging from 90° to room temperature (Solms *et al.*, 1973). This suggests that the reaction requires some sort of activation. Furthermore, the humidification experiments shown by Figure 3 are another indication that no irreversible complex was formed, resulting in 2-propanol retention. Humidification to 75% RH produces almost total loss of retained 2-propanol. This effect can be attributed to the swelling effect of water molecules which permits the loss of the entrapped alcohol.

We may conclude that inclusion of the volatile between the polymer chains and readsorption in the dry layer are the main mechanism of retention. The low levels of retention observed with cellulose can be explained on the basis of the low mobility of the cellulose chains, as compared with starch or other polymeric materials.

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LITERATURE CITED

Bear, R. S., *J. Amer. Chem. Soc.* **64**, 1388 (1944).
Chirife, J., Karel, M., Flink, J., *J. Food Sci.* **38**, 671 (1973).
Chirife, J., Karel, M., *J. Food Sci.* in press (1973a).
Chirife, J., Karel, M., *J. Food Technol.* submitted for publication (1973b).
Colombo, E. A., Immergut, E. H., *J. Polym. Sci. Part C* No. 31, 137 (1970).
Flink, J. M., Karel, M., *J. Agr. Food Chem.* **18**, 295 (1970a).
Flink, J. M., Karel, M., *J. Food Sci.* **35**, 444 (1970b).
Flink, J. M., Labuza, T. P., *J. Food Sci.* **37**, 617 (1972).
Flink, J. M., Gejl-Hansen, F., *J. Agr. Food Chem.* **20**, 691 (1972).
King, J., *Crit. Rev. Food Technol.* **1**, 379 (1970).
Kuge, T., Takeo, K., *Agr. Biol. Chem.* **31**, 257 (1967).
Lauer, K., Ayer, J. E., *J. Polym. Sci.* **26**, 67 (1957).
Le Maguer, M., "Proceedings of the International Symposium on Heat and Mass Transfer Problems in Food Engineering," Vol. 1, Wageningen, Netherlands, Oct 24–27, 1972, p C1-1.
Maier, H. G., Bauer, A., *Die Starke* **24**, 101 (1972).
Osman-Ismail, F., Solms, J., *Die Starke* **24**, 213 (1972).
Rulkens, W. H., Thijssen, H. A. C., *J. Food Technol.* **7**, 79 (1972).
Russell, J. K., Maass, O., Campbell, W. B., *Can. J. Res.* **15**, 13 (1937).
Solms, J., Osman-Ismail, F., Beyeler, M., *Can. Inst. Food Sci. Technol. J.* **6**, A10 (1973).
Staudinger, H., Inder Birken, K. H., Staudinger, M., *Makromol. Chem.* **9**, 148 (1953).
Takashi, K., Takeo, K., *Agr. Biol. Chem.* **32**, 753 (1968).
Thijssen, H. A. C., Rulkens, W. H., *Ingenieur (The Hague)* **80**, 45 (1968).

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Inhibition of Cooked Flavor in Heated Milk by Use of Additives

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Four organic thiolsulfonates and three organic thiosulfates effectively inhibited cooked flavor when added to whole milk, prior to heating, at a level ranging between 0.003 to 0.05%. They are: 2-aminoethyl 2-aminoethanethiolsulfonate dihydrochloride; 5-aminopentyl 5-aminopentanethiolsulfonate dihydrochloride; 2-acetamidoethyl 2-acetamidoethanethiolsulfonate; cystine *S*-dioxide; 2-aminoethanethiosulfuric acid; *S*-sulfocysteine; and *S*-sulfoglutathione. Their inhibitory action is based on their ability to react with the sulfhydryl-containing compounds that form by heat denaturation of β-lactoglobulin. The possible implications of sulfhydryls removal with regard to the stale flavor control are discussed.

The appearance of a cooked off-flavor is the first measurable manifestation of chemical changes that occur in heated milk. The origin of the cooked flavor has been the object of much speculation. The consensus is that it is due to the presence of volatile sulfides and thiols that arise from thermal breakdown of serum proteins, primarily β-lactoglobulin, and of the proteinaceous material associated with the fat globule membrane (Hutton and Patton, 1952). That the cooked flavor is largely associated with sulfhydryl compounds is confirmed by its concomitant appearance with titratable (nitroprusside test) mercapto groups and by the fact that the flavor of milk depleted of mercapto groups becomes indistinguishable from that of unheated milk (Josephson, 1954). However, the chemical path, or paths, leading to formation of sulfides and sulfhydryls from sulfur-containing amino acids is still largely obscure. The hypothesis, as elaborated below, that volatile sulfur compounds in milk are byproducts of the Maillard reaction (Ellis, 1959; Maillard, 1912) has never been proposed but deserves consideration.

The occurrence of the Maillard reaction in dairy products has been extensively demonstrated, along with its nutritional and organoleptic consequences (Ferretti and Flanagan, 1971, 1972; Henry *et al.*, 1948; Patton, 1955). Reductones and dehydroreductones are important intermediate compounds in this reaction (Hodge, 1953). The latter are characterized by the presence of an α-dicarbonyl moiety or by the structure $-CO[C{=}C]_nCO-$, where n is zero or an integer. The dehydroreductones are Strecker degradation (Schönberg and Moubacher, 1952) agents, presumably responsible for the formation of many volatile compounds when systems containing amino acids and reducing carbohydrates are heated (Hodge, 1967). When cysteine is involved, hydrogen sulfide is one of the products (Kobayashi and Fujimaki, 1965; Schutte and Koenders, 1972). When methionine is one of the reactants, methanethiol is one of the main products (Ballance, 1961; Schutte and Koenders, 1972). The mechanism of the reaction has been studied in some detail by Schutte and Koenders (1972). Whereas the formation of dimethyl disulfide can be readily rationalized on the basis of methanethiol oxidation, the mechanism of formation of dimethyl sulfide from methionine (Ballance, 1961) is less obvious. One precursor of dimethyl sulfide in heated milk is an *S*-methylmethionine sulfonium salt, possibly originating from plant material (Keenan and Lindsay, 1968). The involvement of methionine and cystine in the production of volatile sulfur compounds in heated milk has been confirmed by Demott and Gibbs (1966) by using ^{35}S-labeled materials.

In addition to the dehydroreductones that may form by a Maillard-type degradation of carbohydrates, milk has natural constituents which can act as Strecker degradation agents on amino acids resulting from protein breakdown. The most likely candidates for such interaction are vitamins C and K. Milk, immediately after removal from the udder, contains vitamin C in the form of L-ascorbic acid (Hartman and Dryden, 1965), which is a reductone, but is fairly readily converted to the dehydro form. Vitamin K, on the other hand, having a naphthoquinone structure, would be ready to function as a degrading agent.

Gruenwedel and Patnaik (1971) recently reported a quantitative study of the previously known hydrogen sulfide and methanethiol release from L-cysteine and DL-methionine, respectively, by action of pyridoxal catalyzed by metal ions, including iron. Because 70 to 95% of vitamin

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