AN ABSTRACT OF THE THESIS OF

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Title: Edible Food Coatings to Control Potassium Sorbate Diffusion from Surface into Food Bulk: Characterization of the Diffusion Process in Polysaccharide Based Films

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Edible coatings controlling preservative migration from surface to food bulk could inhibit surface microbial growth which is often the main cause of spoilage for many food products. In this project we focused our attention upon methylcellulose, hydroxypropyl methylcellulose, and chitosan as the structural component for such edible films. These films were generally transparent and effective at thicknesses in the order of 20 to 100 μm. We expect them to have little impact on the sensory properties of a food.

Permeability cell measurements were used to evaluate the effect of coating composition. Further film characterization

included film thickness and electron microscopy studies. To gain an understanding of the permeation process, the permeability tests were done at 5, 24, 32, and 40°C.

Among these polysaccharide films, methylcellulose was the most promising diffusion barrier with a permeability constant of 3.4 and 1.4×10^{-8} (mg/sec cm^2)(cm)/(mg/ml) at 24 and 5°C, respectively. These barrier properties were enhanced by the incorporation of lipids into the film formulation.

The permeability of sorbates in methylcellulose and hydroxypropyl methylcellulose emulsified with lauric, palmitic, stearic and arachidic acid was found to depend upon the polysaccharide, the fatty acid chain length, and the number of fatty acid double bonds. Potassium sorbate permeation increased in the following order lauric> palmitic>stearic>arachidic acid. The effect of the double bond type, i.e. cis vs. trans was also determined. The permeability rate of potassium sorbate increased in the order of oleic>elaidic>stearic acid.

The effect of temperature on potassium sorbate permeability was analyzed using an Arrhenius activation energy model for the permeation process. Permeability determinations at four different temperatures showed excellent agreement with this model and suggest that the permeation process is diffusion controlled. Electron microscopy studies showed the absence of pores, channels or other defects which might be introduced during casting, drying, handling or permeability determination. This observation is consistent with our hypothesis that

potassium sorbate permeation is diffusion controlled. Furthermore, our experimental data suggest that the diffusion is controlled by the properties of the solvent embedded in the film. Further studies are required to confirm this hypothesis.

The effect of casting technique was examined by coating a pure polysaccharide film with a fatty acid mixture or bees wax and by laminating a fatty acid mixture or hydrogenated palm oil between two layers of pure polysaccharide films. Unfortunately, most of these films cracked easily and could not be tested in our permeability cell. On the other hand, hydroxypropyl methylcellulose films coated with bees wax showed exceedingly low potassium sorbate permeability values.

These modifications of the polysaccharide film properties reduced the potassium permeability down to 10^{-9} to 10^{-11} (mg/sec cm^2)(cm)/(mg/ml) depending upon temperature, film composition and film casting technique. A simplified procedure previously published was used to evaluate surface microbial stability enhancement. With this information a food processor can select the appropriate film, application procedure and film thickness to achieve the desired shelf life under ambient or refrigerated storage conditions.

EDIBLE FOOD COATINGS TO CONTROL POTASSIUM SORBATE DIFFUSION FROM SURFACE INTO FOOD BULK: CHARACTERIZATION OF THE DIFFUSION PROCESS IN POLYSACCHARIDE BASED FILMS

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INTRODUCTION

One of the oldest methods to control the microbial spoilage of foods is through control of moisture content. Descriptions of preservation by sun drying can be found in the Bible, in ancient Egyptian hieroglyphics and in the journals of Marco Polo (Labuza, 1980). The control of moisture content can be achieved by either water removal or by water binding so that the food becomes stable to both microbial and chemical deterioration (Labuza, 1980). Both approaches reduce the food water activity (a_w). The food will be microbiologically stable as long as $a_w < a_w^*$ where a_w^* is a critical water activity value which depends upon the food properties, the expected microbial load and the storage conditions (Torres, 1984).

Intermediate Moisture Foods (IMF) usually have a a_w of 0.6-0.85 (Quast and Teixeira Neto, 1976). Although this range controls the growth of most microorganisms, particularly

bacteria, there is a need to add fungistatic agents to control the growth of yeasts and molds.

Unsteady state environmental conditions such as temperature fluctuations are common occurrences in processing operations, storage and distribution of foods and can result in microbial spoilage. For instance, a very small change in environmental temperature, can cause a high surface a_w due to condensation of moisture inside the package, particularly of IMF (Torres, 1987; Quast and Teixeira Neto, 1976). This results in a very suitable condition for the surface growth of microorganism even though the average moisture content and a_w of the product are still at a safe level (Torres, 1987). The effect of unsafe high surface a_w can be prevented by high surface preservative concentrations maintained by edible coatings reducing preservative diffusion from food surface to food bulk (Torres *et al.*, 1985a).

A similar surface microbial growth problem affects meat products, specially fresh, refrigerated poultry and fish. Such products have a relatively short shelf-life. For instance, in a retail outlet, a fresh broiler can be expected to have an initial concentration of 10^4 to 10^5 microorganisms/cm_2 and can be stored only for a few days at 3-5°C and still maintain its freshness (Cunningham, 1981). Surface growth of microorganisms causes spoilage usually accompanied by the development of malodors, known as sour smell or tainted smell (Mahadevan, 1970). In most cases surface spoilage of these

products is associated with various species of *Pseudomonas* and *Achromobacter*. Other type of bacteria also involved are *Proteus*, *Flavobacterium* and *Salmonella*. While surface microbial growth leads to an unacceptable product, the bulk of the meat remains essentially sterile (Gill, 1979). These meat products can be stabilized by freezing, but at a high energy cost and lack of consumer convenience.

These observations show that in many food products, including refrigerated meats and IMF's, spoilage is caused by surface microbial growth. Very often it begins the degradation process that ultimately renders the product unacceptable for consumption. These surface microbial stability problems could be controlled by high surface preservative concentrations maintained by edible coatings impermeable to the diffusion of these antimicrobial agents.

In recent years, the use of surface application of chemical preservatives has been investigated as a solution to surface microbial growth. Sorbic acid and its salt have been reported to be an effective antimicrobial agent when applied on the surface of poultry (Cunningham, 1981; Robach and Ivey, 1978; Perry *et al.*, 1964).

A 7.5% potassium sorbate solution in a 70:20:10 propylene glycol-water-glycerin mixture at 60^{o}C extended the shelf life of poultry (Perry *et al.*, 1964). Cunningham (1981) studied the effectiveness of potassium sorbate dip to control bacterial growth and showed that when fresh broiler drumsticks with an

initial bacterial growth count of $10^2/cm^2$ were dipped in 10% potassium sorbate for only 30 seconds they had counts averaging $10^5/cm^2$ after 10 days at 4°C, and $10^6/cm^2$ counts after 12 days. The controls which were dipped in water and also stored at 4°C had counts of $10^7/cm^2$ in 10 days. Robach and Ivy (1978) reported that potassium sorbate reduced the number of the viable *Salmonella*/cm^2 significantly throughout the storage period.

However, shelf-life extension by these surface treatments is limited and microorganisms overcome the preservative effect of sorbate. The disappearance of effectiveness is related to sorbate permeation from the treated surface into the meat bulk. Diffusion results in a reduction of sorbate concentration on the surface where microbial spoilage is occurring.

RESEARCH APPROACH

The approach in our research has been to consider the surface as a separate food region which has specific microbial stability problems. The purpose of this study was then to find an inexpensive polymer to be used as the structural element of edible coatings controlling surface preservative concentration and thus improve the surface bacteriostatic barriers to inhibit surface microbial outgrowth. The antimicrobial agent used was potassium sorbate (K-sorbate). An edible, organoleptically acceptable coating could be used to reduce sorbate diffusion into the food bulk. We focused our attention on polysaccharides such as chitosan, methylcellulose and hydroxypropyl methylcellulose as the barrier retarding K-sorbate diffusion from the surface into the food bulk.

Torres *et al.* (1985a) reported that a zein coating was an effective barrier as shown by permeability and microbial challenge tests. The zein coated samples with bulk a_w of 0.88 stored at 30°C under constant relative humidity (RH) 88% remained stable for over 16 days while uncoated controls were stable for only 2 days. Coated samples with bulk a_w of 0.85 remained stable for more than 28 days. Uncoated controls were stable for only 3 days. However, zein is expensive and is not produced commercially. Polysaccharides such as modified cellulose, methylcellulose, hydroxypropyl methylcellulose and

chitosan are inexpensive and produced commercially for a large number of applications.

RESEARCH GOAL

Our research goal was to evaluate the K-sorbate permeation process through chitosan, methylcellulose and hydroxypropyl methylcellulose films with an understanding of the physical and chemical properties controlling this permeation process. This understanding of the permeation process should aid the design of edible coatings to maintain a high K-sorbate surface concentration.

RESEARCH OBJECTIVES

The objectives of this investigation were:

1) To develop formulations and casting procedures for chitosan, methylcellulose and hydroxypropyl methylcellulose based films to be tested on a K-sorbate permeability cell.

2) To determine the K-sorbate permeability properties of chitosan, methylcellulose and hydroxypropyl methyl cellulose based films.

3) To evaluate the effect of the incorporation of fatty acids, triglycerides and bees wax on the K-sorbate permeability coefficient of methylcellulose and hydroxypropyl methylcellulose based films.

4) To evaluate the effect of casting modifications on the K-sorbate permeability coefficient of methylcellulose and hydroxypropyl methylcellulose based films.

5) To model the effect of temperature on the K-sorbate permeability constant using the Arrhenius activated energy and the Stokes-Einstein diffusion equations.

6) To characterize the physical properties of original and modified methylcellulose and hydroxypropyl methylcellulose and chitosan based films by electron microscopy.

LITERATURE REVIEW

METHYLCELLULOSE AND HYDROXYPROPYL METHYLCELLULOSE

Methylcellulose (MC) and hydroxypropyl methylcellulose (HPMC) are cellulose ethers obtained by substitution of the hydroxyls of the cellulose by methyl or hydroxypropyl groups (Windover, 1962). An idealized formula at a degree of substitution (D.S.) of 1.5 is shown in Fig. 1 (Anonymous, 1987).

The properties of the cellulose ethers will vary widely with the D.S., uniformity of the substitution, and type of substituent group. For example, varying the methoxyl content yields a series of MC ranging from alkali-soluble to organo-soluble. The water-soluble commercial derivatives contain approximately two methoxyl groups per anhydroglucose ring, while the organo-soluble derivatives contain approximately three such groups (Windover, 1962). Products with few methoxyl groups are soluble only in aqueous alkali solutions. Also, a decrease in the molecular weight of the product as indicated by intrinsic viscosity will improve solubility. The introduction of groups other than methoxyl groups can also effect solubility and other properties of cellulose. The other groups include ethoxyl, carboxyl, hydroxyethyl, and hydroxypropyl (Glicksman, 1969).

MC has been approved by the U.S. Food and Drug Administration (FDA) and the Food and Agriculture Organization. MC (U.S.P.) with methoxyl content of 27.5 - 31.5% has been

placed on the list of additives generally recognized as safe (GRAS). Use of MC in meat and poultry products come under jurisdiction of the U.S. Department of Agriculture (Overeen, 1984; Klose and Glicksman, 1972). The FDA defines the food additive HPMC as a cellulose ether containing propylene glycol group attached by an ether linkage and containing, on an anhydrous basis, not more than 4.6 hydroxypropyl groups per anhydroglucose unit.

HPMC is permitted as a food additive for use as an emulsifier, film former, protective colloid, stabilizer, suspending agent, or thickener (Anonymous 1985b; Battista, 1958). Both MC and HPMC are used in many food products, including bakery products, salad dressings, ice cream and other dairy products, whipped toppings, frozen foods, and dietetic foods. They have also a broad application in the pharmaceutical industry (Battista, 1958) such as in bulk laxatives, ointments, lotions, emulsions, suspensions, and tablets. Other industrial uses include paper, textile, cosmetic, adhesive and ceramic products (Croll and Kleinlein, 1986; Anonymous, 1985b, 1982a,b; Ford et al., 1985; Greminger and Krumel, 1980; Glicksman, 1972).

MC was first prepared in 1905 by Suida, who reacted alkali cellulose with dimethyl sulfate. Manufacturing of MC is comparatively simple. Cotton linters or wood pulp of cellulose fiber is used to react and swell with caustic soda to produce alkali cellulose, which is then reacted with methyl chloride to give the methyl ether of cellulose according to the following

reactions (Scheffel, 1968; Greminger and Krumel, 1980):

Main Reaction:

$$R_{cell}OH + NaOH \rightarrow R_{cell}\text{-}ONa + H_2O$$

cellulose radical — alkali cellulose

$$R_{cell}\text{-}ONa + CH_3Cl \rightarrow R_{cell}\text{-}OCH_3 + NaCl$$

alkyl halide — methylcellulose

Side Reactions:

$$CH_3Cl + NaOH \rightarrow CH_3OH + NaCl$$

$$CH_3Cl + H_2O \rightarrow CH_3OH + HCl$$

$$CH_3OH + NaOH = CH_3ONa + H_2O$$

$$CH_3ONa + CH_3Cl \rightarrow CH_3OCH_3 + NaCl$$

For the production of HPMC, propylene oxide is added to cellulose at elevated temperature and pressure which reacts as follows:

Main Reaction:

$$R_{cell}OH + CH_2\text{-}CH(CH_3)\text{-}O \xrightarrow{NaOH} R_{cell}\text{-}O\text{-}CH_2\text{-}CH(OH)\text{-}CH_3$$

Side reaction:

$$CH_2\text{-}CH\text{-}CH_3 \xrightarrow[CH_3Cl]{NaOH} \text{glycols + glycol ethers}$$

The hydroxyl groups present in the side chain is also available for further reaction with the oxide. This results in the formation of side chains containing more than one mole of combined propylene oxide.

The relative amounts of methyl and hydroxypropyl substitution are controlled by the weight ratio and concentration of sodium hydroxide and the weight ratios of methyl chloride and the weight ratios of methyl chloride and propylene oxide per unit weight of cellulose (Greminger and Krumel, 1980). A continuous process for preparing MC is fully described by Meltzer (1976).

1. Solubility

As a cellulose ether is prepared, the gradual increase in D.S. is accompanied by a progress from insoluble cellulose through solubility in aqueous alkali, to solubility in water, and finally to solubility in water-alcohol or hydrocarbon-alcohol mixtures. MC is soluble in cold water at 1.3-2.6 D.S. and increasingly soluble in ethanol above 2.1 D.S. Maximum water solubility of MC occurs at a D.S. of 1.64-1.92 (27.5-31.5% methoxyl) with viscosity principally dependent upon

molecular chain length (Whistler and Daniel, 1985; Greminger and Savage, 1959).

The solubility of MC depends on the D.S., degree of polymerization (D.P.), and the distribution of methyloxyl groups of these. The D.S. appears to be the most important factor (Gloor and Klue, 1955). The generally accepted explanation of the water and aqueous alkali solubility is that the introduction of the methyl groups causes a disruption of the cohesive forces between hydroxyl groups on adjacent cellulose chains, thus rendering free hydroxyl groups accessible for hydration. Solubility in organic solvents is due to an excess of methyl groups over the remaining free hydroxyl groups (Croon, 1963). Hydrophobic polymers dissolve by solution and swelling of their structural layers. This mechanism is very different from the dissolution of other crystalline materials such as common salt (Windover, 1962).

There are three basic steps in making good gel-free solutions of MC and HPMC (Anonymous, 1981): (1) Disperse to allow each particle of cellulose ether to be wet out; (2) Agitate to keep dispersed; (3) Dissolve to hydrate. It is important to disperse the particles before attempting to dissolve them. Good dispersion prevent lumping caused by the formation of a gel. Since the MC are insoluble in hot water, the use of hot water assures wetting of all portions of the particle prior to solution in cold water. If cold water is mixed with the powder first, it creates a gelatinous membrane on

the outside of the particles which causes lumping and slow diffusion of water to the interior of the particle (Windover, 1962).

Another method proposed by Greminger (1959) is to prepare a MC slurry in high-purity methanol, ethanol, or low molecular weight glycols. The slurry will dissolve readily in cold water. Other techniques are dispersion in oils and in other dry ingredients.

The mixed ethers of cellulose containing both hydroxypropyl and methyl substituents are also widely used. Table 1 shows the chemical composition of MC and HPMC (Ganz, 1977).

2. Viscosity

The viscosity of MC depends mainly upon its molecular chain length and D.P. Solutions of commercial products (2%) with molecular weights of 13,000 to 140,000 have viscosities in the range of 10 to 19,000 cP at 20°C. The viscosity is concentration dependent, and it limits the preparation of solutions at higher concentrations (Glicksman, 1969).

Commercial MC and HPMC are available in a wide range of viscosities varying from 5 to 75,000 cP (2% w/w solutions at 20°C). The MC and HPMC viscosity/concentration relationship can be approximated by Philippoff's equation (Greminger and Krumel, 1980):

$$\eta/\eta_o = (1 + 0.125[\eta]c)^8 \quad (1)$$

where:

η = apparent viscosity

η_o = solvent viscosity

$[\eta]$ = intrinsic viscosity, dl/g

c = concentration, g/dl

Effect of concentration

The viscosity of MC and HPMC solutions increases rapidly with concentration and becomes almost a straight-line relationship when plotted on a semilog basis (Figs. 2 and 3). Two viscosity types of HPMC or MC can be blended to obtain an intermediate viscosity. Fig. 4 shows a blending chart, which can be used to determine the result of blending the various amounts of two viscosity types or, conversely, one can determine the amount of two types of known viscosity needed to achieve a desired viscosity. The viscosity of a mixture of a MC (or HPMC) **A** with a MC (or HPMC) **B** can be obtained as follows. The viscosities of the two components **A** and **B** are located on the vertical axis at 0 and 100% **B** concentration, respectively. These two points are then connected with a line. The point corresponding to the desired final viscosity is then located on the vertical scale and a horizontal line is drawn from it to the first line drawn. A vertical line drawn from this intersection to the bottom scale will reveal the percent **B** needed to make

up a blend with the specified viscosity (Anonymous, 1987; Greminger and Krumel, 1980).

Effect of temperature

When a cold solution of MC or HPMC is heated, the viscosity decreases. Fig. 5 shows the effect of temperature on viscosity of a 5% aqueous MC solution of medium viscosity. A temperature is eventually reached at which small increases in temperature produce rapid increase in viscosity. Within the range of a very few degrees, the solution transforms to form gel. The temperature at which this occurs is the gelation temperature. By lowering the temperature below this point, and stirring, the gel can be returned to the original smooth flowing solution (Mantell, 1947).

Effect of pH

MC and HPMC are nonionic polymers. Therefore the viscosity of water solutions is not affected by a change in pH. In general the viscosity of these type polymer solutions is stable within a pH range of 3-10 with no apparent changes in the color, solubility, or surface tension properties of the solution (Anonymous, 1987; Glicksman, 1969; Mantell, 1947). However, the best viscosity stability is obtained when the pH is hold between 6.0 and 8.0, and when the solutions are protected from light, heat, and the action of microorganisms (Anonymous, 1984, 1976).

3. Thermal gelation

A thermally reversible gelation of aqueous solutions of macromolecules is characterized by the formation of a coherent continuous three-dimensional crosslinked network structure (Morris, 1986). It is well known that reversible crosslinks must exist in any reversible gel. Hydrogen bond, hydrophobic bonds and dipolar interactions between polymer chains are candidates for reversible crosslinks (Kato *et al.*, 1978). Since this sol-gel transformation is reversible within a narrow temperature range, it does not involve the making or breaking of any covalent bonds. When the temperature is decreased or increased beyond the gelation temperature, and at suitable concentrations, the polymer begins to reconstitute the original solid-state structure. Gelation is therefore an intermediate nonequilibrium metastable state in which a three-dimensional network structure is formed due to secondary valence forces (Sarkar, 1979).

Gelation of MC and HPMC solution is primarily caused by the hydrophobic interaction between molecules containing methoxyl substitution (Fig. 6). At lower temperatures, molecules are hydrated and there is little polymer-polymer interaction other than simple entanglement. As the temperature is increased, the molecules gradually lose their water of hydration, which is reflected by a drop in viscosity. Eventually, when a sufficient but not complete dehydration of the polymer occurs, a polymer-polymer association takes place and the system

approaches an infinite network structure reflected by a sharp rise in viscosity. These gels are completely reversible in that they are formed upon heating yet will liquefy to the original consistency upon cooling (Roots *et al.*, 1980; Sarkar, 1979; Gloor *et al.*, 1955).

The gelation temperature depends on the type of ethers and the D.S. Table 2 shows gel temperatures for various MC and HPMC types. In general, the gelation temperature increases with the decrease in methyl substitution which can be interpreted as resulting from increased hydrophobic interactions (Vacher, 1940). The gel point can be raised by the introduction of hydroxypropyl groups. Depending on the amounts and the ratios of methyl and hydroxypropyl groups, the gel point can be raised up to 85^{o}C (Klose and Glicksman, 1972).

Applications of the thermal gelation phenomenon

The thermal gel property of MC and HPMC has a large number of applications in products such as bakery and pie and pastry fillings (Anonymous, 1982b,d). The advantage of cellulose ethers is that they thicken (gel) at cooking temperatures, while starches typically thin down. These hydrocolloids help to maintain normal consistency through the cooking phase. In many adhesive and coating techniques the temporary gelation of these cellulose ethers provides viscosity control during high temperature curing (Sarkar, 1979).

Effect of viscosity and concentration

The viscosity of MC and HPMC gums has little effect on gel temperature. However, increasing the concentration of the solution lowers the gel temperature (Anonymous, 1987, 1986c). Fig. 7 shows gel temperature as a function of concentration of selected cellulose ethers.

Effect of heating rate and agitation

A fast rate of heating tends to raise the gel temperature of solutions of MC and HPMC. Increasing agitation of the solution (a high shear rate) also raises the gel point. In some instances, continued rapid agitation can alter both the strength and texture of the gel (Anonymous, 1987).

Effect of additives

Thermo-gelation properties is affected by most additives such as, electrolytes and non-electrolytes (Anonymous, 1987; Saarnivaara and Kahela, 1985). Electrolytes such as sodium chloride, magnesium chloride, sodium carbonate and sodium phosphate, as well as non-electrolyte such as sorbitol, sucrose, polyethylene glycol, and glycerol depress the gelation point of MC and HPMC solutions (Anonymous, 1987; Saarnivaara and Kahela, 1985; Iso and Yamamoto, 1970). Most likely these solutes have a greater affinity for water and dehydrate the cellulosic polymer.

On the other hand ethanol, propylene glycol and polyethylene glycol 400 raise the gel point. Although these compounds have

greater affinity for water than the cellulosic polymer, they act as solubilizers due to their solvent power and thus increase the gel temperature (Anonymous, 1987).

Fig. 8 shows that 5% ethanol decreases by 9 and 6°F the gel point of 2% MC (Methocel A4M) and HPMC (Methocel F4M), respectively. Fig. 9 shows that the same amount of propylene glycol required to increase the thermal gelation temperature of a MC solution by 7°F will increase the one for a HPMC by 18°F (Anonymous, 1987, 1986d).

Textural characteristics

The type of cellulose ether determines the texture of the thermal gel structure and the temperature at which the solution gels. As shown in Table 2, strong and elastic gels are obtained with MC while HPMC yields softer gels (Anonymous, 1986d).

4. Rheological properties

MC and HPMC solutions have typical non-newtonian properties. Their behavior is primarily pseudoplastic because the long-chain molecules tend to orient themselves in the direction of flow. Therefore, as the shear stress is increased, the resistance to flow decreases. Solutions of low viscosity, i.e. low molecular weight are less pseudoplastic than solutions of high viscosity polymers (Glicksman, 1969, Greminger and Krumel, 1980).

5. Compatibility

The compatibility of MC and HPMC with other additives used in film formation is important for the optimization of the film formulation and casting conditions (Sakellarious *et al.*, 1986).

MC and HPMC are non-ionic and thus not affected by ordinary concentration of electrolytes. Table 3 shows the concentrations of additives that will still allow a 2% solution of MC or HPMC to gel. Above these concentrations a salting-out phenomenon is observed (Anonymous, 1987). This results in some decrease in viscosity and the appearance of cloudiness in the solution. In borderline cases, this salting-out may not be immediately apparent, but may occur upon standing (Anonymous, 1976).

Because of the difference in the amount of organic substitution, solutions of HPMC are less affected by salts than MC solutions (Anonymous, 1987). Certain ions substantially reduce the gel/sol transition temperature. This implies that the strength of the hydrogen bond which cross-links the polymers is weakened and that the dissolved ions decrease the hydration of the cellulose ether (Abraham and Ketteerson, 1986).

Univalent ions such as thiocyanates, iodides, bromides, and the chlorides have less effect on HPMC and MC solutions as compared to polyvalent ions such as carbonate, sulfate or phosphate which can cause polymer precipitation (Mantell, 1947). Precipitation can be reduced by modification of MC with

sodium carboxymethoxyl or alkoxyl (Greminger and Savage, 1959).

MC or HPMC are compatible with most water-miscible organic solvents. Water solution of MC and HPMC can be diluted infinitely with alcohol without precipitation. Dry MC and HPMC will not disperse in an aqueous alcohol mixture containing in excess of 40% alcohol. They must be first dissolved in water. Stable dispersions can be frequently prepared in this manner (Mantell, 1947).

6. Resistance to microorganisms

MC and HPMC are resistant to attack by microorganisms. In general, the compounds with higher D.S. are more resistant to microbial attack (Anonymous, 1987).

7. Food applications

As shown in Table 4 MC and HPMC have a broad range of use in several industries (Greminger and Krumel, 1980; Berger, 1962). As shown in Table 5 food application include their use as emulsifying, thickening, binding, lubricating and foam stabilizing agents (Morley, 1984; Greminger and Krumel, 1980; Ward and Seib, 1970). Numerous other food applications have been developed on the basis of their film forming abilities (Ward and Seib, 1970).

MC and HPMC dissolve in cold water and yield smooth, clear solutions. The most important physical property of MC and HPMC is their stability when used as thickeners of aqueous solutions.

MC and HPMC are not digestible and make no caloric contribution to the diet (Whistler and Daniel 1985). Another nutritional advantage is that they do not contain sodium in their chemical structure (Greminger *et al.*, 1959). They produce a feeling of fullness or satiety due to their ability to hold many times their own weight in water.

The thickening and texture-modifying properties of nonionic cellulose ethers (MC and HPMC) have been used in gravies, dry milk shake mixes, synthetic breakfast drinks, dry foods, and sherbets (Krumel and Sarkar, 1975).

In pie and pastry fillings, MC and HPMC improve the consistency, texture, and shelf life of the product (Anonymous, 1982b; Davidson *et al.*, 1969). They provide excellent freeze-thaw stability, reduce syneresis and promote growth of smaller, more uniform ice crystals. Furthermore, they do not mask fruit flavors and avoids the starchy taste so often present in all-starch fruit fillings. In pie and pastry filling, MC and HPMC significantly improve freeze-thaw stability. All-starch fillings tend to retrograde at low storage temperature. Addition of MC and HPMC reduces retrogradation and ensures good, consistent product quality through both freezing and thawing (Anonymous 1982b).

MC and HPMC gels have the very desirable property of stability at temperatures near the freezing point and do not undergo separation into phases upon freezing. This property is used in mixtures of meat, fish, or fowl with vegetables, sauce, fruits, and vegetables (Anonymous, 1987; Whistler and Daniel, 1985). Combinations of frozen fruits and vegetables are also possible. MC is used to hold these patties together by gelation during frying and to provide the moisture required to prevent burning of the vegetables (Greminger and Savage, 1959).

In food products such as tomato based sauces, fruit pie fillings, and certain pet foods which are acid and processed by retorting, the thickener/stabilizer must be able to function even under the severe conditions of low pH values (<4) and high temperature/long time (140^{o}C, >30 minutes) processing conditions (Anonymous, 1982c). Fig. 10 shows that MC solutions are more resistant to viscosity loss than other thickening agents commercially used.

The important functional properties of MC in bakery products are thermal gelation, moisture and gas retention, freeze-thaw stability, surfactancy, insolubility in fats and oils, and thickening. MC in baked food increases water absorption and retention and confers a degree of resistance to oil absorption in deep-fried items, such as doughnuts (Whistler and Daniel, 1985).

The gelation properties of MC and HPMC are used as dough strengthener for low-gluten bakery products (Sarkar, 1979). MC can improve the baking properties of nonwheat or gluten free wheat flours. MC, specially with 4,000 cp increases gas retention in sorghum bread and improves the texture of both sorghum and barley bread (Hart *et al.*, 1970).

Some of the properties of the MC are similar to the gluten present in flour (Hart et al., 1970), but they are not affected by proteases, mineral salts, or acids, and are affected only mildly by common oxidizing agents. In addition MC gels during elevated baking temperature, thus assisting gas retention (Nishita *et al.*, 1976; Hart *et al.*, 1970) during baking without increasing the toughness of the finished product and resulting in an increased product volume and a more uniform texture (Hart *et al.*, 1970; Glicksman, 1969).

Thermal gelation property of MC reduces boil-over during baking due to the formation of a gel structure at elevated temperatures. This gel formation is a reversible phenomenon. Upon cooling, the pie filling returns to its usual consistency. A combination of both thermal gelation and moisture retention properties of MC, inhibits moisture migration from the filling to the crust during baking (Lindsay, 1985; Anonymous, 1982a).

The synergistic effect of MC and HPMC on the viscosity of modified waxy maize starches is depicted in Figs. 11, 12 and 13 (Anonymous, 1982a). Fig. 11 shows how a low concentration of MC

used in combination with starch produces an increase in viscosity over the product with starch alone. Because of this synergistic relationship, it is possible to reduce starch levels in many food formulations (Meltzer, 1976). It is also important to note the low initial viscosity which allows easier mixing and less damage to the fruit (Anonymous, 1986b; 1982c). Fig. 12 shows the same phenomenon when HPMC is used instead of MC. Fig. 13 demonstrate another application possibility. In this case the MC is added when the product is hot and does not solubilize. Solubilization occurs later during the cooling period. This has significant advantages in retort processing.

The combination of hydrophobic and hydrophilic regions in MC and HPMC reduces surface tension and interfacial tension of aqueous systems to values of 41 to 55 dyne/cm and 18 to 28 dyne/cm, respectively, and thus can be considered moderate emulsifiers. Since they are polymeric materials, they are active surfactants at very low use levels ranging from 0.001 to 1.0% w/w (Anonymous, 1987; Davidson, 1980). This allows the development of cake mixes with partial egg content replacement with MC (Anonymous, 1982a; Glicksman, 1969; Weaver *et al.*, 1957).

The heat-gelation, adhesive, and grease-barrier properties of MC and HPMC provide a combination of effects especially desirable in dipping batters for deep fried breaded foods (Sarkar 1979). A 1% concentration of HPMC based upon the total

weight of the wet batter has been recommended as an appropriate usage level. In the case of prefried foods MC and HPMC have the dual role of holding moisture in the product and at the same time reducing oil migration into the product (Anonymous, 1986b; 1982e; Whistler and Danniel, 1985). The HPMC can also be used in a conventional batter to control viscosity and to permit dipping at lower batter solids (Greminger and Savage, 1959). MC reduces by 7 to 10% the absorption of cooking oil into fresh cut potatoes (Anonymous, 1982e).

8. Edible film forming applications

Cellulose and its derivatives yield high-quality films and fibers because of the linear structure of the polymer backbone. The food approved nonionic cellulose ethers all yield water soluble films. MC films are tough and flexible (Krumel and Lindsay, 1976; Row, 1976). Edible film prepared of MC and HPMC have been utilized in protective coating for various food applications, such as meat, bakery, cereal, fruit and nuts, jelly, candy and by the confectionery and pharmaceutical industry (Saarnivaara and Kahela, 1985).

The film formation and thermal gelation of MC act together to provide a barrier against excessive penetration of frying oil and the result is less greasy fried food (Anonymous, 1986e; Daniel, 1985; Whistler and Glicksman, 1969). Similar results have been observed during frying of extruded and frozen french

fries and onion rings. Although both polymers, MC and HPMC are water soluble, HPMC is more easily hydrated because of the increase in size of the substituents opening up the molecular structure (Row, 1976). MC being the least hydrophilic of the water-soluble cellulose ethers, can be expected to be the most resistant to passage of water vapor (Kester and Fennema, 1986). In a study of the barrier properties of MC films, it was noticed that adding polyethylene glycol 400, improved its water-vapor barrier properties (Kamper and Fennema, 1985).

A number of additives can be added to improve the protective properties of edible films, which is generally poor compared to that of non-edible materials. One possibility is to add antimicrobial agents such as sorbic acid, pimaricin or by adding antioxydant agents such as tocopherols or ascorbyl palmitate. Using an external layer of high antimycotic or antioxidant concentration and a diffusion barrier leads to high and long lasting surface concentrations. Therefore the diffusivity of the additive within the film and the food is of particular importance (Guilbert, 1986).

The coating solution can be applied by dipping, misting, spraying or other methods. When water-alcohol mixtures are used as the solvent for MC, HPMC and fatty acid mixtures, the alcohol speeds up the evaporation to produce a non-greasy, almost transparent, shiny film, controlled by the amount and type of fatty acid present in the emulsion.

MC and HPMC can be used in confectionery products and candy items to maintain the desirable levels of moisture. The moisture barrier retains desirable moisture within the food, and excludes absorption of external moisture which would result in undesirable stickiness and recrystallization.

Edible coatings can also help retain desirable nutrients. For example, Peil *et al.* (1981) designed a rice-fortification method using MC and HPMC to retain significant quantities of added nutrients during the common practice of boiling rice in excess water. This polymer system was used to fortify rice with thiamine, niacin, riboflavin, vitamin A, and iron. When samples of this rice were cooked in excess water, retentions were: thiamine 18%; niacin, 18%; riboflavin, 21%; vitamin A, 70%; iron, 100%. This approach could be an attractive means of preventing nutrient deficiencies because it is relatively inexpensive, provides the opportunity for simultaneously adding several micronutrients and it requires only passive consumer participation (Peil *et al.*, 1981). Their data indicated that release of nutrients was primarily via diffusion through the polymer. The high retentions of vitamin A and reduced iron, both of which are not significantly water-soluble, are consistent with such a mechanism.

Edible films composed of a blend of HPMC and lipids were studied by Kamper and Fennema (1985, 1984a,b). Of particular interest were bilayer films consisting of either stearic or a

blend of stearic and palmitic acid as one layer and HPMC as the other. Films were tested for resistance to water vapor permeability using a relative humidity differential of 85%, and found to be excellent barrier to water vapor transmission. They noted also that a C18-C16 emulsion-film provided good barrier properties to the transfer of water vapor if the hydrophilic side of the film is maintained in a dry condition. This film exhibited permeability values (g mil m^{-2} day^{-1} mm Hg^{-1}) of 0.5 at 40°C, 0.3 at 25°C, 1.7 at 5°C, and 6.0 at -19°C. These films might be useful in retarding the transfer of moisture in foods having two or more layers with different water activities.

For example, crackers and tomato paste were studied because of their large difference in a_w, and their similarity to existing products (for example, pizza crust/pizza sauce). The bilayer film substantially slowed down the transfer of water from the tomato paste to the crackers during 14 days at 25°C and 21 days at 5°C. During 70 days at -20°C, the film essentially stopped the transfer of water from the tomato paste to the crackers (Kamper and Fennema, 1985).

Another possible use for such films is as a barrier to the transport of gases such as oxygen and carbon-dioxide which significantly affect storage stability of food products.

Adhesion is another important coating design factor, particularly in the case of battered and breaded products.

Suderman *et al.*, (1981), have studied the adhesion of a commercial breading mix to poultry skin. Protein sources used were whey, soy, nonfat milk, egg albumen and gelatin. Additives studied were sodium alginate, carboxymethyl cellulose (CMC), guar, tragacanth and xanthan. Among the proteins, gelatin and egg albumen most effectively improved adhesion. Among the hydrocolloids studied only CMC improved adhesion. They reported no significant adhesion difference between egg white and HPMC.

Cellulose ether films have also been used in the bakery industry to prepare cold-water dissolving edible pouches containing small amount ingredients such as dough conditioners and vitamin supplements. The pouch can be made by a coating process using the cellulose ether mixed with various food grade plasticizers that improve its film-forming characteristics and allow it to be heat sealed. These pouch packs, distributed in a convenient dispenser carton, could save the baker time and eliminate waste in measuring ingredients from bulk quantities without the trouble of opening small unit packages (Glicksman, 1969).

9. Pharmaceutical applications

As shown in Table 6, MC and HPMC have a large number of applications in the pharmaceutical industry (Raw and Kayes, 1984; Greminger and Krumel, 1980). These polymers are used

extensively in the coating of tablets and the fabrication of capsules (Sakellariou *et al.*, 1985; Saarnivaara and Kahela, 1985; Aulton *et al.*, 1984; Row, 1985, 1982b, 1981, 1980, 1976; Row *et al.*, 1980; Entwistle and Row, 1979). Films and coating can be prepared by casting, rolling or extrusion of MC or its derivatives in solutions in water or water-ethanol mixtures. After solvent removal, transparent and smooth films are formed which can function as moisture barrier or solute diffusion barrier. They are also impervious to fat, oils, grease and most organic solvents.

Since MC and HPMC are available in a wide range of molecular weights with varying physical properties, such variation will influence the properties of the coatings, offering the formulator an opportunity to develop coatings with specific characteristics without greatly changing the chemical properties of the film former (Row, 1976). A general use guideline for MC and HPMC is that the low-viscosity products are used when film formation is required, and the higher-viscosity products are used when thickening and bulking action is required. Furthermore, compatibility with organic products and solubility in organic solvents improves as degree of esterification increases (Greminger and Krumel, 1980).

The release of solute from coated tablets is proposed to take place by two mechanisms (Row, 1986a; Donbrow and Sanuelov,

1980; Donbrow and Friedman, 1975):

a. Transport of the solute or drug through a network of capillaries filled with dissolution medium. This is applicable only if the water soluble component of the film is leached out of the matrix.

b. Transport of the drug through a hydrated swollen film. This is applicable only if the water-soluble component is retained within the matrix.

c. Transport of the drug through flaws, cracks and imperfections within the matrix.

In the first two mechanisms the assumption is made that the film has no flaws or cracks (Row, 1986a, 1982b, 1981; Row and Frose, 1980). One theory for the origins and causes of flaws and cracks in the film coatings involves the presence of residual internal stress within the film coating (Row 1986a, 1981). These are created by the shrinkage of the film on evaporation of the solvent and by differences in the thermal expansion of the coating and the substrate. If these stresses exceed the cohesive strength of the film, cracking will occur and film integrity will be lost. It follows that the incidence of such defects will depend on the mechanical properties of the polymer and its molecular weight.

Films prepared from low molecular weight polymers with short chains are relatively weak, but as the chain length and hence molecular weight is increased the mechanical properties of the films also improve until at some critical molecular weight there

is no further improvement (Row, 1986a). A film must be hard, tough and yet very extensible (Okhamafe and York, 1983). Based on a study on coated tablets, high molecular weight coatings are harder, less elastic, more resistant to abrasion, and with higher disintegration time and crushing strength (Row, 1976).

The addition of HPMC in coatings will generally improve flexibility and toughness, and reduce water resistance and the tendency to crack. Coatings should be hard and tough without being brittle. These properties are reflected in a high tensile strength value, a high elasticity modulus and a substantial elongation percentage at break point (Aulton *et al.*, 1984).

A common method to enhance the film forming properties of MC and HPMC is by the addition of a plasticizer (Sakellarious *et al.*, 1986a). Plasticizers such as phthalate, caster oil, glycerin, propylene glycol and sorbitol increase flexibility, maximum elongation before break strength, and provide desirable lubrication (Sakellariou *et al.*, 1986; Aulton *et al.*, 1984; Row, 1984; Entwistle and Row, 1978a,b).

10. Use of other natural ingredients for film formation

The ability of certain macromolecules to form rheologically desirable films can be used for the development of casings, microencapsulations, edible wrappers and coating applications (Szczesniak, 1986). Polysaccharides, proteins, lipids or their

derivatives can be used as a gas, moisture, and solute barrier to extend the food product stability.

Examples of polysaccharides which can be used as coating are cellulose, starch, dextrin, modified cellulose, such as MC, HPMC, and carboxymethyl cellulose, carrageenan, alginate, pectin, dextran, scleroglucan, pullulan, curdlan (Guilbert, 1986; Kennedy *et al.*, 1984). Some of these polymers have been discussed in the previous section.

Proteins which have capability to form films, include collagen, ovalbumin and serum albumin and wheat gluten (Anker, *et al.*, 1972). Zein, the alcohol soluble protein extracted from corn gluten is another film forming protein (Torres, 1987; Kaning and Goodman, 1962). Other examples are casings made from collagen for sausages or other meat products. Although this material is edible it is not water soluble and thus it is generally necessary to remove it before consumption. Gelatin has good coating properties and it can be used without any restrictions in fabricated foods. Gelatin films are strong and clear but have very poor water barrier properties (Guilbert, 1986).

Ovalbumin and serum albumin can be used as film formers but these films have very poor mechanical and water barrier properties. Casein films are prepared by neutralization of an alkaline solution of casein prior to drying. These films are relatively water resistant. Soya protein film is a traditional

ingredient in oriental foods and is formed on the surface of heated soybean milk. The films are rather bland, water resistant and flexible if plasticizers are used. Soya protein films made directly from soya isolates, have very good mechanical properties but rather poor water barrier properties (Guilbert, 1986).

Protein films can be modified by denaturing, cross-linking, and by addition of tanning agents such as tannic acids, tri-or divalent cations, etc. Guilbert (1986) found that these modified films were more water resistant but less flexible and transparent.

Gelatin has been applied on the surface of poultry meat prior to frozen storage (Klose *et al.*, 1952). This film provided a limited degree of protection against the development of oxidative rancidity. Film effectiveness was increased by the incorporation of an antioxidant in the film formulation.

Waxes, lipids and derivatives as coatings have been in use for a long time. Coating of fresh oranges and lemons with wax to retard desiccation was practiced in China in the 12th and 13th centuries (Kester and Fennema, 1986). Enrobing foods in fat, called as "larding" was used in 16th century in England to slow the rate of moisture loss from the product (Kester and Fennema, 1986). Beeswax has been shown to reduce the water permeability 10 times more than lecithin or acetostearin films, 25 times more than common oils and 100 or 200 times more than casein or pectin films (Guilbert, 1986).

Apples stored at low temperatures are susceptible to a physiological disorder called "soft scald". Fruits covered with a fatty acid methyl ester or various other edible fats and oils immediately after harvest show a decrease incidence of soft scald. These can be applied on the fruit surface as either an ethanol solution or an oil-in-water emulsion. Compounds used include methyl esters of lauric, palmitic, stearic, oleic, linoleic, and linolenic acids, as well as palm oil, sunflower oil, safflower oil, coconut oil, lard, and lecithin (Kester and Fennema, 1986).

Acetoglycerides, which are flexible, waxlike solids (Feuge, 1955) can be used to coat meat products, cheeses, raisins, nuts, candies, chocolate-pieces, etc. These films are relatively moisture and oxygen impermeable.

Composite films prepared using a blend of various molecules could be used to take advantage of cooperative functionalities. For example a complex formation has been shown to form between corn starch and fatty acids. X-ray diffraction studies have shown that the complexing agent is entrapped within the helical amylose chain in a similar form to the blue amylose-iodine complex (Whittam *et al.*, 1986). The complex behavior depends both on the added lipid concentration and the fatty acid chain length. Similar observations have been done by Kamper and Fennema (1984) in films composed of HPMC and fatty acids.

An example of a protein-polysaccharide film is a combination of gelatin and acacia (gum arabic). Acacia is a hydrocolloid derived from plant sources. By mixing gelatin aqueous solutions with acacia a complex coacervate will form because of the change attraction between the negatively charged acacia and the positively charged gelatin. This film can be made insoluble by using crosslinking agents such as ionized calcium (Kester and Fennema, 1986; Deasy, 1984).

Composite films consisting of a blend of stearic, palmitic acid and carnauba wax as one layer and gelatin or casein as the other have also been developed. Guilbert (1986) observed that the water barrier properties were very good but that the films were rather brittle, opaque and had a waxy taste.

USE OF SORBATES AS FOOD PRESERVATIVES

Since the early 1950's, sorbates, i.e., sorbic acid and its salts have been widely used in many foods as a broad-spectrum, antimicrobial agent (Huhtanen *et al.*, 1981; Kanuch and Staff, 1980). They have been used in foods as effective inhibitors of fungal growth, including those genera that possess a mycotoxin producing capability (Robach, 1980; Baldock *et al.*, 1979). Recent studies have demonstrated that sorbates are also effective antimicrobial agents against the growth of *Clostridium botulinum, Staphylococcus aureus,* and *Salmonella*, all potent food-poisoning organisms. This has lead to research on the use of sorbates as a partial replacement for sodium nitrite in meat products (Robach, 1980). Food applications include dairy, meat, seafood, bakery and confectionery products; beverages, processed fruits and vegetables, and in fermented products such as sausages, pickles and cheese (Chung and Lee, 1982; Kaul *et al.*, 1981; Sofos and Busta, 1981; Huhtanen and Feinberg, 1980; Baldock *et al.*, 1979; Varga *et al.*, 1979). We should also mention the use of sorbates in sausage casings, food wrappers and edible coatings.

Sorbic acid was first isolated from pressed unripened rowan or mountain ash tree berries in 1859 by the German chemist A.W. Hoffman (Sofos and Busta, 1980). The antimicrobial action of sorbic acid was discovered in 1939-40 (Gooding, 1945). The

awareness that this preservative had an impressive number of applications in foods, drugs and cosmetics stimulated the research on its physiological safety, the mechanism of inhibition of fungal and bacterial growth. This has led to its numerous applications.

Sorbic acid or 2,4 trans,trans hexadienoic acid is a straight chain monocarboxylic acid and has the chemical formula $C_6H_8O_2$. Saturated fatty acids are less active than unsaturated ones of the same length. Table 7 shows sorbic acid and other organic acids used as antimicrobial agents. We should note that sorbates have a wider spectrum of activity. For example, propionates are effective against mold but have limited antibacterial activity and essentially no activity against yeast. Although benzoates are effective against yeast, mold and bacteria, they are not recommended for bacterial control because their use level is restricted to 0.1%. Moreover, their activity is poor above pH 4, where bacterial growth is the main problem. Its primary usage is in acid food products (Sauer, 1977). Another disadvantage of benzoates is their adverse flavor effect, usually described as a burning throat sensation detected at higher concentration levels (Sauer, 1977). We should also mention that sorbates have been confirmed as GRAS with no tolerance level, while benzoates have a tolerance level of 0.1%. No tolerance levels have been defined for sorbate, however the maximum usage level is very often limited by standard of identity regulations (Sauer, 1977).

The resistance of sorbates to the effect of physical and chemical agents is well documented and is another reason for their large number of applications (Torres and Karel, 1987; Deak and Novak, 1972; Boyd and Tarr, 1955; Melnick *et al.*, 1945a; Schelhorn, 1954).

1. Physical and chemical properties

Potassium sorbate is a white powder, (m.w. = 150.22), very water soluble and relatively tasteless and odorless (Sofos and Busta, 1981). Solubility of potassium sorbate in water is 139.2 g/100 ml water at 20°C while sorbic acid, a white crystalline powder (m.w. = 112.12), is only slightly soluble in water, 19g/100ml at 20°C. Solubility in water increases with pH and temperature (Sofos and Busta, 1981). Therefore, dip and spray food applications which require high aqueous preservative applications are only possible by using the salt form of sorbic acid.

Solubility of potassium sorbate and sorbic acid in alcohol at 20°C is 2.0 g/ml and 14.8 g/100ml, respectively (Chichester and Tanner, 1972). Additional solubility information is presented in Table 8.

Although unsaturated fatty acids undergo oxidation through free-radical mechanisms, it has been reported that sorbate is resistant to oxidation (Melnick *et al.*, 1954a).

2. Antimicrobial effectiveness of sorbates

The antimicrobial effectiveness of sorbates and other lipophilic acids has been found to be pH-dependent (Eklund, 1983; Blocher, *et al.*, 1982; Kabara, 1981; Jay, 1978). Work by Freese *et al.* (1973) have shown that the undissociated molecule is the effective agent, whereas any activity of the anions is usually of lower order. For this reason the dissociation constants of these acids are important. Sorbate is more effective at pH values approaching its dissociation constant (pK_a) which is 4.75. At this pH level, 50% of the acid is in the effective undissociated form (Sofos and Busta, 1981; Anonymous, 1978; Sauer 1977). This has been attributed to the effect of pH on the amount of undissociated sorbic acid present in solution (Blocher *et al.*, 1982; Sofos and Busta, 1981; Sofos *et al.*, 1980). Table 9 shows the effect of pH on the dissociation of sorbate, benzoate and propionate. Since the antimicrobial activity in the neutral pH range is greater than can be accounted for by the concentration of the undissociated acid, some activity is attributed to the anion (Sauer, 1977). For instance, in the pH range 5.0-6.0 where the acidity itself is not inhibitory, the fatty acid has a marked inhibitory effect (Kabara, 1981).

When compared with sodium benzoate and sodium or calcium propionate at pH 4 and higher, the antimicrobial activity of sorbates is higher (Kabara, 1981). This may be partly explained

by considering their pK_a values. Sorbate was found at least three times more effective than benzoate in preserving cheese, fish and bakery products (Sofos and Busta, 1981; Boyd and Tarr, 1955; Gooding *et al.*, 1955). Sorbate is also considered a more potent preservative agent than propionate. Sorbate was about four times as effective as propionate in preserving cheese, fish and bakery product (Sofos and Busta, 1981; Gooding *et al.*, 1955). It should be emphasized that the fat-to-water partioning of these preservative is very important to the extent of their activity (Sheu *et al.*, 1975). At pH range of 2.5, 3.0, sorbates are still somewhat more effective than sodium benzoate as a yeast and mold inhibitor and more than twice as potent as propionates. Sorbate can also replace benzoate, partially or totally, in more acidic foods to avoid off-flavors and extend the spectrum of microorganisms inhibited. Benzoates function up to pH 6.5 but are relatively ineffective at pH 7.0 and above (Sofos and Busta, 1981; Anonymous, 1978). The relation of pH to the antimicrobial activity of sorbate respect to a few microorganisms is illustrated in Fig. 14.

It has been reported that in the formulation of a high acid, low a_w food products preserved with K-sorbate, the addition of citric acid could potentiate the antimycotic effectiveness of sorbate, either by extending the lag phase or reducing the growth rate (Restaino *et al.*, 1982). Restaino *et at.* (1981) reported that acids combined with sorbates

synergistically delay growth initiation, reduce growth rate and/or lower the total population of various yeasts and bacteria. However, in a study of the effects of five acids, hydrochloric, citric, lactic, phosphoric and malic acid, in combination with potassium sorbate on the growth of two osmophilic yeasts, *Saccharomyces rouxii* and *S. bisporus*, only acetic acid/potassium sorbate combinations synergistically delayed or retarded the growth rate (Restaino *et al.*, 1982). This result indicates possible different inhibition sites for sorbates and acids between bacteria and yeasts.

3. Synergistic effects of other preservation methods

Elliotte and Gray (1981) determined the combined effect of potassium sorbate, atmosphere composition and medium pH on the growth of *Salmonella enteriditis*. Their results have shown the potential of a synergistic relationship between CO_2 modified atmosphere, sorbate concentration and pH. They demonstrated a synergistic inhibition of *Salmonella enteriditis* when sorbate and CO_2 concentrations increased and pH values decreased. Later, Elliott *et al.* (1982) also studied the effect of these parameters on the growth of *Staphylococcus aureus*. They reported that in the presence of potassium sorbate all tested atmospheres, 100, 60, 20% CO_2 atmosphere, vacuum or air control were inhibitory, with increased inactivation occurring at higher concentrations of

sorbate and lower pH. The inhibition of *S. aureus* was optimal at pH 5.5, 1.5% sorbate and 100% CO_2 atmosphere.

Synergistic sorbate-temperature interactions have also been reported (Beuchat, 1981; Sofos and Busta, 1981). A mild heat treatment (49°C) and a sorbate concentration of 0.06-0.12% increased greatly the shelf-life of fruit products. Also at low temperature (1.1°C) sorbate increased the storage life of grape juice (Sofos and Busta, 1981). It has also been reported that sorbic acid inhibited the cellular repair mechanisms after heating of *Escherichia coli* and *Candida utilis*, and thus enhanced the destructive effects of heat treatment on the cell (Shibasaki and Tsuchido, 1973).

Salting is one common technique for preserving fish meat. The stability of salted minced fish with a_w of 0.745 at storage temperatures as high as 35-40°C is unexpected since halophilic microorganism and some molds can grow at this temperature range (Varga *et al.*, 1979). The limiting a_w for these organisms was reported to be 0.70, which is lower than the a_w of salted minced fish. The growth of this organisms can be controlled synergistically by additives such as sorbate (Varga *et al.*, 1979; Doesburg *et al.*, 1969).

Elevated salt and or/sugar concentration and pH act synergistically to enhance the antimicrobial properties of sorbate (Bills *et al.*, 1982). Robach and Stateler (1980) showed that the antimicrobial effects of potassium sorbate against the growth of certain strains of *Staphylococcus*

aureus were potentiated by the addition of salt. Other investigators showed that acids, butylated hydroxyanisole (BHA) and tertiary butylated hydroxy quinone (TBHQ), nitrite, and sodium chloride combined with sorbate synergistically to extend lag phase, reduce growth rate, and/or lower the total population of various yeasts and bacteria (Restaino *et al.*, 1982; Davidson *et al.*, 1981; Restaino *et al.*, 1981; Robach, 1980; Sofos *et al.*, 1979; Costilow *et al.*, 1955).

Low pH may depress the solubility of sorbate in water, but, in general, it increases its antimicrobial activity (Sofos and Busta, 1981).

Low pH, low a_w, and sorbate could have interdependent inhibition against microorganisms. For example, with increasing sucrose concentration, decreasing pH and increasing potassium sorbate concentration, the antimicrobial action on the growth of several yeast strains increased (Bills *et al.*, 1982). Restaino *et al.* (1981) showed that citric or lactic acids and potassium sorbate synergistically delayed the growth or restricted the total growth of *Salmonella* group D, *Yersinia enterocolitica, Pseudomonas fluorescens*, and two lactic acid bacteria strains. The citric acid/sorbate (pH 5.5 at 0.2% sorbate level) combination had the broadest effect on the above mentioned bacterial strains.

4. Other considerations for the use of sorbates

Although sorbates have a broad spectrum of activity against yeasts, molds and many bacteria of public health significance, consideration of other factors play an important role in its selection as a preservative (Kanuch and Staff, 1980). Among them we have the effect of sorbates on the type of microflora found in the raw materials and the final product, expected contamination during product storage and distribution, expected contamination levels, pH and water activity of the product, storage and distribution temperature and mode of application (Sofos and Busta, 1981). We should also consider interactions with other food constituents that may lead to deleterious food changes, particularly flavor. Finally, it is important to consider also the ease of handling, cost and legal use restrictions.

5. Metabolism and resistance

Sorbate preserved foods do sometimes show evidence of microbial growth accompanied by a decrease in the concentration or even the disappearance of sorbic acid (Daley *et al.*, 1986; Bolin *et al.*, 1980; Deak and Novak, 1970; Troller, 1965; Melnick and Luckman, 1954; Melnick *et al.*, 1954a; Costilow *et al.*, 1955). This phenomena has been noticed in products with strong microbial activity and/or high initial cell counts (e.g. pickles). Deak and Novak (1972) have shown losses

of 30 to even 100% of added sorbic acid during the fermentation process of pickled cucumbers. The reduction has been attributed to sorbates being metabolized by microorganisms.

Saccharomyces bailii, which causes fermentative spoilage of contaminated beverage and other food product grows in the presence of high concentration of sorbic, benzoic and other short-chain monocarboxylic acids commonly used as preservative (Warth, 1977) even at low pH. *S. bailii* seems to be permeable to the undissociated form of low to medium molecular weight organic lipophilic acids. Consequently, cells with an internal pH appreciably grater than the pk_a of the acid and the external pH must either concentrate the anion or expend energy in opposing the equilibrium. A third option would be the destruction of intracellular preservative.

Freese *et al.* (1973) noted that the resistant organisms seem to prevent the accumulation of the inhibitor on the transporting cell membrane. The disappearance of sorbic acid from cheese wrapped with a sorbic acid-treated film has been shown to be caused by metabolic oxidation of the sorbic acid. Sorbic acid seems to be metabolized by the same mechanisms operating in animals, i.e. beta-oxidation to the next lower fatty acid with an even number of carbon atoms and finally to carbon dioxide and water. Unsaturated fatty acids, such as sorbic acid, are normal transitory metabolites in the oxidation of saturated fatty acids by molds (Melnick *et al.*, 1954).

Metabolic studies using *Candida claussenii*, *C. utilis*, *C.*

pseudotropicalis, *Procandida albicans* and *Pc. tropicalis*, showed a sorbic acid concentration decrease and subsequent cessation of its growth inhibitory action (Deak and Novak, 1972, 1970, 1968; Deak and Tuske, 1967; Deak *et al.*, 1970). This phenomenon did not occur in cultures of *Saccharomyces cerevisiae*, *S. carlsbergensis*, *C. beverwijkii*, and *C. krusei*. These researchers concluded that certain yeast species are capable of assimilating sorbic acid and utilizing it for growth.

It is further reported although sorbic, benzoic and other aliphatic fatty acids can be metabolized, the rapid rate at which the undissociated preservative enters *S. bailli* makes it unlikely that modification or degradation could be fast enough to significantly lower the steady state concentration of preservative in the cell. An important observation is that adaptation to growth in one preservative increases the resistance to other preservatives (Warth 1977).

Due to the greater difference between the intracellular pH and the pk_a of the preservative, preservatives with low pk_a accumulate to higher intracellular concentrations. Operation of a pump mechanism may therefore be more effective in protecting the organism against preservatives with low pk_a than against preservatives with higher pk_a (Warth, 1977).

Since adapted cells are more resistant to preservatives it would be advisable to prevent inoculation of food products with adapted cells. It would be necessary then to avoid prior

exposure not only to the preservative in use but to others, including acetic and butyric acids, which may be natural constituents of the product (Warth, 1977).

Methods adopted by organisms to gain resistance to cytotoxic substances include cellular impermeability, detoxification, modification of the sensitive site and compensation for its inhibition. Another mechanism is the use of an active transport system as used by *Saccharomyces bailii* (Warth 1977). However this is uncommon among microorganisms. *S. bailii* shows a higher resistance to the effect of preservatives in the presence of higher glucose concentration which is consistent with the energy demands of an active transport system. As operation of the pump requires a high energy input, *S. bailii* will benefit from the pump only when a sufficient energy source is available. Consequently, resistance to preservative will be greatest for high glucose concentrations. Stimulation of fermentation and reduction in growth yield by preservatives are also consequences of the energy demand of the preservative pump. Hence relatively low numbers of cell give high CO_2 production during the spoilage of beverages containing preservatives.

Other studies seem to indicate that the mechanism of resistance of *S. bailii* to benzoic, sorbic and other weak acid results primarily from an inducible, energy requiring system (enzyme linked active transport system). This mechanism is induced when the organisms are previously grown in the

presence of low concentrations of the preservative (Sofos and Busta, 1981; Warth, 1977). The energy required is produced by oxidation of a fermentable substrate such as glucose.

Penicillium species degrade sorbate via β-oxidation and this degradation is enhanced by a nutritious substrate and retarded by a poor medium (Sinskey, 19??; Marth *et al.*, 1966). An off-flavor development associated with the use of sorbic acid during feta cheese maturation has been reported (Daley *et al.*, 1986; Horwood *et al.*, 1981). The off-flavor is shown to be caused by a volatile compound with a hydrocarbon-like odor identified as 1,3-pentadiene (Daley *et al.*, 1986; Marth *et al.*, 1966). Marth *et al.* (1966) reported that the molds degrade sorbate through decarboxylation. They concluded that *Penicillium* spp., by decarboxylation, convert the inhibitory sorbic acid to the inactive 1,3-pentadiene. The reactions believed to occur in the medium are (Sofos and Busta, 1981):

K-sorbate + tartaric acid → K-tartrate + sorbic acid

sorbic acid decarboxylation → 1,3-pentadiene + CO_2

The uptake of sorbic acid and sorbates by a cell can be represented to occur according to the following triple equilibrium:

$$SA^- \rightleftharpoons SA \underset{\text{cell membrane}}{\rightleftharpoons} SA \rightleftharpoons SA^-$$

where:

SA = undisssociated sorbic acid

SA^- = dissociated sorbic acid

The following factors would then affect the sorbic acid uptake (Deak and Novak, 1972; Oka, 1960a,b, 1962):

1) extracellular $SA:SA^-$ ratio which is pH dependent
2) extra- and intracellular pH difference
3) intracellular adsorption of dissociated or undissociated sorbic acid
4) existence of a transport system which could actively promote the uptake of sorbate even at low concentrations of undissociated sorbic acid
5. increasing the uptake of sorbic acid will result first in a faster growth rate which after having reached a maximum will decrease and finally growth will stop because of the inhibitory effect of the intracellular sorbic acid dissociation which lowers intracellular pH

6. Selective inhibition

Several reports have suggested that sorbic acid exerts a selective inhibition against all types of catalase-positive

microorganisms. Thus it could be used as a selective agent for catalase-negative lactic acid bacteria and clostridia (Sofos and Busta, 1981; Emard and Vaughn, 1952. The pH of the medium affected the selective power of sorbic acid. However, there is information that contradicts these observations. For instance, some catalase-positive strains of *Staphylococcus aureus* as well as catalase-negative *Lactobacilli* have grown in the presence of sorbates (Sofos and Busta, 1981). On the other hand, Hamdan *et al.* (1971) showed that growth and acid production by *Streptococcus thermophilus* and *Lactobacillus* (both catalase-negative) were reduced by 0.05-.01% sorbic acid.

7. Mechanism of inhibition

The antimycotic activity of sorbic acid and its salt is attributed to the observation that molds are unable to metabolize aliphatic chains with α-unsaturated dienes. This mechanism is not operative in higher animals, and sorbic acid is thought to be metabolized in a manner similar to longer chain fatty acid (Sinskey, 19??; Deuel *et al.*, 1954b).

Possible targets for the antimicrobial action of relatively simple food preservatives such as benzoate, propionate, and sorbate can be classified as (Eklund, 1980):

1) The cellular membrane

2) Genetic material

3) Enzymes

Since lipophilic acids could inhibit growth either by their effect on the cell membrane or after they have penetrated it, their inhibitory strength should be related to their solvent partition coefficient in the membrane (Sheu *et al.*, 1975). Most studies show that the cellular membrane seems to be the primary target for chemical food preservatives (Eklund, 1980; Freese *et al.*, 1973; Sheu and Freese, 1972; Sheu *et al.*, 1972). The inhibition could be caused by alteration of the membrane structure or to effects on specific types of proteins within it, e.g. those proteins used for ATP regeneration or the ones needed for transport of certain compounds into the cell (Freese and Levin, 1978; Sheu and Freese, 1972). Weak acids could inhibit NADH oxidation and/or cellular O_2 consumption. Although saturated fatty acids inhibit cellular oxygen consumption, they do not inhibit the NADH oxidation by isolated membranes, that is by the cytochrome-linked electron transport system. Therefore, the inhibition of oxygen consumption in whole cells must result from the deficiency of compounds that enter electrons into the electron transport chain. This deficiency would result from the inhibition of transport of the necessary substrates into the cells (Freese *et al.*, 1973). The undissociated form of the preservative can cross the membrane due to its high solubility in the phospholipid membranes and dissociate inside the cells. This would eliminate the transport-driving pH difference between the two

sides of the membrane and is one of the mechanism proposed to explain the antimicrobial action of lipophilic acids (Eklund, 1983). The growth inhibiting effect of sorbates increases when the pH is lowered from neutrality suggesting that inhibition results from the undissociated acid (Eklund, 1981).

The effect of lipophilic acids at pH = 6.5 on growth of *Bacillus subtilis* and serine uptake by *B. subtilis* vesicles has been quantified as a function of preservative concentration by defining an inhibition index, where 0 means no growth inhibition, 1 means 100% inhibition and values above 1 indicate cell lysis (Freese *et al.*, 1973). Fig. 15 shows that the concentration of fatty acids needed to produce a certain amount of growth inhibition of *Bacillus subtilis* coincides with the concentration needed to inhibit serine uptake. The value for this critical concentration decreases when the chain length of the lipophilic acid increases with saturated and unsaturated fatty acids of chain lengths of up to 10 carbons being about equally effective. It is interesting to note that the inhibition of active transport has been observed also for other amino-acids such as L-leucine and also for L-malate. The uptake of all these compounds is energized by glycerol-phosphate or NADH (Freese *et al.*, 1973). These findings are consistent with the observation by Kabara (1981) that the germicidal titer of fatty acids and their salts increases to a maximum for a carbon chain length of about 12 and then decreases as a function of molecular weight. The chain

length at the inflection point varies with pH and the specific microorganism. One reason for the inflection is that the hydrophilic property of the free fatty acid and its salts decreases as the molecular weight increase (Kabara, 1981). The more lipophilic long chain compounds are more effectively partitioned into the membrane. This membrane attachment is reversible, because when cells which have been inhibited by fatty acids from acetic to linoleic acid, are resuspended in fresh medium, growth resumes immediately at the uninhibited rate (Freese *et al.*, 1973).

Lipophilic acids (including sorbates), somehow uncouple, both substrate transport and oxidative phosphorylation from the electron transport system. In whole cells these two inhibitory phenomena are strictly correlated, whereas the concentration of ATP does not necessarily decrease. In contrast to saturated fatty acids and 2.4.-dinitrophenol (DNP), which only uncouples, unsaturated fatty acids inhibit also the electron transport system itself (Sheu *et al*, 1975; Freese *et al.*, 1973). Most likely, the double bond of unsaturated fatty acids sets up pressures which lead to steric disorganization and interference with the transport mechanism (Sofos and Busta, 1981).

Eklund (1980) observed that the preservative action of esters of p-hydroxy benzoic acid which remain undissociated within physiological pH limits (pk_a = 8.5 vs. 4.2 for benzoate). Their interference with transport phenomena in cell vesicles has also been shown to closely correlate with their

effect on whole cells. This indicates that the action of these type of molecules is closely associated with their solubility in the cell membrane.

Studies with membrane vesicles prepared from *Bacillus subtilis* indicated that inhibition of amino acid transport is also a primary antimicrobial effect of aliphatic diols and their esters (Akedo *et al.*, 1977; Sinskey, 1976). Long fatty acids and diols have some similarities in their structure. Both have hydrophilic ends of carboxyl groups or hydroxyl groups and hydrophobic ends of long chain hydrocarbon residues. These compounds are known as "amphipatic" molecules and are easily partitioned into the phospholipid membrane bilayers. Consequently, they may cause some alterations in membrane structure and function (Akedo *et al.*, 1977).

Although lipophilic acids and aliphatic diols seem to prevent growth by inhibiting the transport of substrate molecules into cells there are other mechanisms that might also be involved. Increasing the membrane permeability, affecting the electrical components of the proton-motive force, depletion of the cell ATP content are also mechanisms of inhibition which should be considered. A proton or charge gradient is involved in energizing the membrane transport system. The undissociated form of sorbic and other lipophilic acids could discharge this gradient by diffusing through the membrane and ionizing when it reaches an interior compartment of the cell with a higher pH (Sofos and Busta, 1981; Hunter and Segel, 1973).

It has also been postulated that fatty acids form a monolayer around the bacterial cells which result in growth inhibition by either blocking the transport of nutrients into the cell or by increasing the leakage of essential metabolites from the cells (Branen *et al.*, 1980). Galbraith and Miller (1973a) showed that leakage of metabolites from fatty-acid treated cell is not the growth inhibitory system. Therefore blockage of transport seems to be the most logical explanation.

Preservatives such as sorbic acid have more antimicrobial action against yeast and molds and gram positive (G^+) than gram negative bacteria (G^-, Greenway and Dyke, 1979). Freese *et al.* (1973) reported that G^- bacteria metabolize these preservative to a greater extent than G^+ bacteria and thus overcome the inhibition better. A more likely explanation, however, is that the lipopolysaccharide layer which typically surrounds the cell wall of G^- organisms can screen out the fatty acids while the cytoplasmic membrane is very permeable (Sofos and Busta, 1981). The lipids are thus prevented from accumulating on the transporting cell membrane and reaching the inner, sorbate-sensitive cytoplasmic membrane of G^- bacteria (Branen *et al.*, 1980; Greenway and Dyke, 1979; Sheu *et al.*, 1975; Freese *et al.*, 1973; Galbraith and Miller, 1973; Sheu and Freese, 1973).

On the basis of reports that sorbate is a selective inhibitor against catalase-positive bacteria it has been suggested that its inhibitory effect against molds might be the

inhibition of the activity or synthesis of the enzyme catalase. Studies have shown that sorbate inhibits the germination of *Aspergillus niger* and catalase activity. Inhibition of catalase would result in increased hydrogen peroxide concentration to a point where it could act on certain vital metabolic processes and prevent spore germination (Sofos and Busta, 1981).

Sorbic acid is susceptible to autoxidation deterioration which might lead to the accumulation of sorbyl peroxide. This peroxide may inactivate catalase or inhibit the activity of other enzymes or co-enzymes vital to mold cell development (Sofos and Busta, 1981).

Potassium sorbate has also been shown to contribute synergestically to the heat inactivation of fungal spores and cells. Low preservative concentrations increased heat sensitivity possibly as a result of increased permeability as the cell wall and cytoplasmic membrane expanded at elevated temperatures. It can be hypothesized that during these conditions the preservative enters more easily into the cells due to a weakening of the physical barriers present in the cell wall and membrane (Beuchat, 1981; Branen *et al.*, 1980). Chilling or the addition of a chelating agent is also likely to increase cell permeability, allowing sorbate and lipid derivatives to enter the bacterial cell membrane (Branen *et al.*, 1980).

No evidence has been found that lipohilic acids used as

preservative have a direct effect on the genetic material of microorganisms (Eklund, 1980). However, several enzyme systems have been found which are sensitive to preservatives (Troller, 1985; Beuchat, 1981; Whitaker, 1959). As early as 1954 it was postulated that sorbic acid inhibits certain dehydrogenases which are involved in the β-oxidation of fatty acids. α,β-unsaturated fatty acids are intermediate products in the oxidation of fatty acids by molds. An accumulation of α,β-unsaturated fatty acid, such as that occurring by addition of sorbic acid would prevent the function of the dehydrogenase enzymes, and therefore would inhibit metabolism and growth of the molds (Sofos and Busta, 1981).

Those enzymes which are reported as probable sites of inhibition, include the sulfhydryl-containing enzymes, fumerase, aspartase, succinic dehydrogenase and yeast alcohol dehydrogenase, ficin and enolase. These could be the sites of inhibition of oxidative metabolism of catalase-positive bacteria, yeasts and molds (Sofos and Busta, 1981; Harada *et al*, 1968).

The inhibition of sulfhydryl enzymes has also been associated with sorbic acid and it has been suggested to occur because sorbic acid might react slowly with cysteine through an addition reaction with the thiol group of cysteine. It is also possible that the action of sorbate is similar to that of maleic acid, which forms stable complexes with sulfhydryl-containing enzymes through thiohexenoic acid derivatives

(CH_3-CH=CH-RSCH-CH_2-COOH).

As reported by Martoadprawito and Whitaker (1963), potassium sorbate inhibits yeast alcohol dehydrogenase, maximally at the pH optimum of the enzyme (pH 8.5). It was found that potassium sorbate competes with NAD and ethanol for a site on the enzyme but in an irreversible manner, by either the formation of a covalent bond between the reactive $ZnOH^-$ or SH^- groups of the enzyme and the δ and/or β carbons of the sorbate ion. Harada *et al.* (1968) found that the respiration was inhibited by sorbate by competing with acetate on the site of acetyl-CoA formation. This mechanism of the sorbate inhibition could also be applied for the inhibition by common straight chain fatty acids.

Although all these reports suggest enzyme systems as the site for the inhibitory action of sorbates they do not prove that they are the inhibition mechanism at the whole cell level.

8. Safety and regulatory aspects of the use of sorbates

The U.S. Food and Drug Administration (FDA) lists sorbate as "generally recognized as safe" (GRAS) (Huhtanen *et al.*, 1981; Namiki *et al.*, 1980; Sofos and Busta, 1980; Deuel *et al.*, 1954a). Sorbates are cited in paragraph 182.3089 and 182.3640 of the Code of Federal Regulation (Anonymous, 1977). The only limitation to the use of GRAS ingredients is that they should be used in amounts no higher than necessary to accomplish

their intended effects. Upper limits are imposed only for foods defined under Federal Standards of Identity. For example, in cheese sorbate may not exceed 0.3% by weight. The maximum is set up at 0.2% for pasteurized blended cheese, pasteurized process cheese, pasteurized process cheese food and spread, pasteurized cheese spread and cold pack cheese (Chichester and Tanner, 1972).

When a preservative is used in a food product the Code of Federal Regulation, 21 CFR 101.22 requires that its common name (i.e. sorbic acid, potassium sorbate) be listed on the product label. An explanatory description can also be appended to describe its function, i.e. "To maintain freshness", "To extend shelf life", "To retard spoilage", "To help protect flavor" or "A mold inhibitor" (Anonymous 1978). Table 10 lists other countries where the use of potassium sorbate has been approved.

Extensive studies evaluating the carcinogenicity of sorbates have provided irrefutable proof of its safety and non-carcinogenicity (Robach *et al.*, 1980). Due to safety of using sorbate, the United State Department of Agriculture (USDA) published on May 16, 1978 a regulation proposal to reduce the level of sodium nitrite used in bacon processing from 120 ppm to 40 ppm combined with 0.26% potassium sorbate (Robach *et al.*, 1980). The objective was to reduce nitrosoamine formation by partial replacement of nitrite for botulism control in meat products (Sofos *et al.*, 1980; Pierson *et al.*, 1979).
Meat products processed with the new formulation, was not

significantly different from products formulated with 120 ppm of sodium nitrite and no potassium sorbate with respect to color and sensory qualities (Paquette *et al.*, 1980).

The simple chemical structure of sorbic acid explains its safety record. They are be metabolized by humans the same way as any other fatty acid (Sofos and Busta, 1981; Deuel *et al.*, 1954a; 1954b). Its LD_{50} of 5g/kg body weight makes it safer than table salt (Bolin *et al.*, 1980).

9. Food applications

Many food products can be preserved by the adequate use of sorbates and at the levels used they do not unfavorably alter the taste or flavor of foods (Huhtanen *et al.*, 1981). Table 11 suggests usage range levels for different food products.

In dairy products, natural and processed, sorbates may be added directly into the product during processing, or by spraying and dipping during or after processing. In the case of solid cheese they can also be incorporated in the packaging film (Guilbert, 1986; Smith and Rollin, 1954). Sorbic acid decreases the growth rate of psychrotrophic bacteria which is crucial in the cheese industry. Psychrotrophic bacteria can destroy diacetyl, an important flavor component in cottage cheese. Loss of diacetyl results in a flat-tasting, less aromatic product. Sorbic acid can inhibit these bacteria and thereby prolongs the original fresh flavor of cottage cheese.

In bakery products, sorbates are usually added with the flour or by direct addition during mixing. Since post baking contamination occurs on the surface and mold growth is a surface event, it is recommended to dust sorbates on the surface or to incorporate them with the wrapping materials (Robach, 1980). Moreover, by using a surface application technique, a possible interruption of fermentation can be prevented. Use of melt-coated sorbic acid is another approach to prevent the preservative from coming into contact with added yeast and other materials during dough rising. In this latter method granular sorbic acid or sorbates are coated with a solid fat. The preservative is released into the baked product upon heating (Sato *et al.*, 1981).

A sorbic acid solution of 0.1% sorbic acid prevents the growth of surface yeast in cucumber fermentations without interfering with the desirable acid fermentation (Sofos and Busta, 1981).

In meat products, including poultry and all types of smoked fish and fresh fish, sorbates may be applied as a dip or spray (Cunningham, 1981; D'Aubert *et al.*, 1980; Olson *et al.*, 1981; Robach, 1979). Dry sausages may be dipped in a K-sorbate solution after stuffing and drying or the casing may be dipped into a 2.5% potassium sorbate solution.

Table 1.

CHEMICAL COMPOSITION OF METHYLCELLULOSE AND HYDROXYPROPYL METHYLCELLOSE

Methoxy %	Hydroxypropoxyl %	Soluble in	Nominal Gelation Temperature, ^{o}C
27.5-32.0	0	water	54-56
26.0-30.0	7-12	water and organic solvents	60
27.0-29.0	4-7.5	water	65
19.0-24.0	4-12	water	70-90

Table 2.

GELATION PROPERTIES OF COMMERCIAL CELLULOSE ETHERS

Cellulose ether	Viscosity[1] cP	Gelation temperature °C	Gel strength
a. Methylcellulose			
A15-LV	15	50-55	Firm
A4C	400		
b. Hydroxypropyl methylcellulose			
E5	5	58-64	Semi-Firm
E15-LV	15		
E50-LV	50		
E4M	4,000		
F50-LV	50	62-68	Semi-Firm
K3	3	70-90	Soft
K35	35		
K100-LV	100		
K4M	4,000		
K15M	15,000		

Table 3.

TOLERANCE OF 2% SOLUTIONS OF MC AND HPMC TO ADDITIVES[1]

	MC		HPMC	
Additive	15 cP[2]	4,000 cP	50 cP	4,000 cP
NaCl	11	7	17	11
$MgCl_2$	11	8	35	25
Na_2SO_4	6	4	6	4
$Al_2(SO_4)_3$	3.1	2.5	4.1	3.6
Na_2CO_3	4	3	5	4
Na_3PO_4	2.9	2.6	3.9	3.5
sucrose	100	65	120	80

1 Number of grams that 100 g of solution will tolerate without a salting-out phenomena

2 Viscosity of a 2% solution without additives at 20°C

Table 4.

COMMERCIAL APPLICATIONS FOR MC AND HPMC

Percent methoxyl: Percent hydroxypropoxyl:	MC 26–33 —	HPMC 28–30 7–12	HPMC 27–30 4–7.5	HPMC 16.5–20 23–32	HPMC 19–24 7–12
Adhesives:					
Leather pasting	4,000	—	4,000	—	—
Temporary binding agent for glass fibers	15	—	—	—	—
Thickener in phenolformaldehyde adhesives	4,000	—	—	—	—
Stationery adhesives	25	—	—	—	—
	400				
	1,500				
Wallpaper	1,500	—	4,000	12,000S	15,000
	4,000			75,000S	—
General adhesives	4,000		4,000	75,000S	—
Agriculture:					
Dispersing agent for wetting powders	15	50	—	—	—
Dust stickers	—	—	4,000	—	—
Seed stickers	15	—	—	—	—
Spray drift control	25	—	—	75,000S	—
Spray stickers	—	—	4,000	12,000S	—
Weed killers	1,500	—	—	—	—
Asphalt:					
Asphalt emulsion	—	—	—	12,000S	4,000
				75,000S	
Release coating	15	—	—	—	35
Caulking compounds	—	—	4,000	5,000S	4,000
				12,000S	15,000
Ceramics:					
Refractory mortars	4,000	—	4,000	—	—
Glaze slips	15–25	—	—	—	—
Hi-temp glaze slips	4,000	—	4,000	5,000S	4,000
Porcelain enamels	15–25	—	—	—	—
Cements	4,000	—	4,000	—	—
Tile mortars	4,000	—	4,000	5,000S	4,000
Plastic mixes	4,000	—	4,000	—	—
Chemical specialties:					
Aerosols	25	50	50	—	—
Cleaning and polishing compounds	1,500	—	—	5,000S	4,000
Insecticides	15	—	—	—	—
Sanitizers	4,000	—	4,000	—	—
Construction products:					
Drywall joint cements	—	—	4,000	5,000S- 75,000S	4,000- 15,000
Masonry mortars	—	—	4,000	5,000S- 12,000S	4,000- 15,000
Pumpability aids	—	—	—	75,000S	15,000
Release coatings	15	—	—	—	—
Stuccos	—	—	4,000	5,000S- 12,000S	4,000- 15,000
Tile grouts and adhesives	—	—	4,000	5,000S 12,000S	4,000 15,000
Cosmetics:					
Creams and lotions	—	4,000	—	†	4,000
Deodorants				†	15,000
Hair dressings	—	4,000	—	†	—

* These numbers refer to proximate viscosity in cP of 2% solutions (20°C) of the product types shown. To convert to Pa·s, multiply by 0.001.

† Viscosities dependent on application need. Consult supplier.

Table 4. (continued)

Shampoos	—	4,000	—	—	—
Toothpastes	—	—	4,000	†	4,000
Foods:					
Baked goods	4,000	—	4,000	—	4,000
Breading batters	25	—	50	—	100
Dietetic foods	†	†	—	—	†
Milkshake drinks	15	50	50	—	—
Pie fillings	150	—	4,000	—	4,000
Salad dressings	—	—	—	—	4,000
Snack foods	†	†	†	—	†
Whipped toppings	—	50	50	—	100
Latexes:					
Creaming of natural rubbers	4,000	—	4,000	5,000S 12,000S	4,000 15,000
Protective colloids	25	50	50	5,000S 12,000S	100
Thickeners	—	—	—	75,000S	—
Leather:					
Finishings	4,000	—	—	5,000S 12,000S	— —
Pasting adhesives	4,000	—	4,000	—	—
Paints:					
Acrylics, polyvinyl acetate, styrene-butadiene	—	—	—	5,000S 12,000S 20,000S 75,000S	4,000 15,000S
Cement paints	—	—	4,000	5,000S 12,000S 20,000S 75,000S	4,000 15,000S
Multicolor lacquers	25–4,000	—	50–4,000	—	—
Texture paints	—	—	4,000	5,000S 12,000S	4,000 15,000
Paint removers§	—	4,000	4,000	—	15,000
Paper:					
Adhesives	15–400	—	50–400	—	100–400
Barrier coatings	15–400	—	50–400	—	100–400
Dielectric papers	15–400	—	—	—	—
Release coatings	15–400	—	50–400	—	100–400
Pencils and crayons	25–400	—	50–400	—	—
Pharmaceuticals:					
Bulk laxatives	—	—	—	—	15,000
Creams and ointments	—	4,000	4,000	—	4,000
Ophthalmic preparations	1,500 4,000	—	4,000	—	4,000
Suspensions	1,500 4,000	4,000	4,000	—	4,000
Tablet binders	15–25	50	50	—	100
Tablet film coats	—	15–50	—	—	—
Plywood control of glue viscosity	4,000	—	4,000	5,000S 12,000S	15,000
Printing inks (Water-based inks)	25–4,000	15–4,000	50–4,000	5,000S 12,000S	100–4,000
Polyvinyl chloride	15–25	15–50	50	—	35–100
Resins:					
Emulsion coatings	—	4,000	4,000	5,000 12,000	4,000 15,000
Mold-release agents	15–25	—	50	—	35–100

† Viscosities dependent on application need. Consult supplier.

Table 4. (continued)

Percent methoxyl: percent hydroxypropoxyl:	MC 26–33 —	HPMC 28–30 7–12	HPMC 27–30 4–7.5	HPMC 16.5–20 23–32	HPMC 19–24 7–12
Rubber:					
Latex stabilizers and thickeners	4,000	—	4,000	5,000S 12,000S	4,000
Mold release	15–25	—	50	—	35–100
Textiles:					
Adhesives	400	—	50	—	35–100
Carpet backsizing	—	—	—	75,000S	15,000S
Dye thickening	1,500	—	4,000	—	4,000
	4,000	—	—	—	15,000
Flocking adhesives	—	—	4,000	5,000S	4,000
				12,000S	15,000
Latex coatings	15–25	—	4,000	12,000S	15,000
	4,000				
Printing pastes	1,500	—	4,000	5,000S	—
	4,000	—	—	12,000S	—
	10,000	—	—	75,000S	—
Warp sizes	15	—	50	—	100
Tobacco:					
Reconstituted sheet	400	—	4,000	—	—
	15–4,000	—	—	—	—
Viscosity control	—	—	—	—	15,000

Table 5.

FOOD USES OF MC AND HPMC

End Use	Functions
Frozen pastries	No syneresis on freezing, thermal gelation on baking
Frozen meat patties	No syneresis on freezing, thermal gelation on baking
TV dinners, gravies	No syneresis on freezing
Frozen fish products	No syneresis on freezing, binding
Breading batters	Improved stability to spoilage
Additive to low-gluten flours	Gas retention, stronger cell structure, thermal gelation, water retention
Food dressings	Surface activity, thickening
Whipped toppings	Surface activity, thickening, foam stabilization
Frozen desserts	Surface activity, crystal modification
Dairy mixes	Whipping action, thickening
Flavor emulsions	Surface activity
Glazes	Binding, whipping action
Doughnut mixes	Gas and moisture retention, reduced oil uptake
French fried potatoes	Reduced oil uptake
Food coatings	Binding, reduced oxygen transmission
Pie fillings	Water retention on baking via thermal gelation
Specialty sauces	Thickening, inhibition of phase separation
Cake mixes	Gas and moisture retention, improved volume
Dietetic syrups	Viscosity control, physiologically inert
Condiment carrier	Water retention
Meringue	Whipping action, foam stabilization
Extruded potato shapes	Reduced oil uptake, binding, lubricity

Table 6.

PHARMACEUTICAL AND MEDICAL APPLICATIONS OF MC AND HPMC

End Use	Functions
Control of diarrhea	Nonmetabolized bulk, film forming, water retention
Control of constipation	Nonmetabolized bulk, water retention
Surgical jellies	Lubricity, thickening
Ointments and lotion	Thickening, lowered surface tension
Suspensions	Thickening, dispersing activity
Burn therapy	Film forming, hydrophilic gel
X-ray contrast media:	
Gastrointestinal	Filming, pigment dispersing, bulking, water retention
Bronchography	Filming, hydrophilic coating
Ophthalmic medicinals	Non-ionic, bland, ointment, good wetting of cornea
Tissue culture	Reduces coating of cells on glassware, inert
Microscopic diagnostic techniques	Thickening, physiologically inert, slows movement of protozoa
Tablet binder	Adhesiveners, non-ionic
Tablet coatings	Filming, soluble in both water and organic solvents
Nose drops	Thickening, hydrophilic film spreads easily
Dental medicinals	Inert to calcium salts
Crystal modification	Absorption on surfaces
Vitamin emulsions	Surface activity, thickening
Nonglycogenetic medicinals	Not metabolized, thickening
Dietetic foods for phenyl ketone via therapy	Improved gas retention in doughs, thermal gelation, filming
Control of bleeding	Filming, thickening, hydrophilic gel
Surgical casts	Controls set of plaster of paris

Table 7.

SOME COMMON ANTIMICROBIAL AGENTS

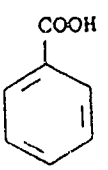
Agent	Formula	Structure
acetic acid	$C_2H_4O_2$	CH_3-COOH
propionic acid	$C_3H_6O_2$	CH_3-CH_2-COOH
butyric acid	$C_4H_8O_2$	CH_3-CH_2-CH_2-COOH
sorbic acid	$C_6H_8O_2$	CH_3-CH=CH-CH=CH-COOH
benzoic acid	$C_7H_6O_2$	

Table 8.

PHYSICAL PROPERTIES OF SORBATES

	K-sorbate	Sorbic acid
Molecular weight	150.2	112.13
Water solubility		
at 20°C	58.2	0.16%
at 30°C	-----	0.28%
at 40°C	-----	0.44%
at 60°C	-----	0.67%
Stability in various organic compounds at 20°C		
Ethanol, 100%	2.0	12.9
Ethanol, 95% w/w	6.5	12.6
Ethanol, 50% w/w	45.3	-----
Ethanol, 20% w/w	54.6	0.29
Ethanol, 5% w/w	57.4	0.16
Ethyl ether	0.1	5.0
Propylene glycol	19	5.5
Acetic acid, glacial, 100%	-----	11.5
Acetic acid, 75% w/w	-----	5.4
" , 60% w/w	-----	2.6
" , 40% w/w	-----	1

Table 9.

EFFECT OF pH ON THE DISSOCIATION

OF WEAK ACIDS USED AS PRESERVATIVES

	% undissociated acid		
pH	sorbic	benzoic	propionic
3	98	94	99
4	86	60	88
5	37	13	42
6	6	1.5	6.7
7	0.6	0.15	0.7
(pk_a)	(4.67)	(4.19)	(4.87)

Table 10.

EXAMPLES OF COUNTRIES WHERE

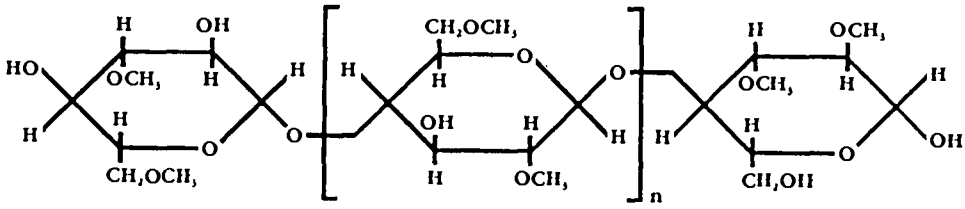
THE USE OF SORBATES HAS BEEN USED

Austria	Germany	Norway
Belgium	India	Pakistan
Canada	Iran	Philippines
Chile	Italy	South Africa
Denmark	Japan	Sweden
England	Netherlands	Switzerland
Finland		

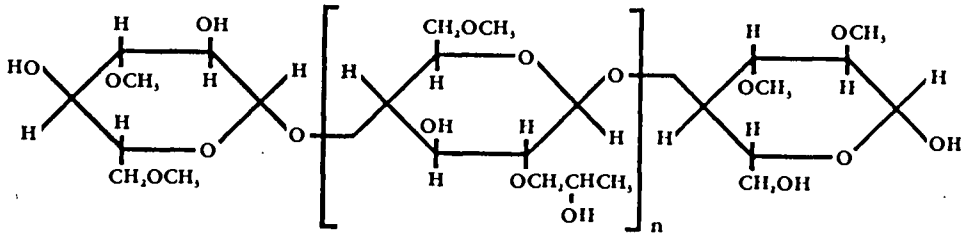
Table 11.

SORBATE APPLICATION MODE AND USE LEVELS IN VARIOUS FOODS

Product	Spray or dipping solution concentration	Use level %
1. Dairy Products		
Cheddar, Colby,		
Monterey Jack, Blue, etc.	20-40%	0.1-0.2
Swiss, Gruyere	20-40%	0.1-0.3
Cottage cheese	direct addition only	0.05-0.075
2. Bakery Products		
a. Cakes		
Angel Food	dry blend	0.03-0.05
Cake mixes	"	0.05-0.1
Fruit cake	"	0.1-0.4
b. Pies, Fillings, doughs		
Filling, Icing, Topping	direct addition	0.05-0.1
Pie Crust Dough	dry blend	0.05-0.1
Doughnut mixes	"	0.004-0.1
c. Yeast-Raised Bakery Product	sprey on surface	
Variety Bread	1.0-1.5	0.016
Hamburger Buns	1.0-2.0	0.02
English Muffins	6-12	0.2
Flour tortillas	3-6	0.1-0.2
3. Dried Fruit		
Raisins	5%	0.25
Prunes	2.5-5.0%	0.02-0.05
Figs	2.5-5.0%	0.05-0.10
4. Meat Products		
Smoked Fish	5.0%	0.05-0.15
Dry sausages	dip sausage or casing prior to stuffing in 2.5% K-sorbate solution	
5. Miscellaneous Food Products		
Fresh-packed salads	direct addition	0.05-0.15%
Pickles, Relishes, Olives	"	0.05-0.1%
Fruit Beverages, table syrup	"	0.025-0.075
Margarine	"	0.05-0.1%



(a)



(b)

Figure 1. Structural formula for methylcellulose (a) and hydroxypropyl methylcellulose (b).

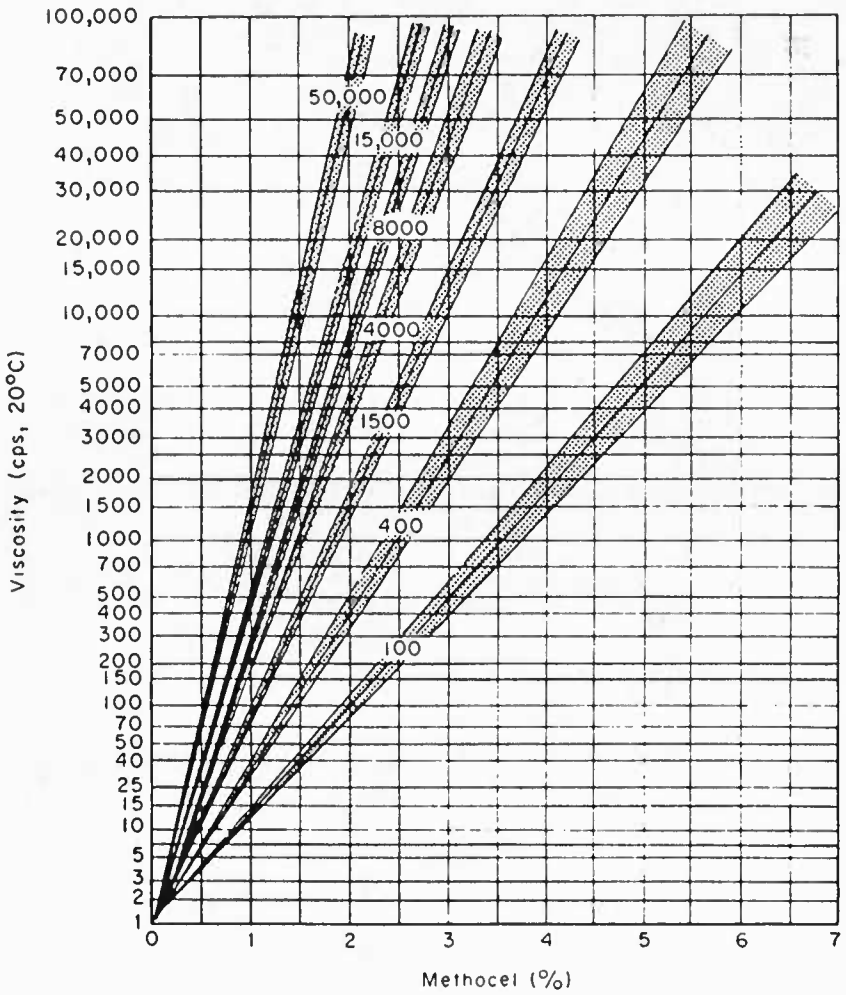


Figure 2. Effect of concentration on the viscosity of high-viscosity methylcellulose.

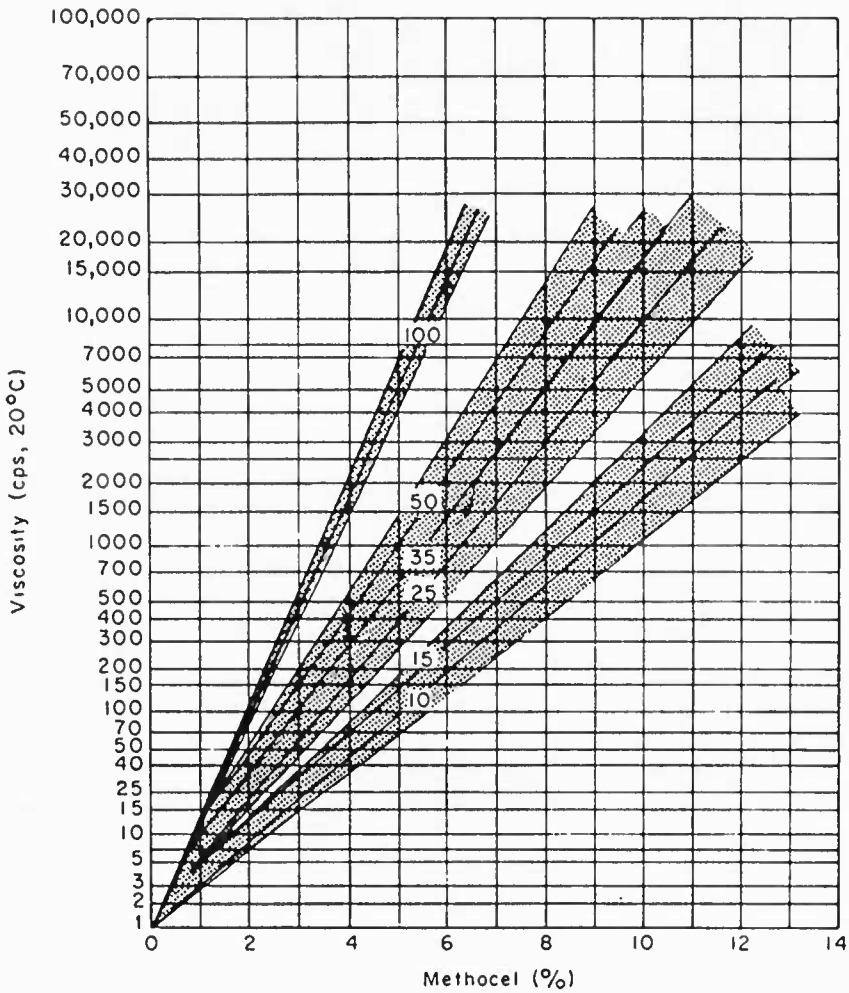


Figure 3. Effect of concentration on the viscosity of low-viscosity methylcellulose.

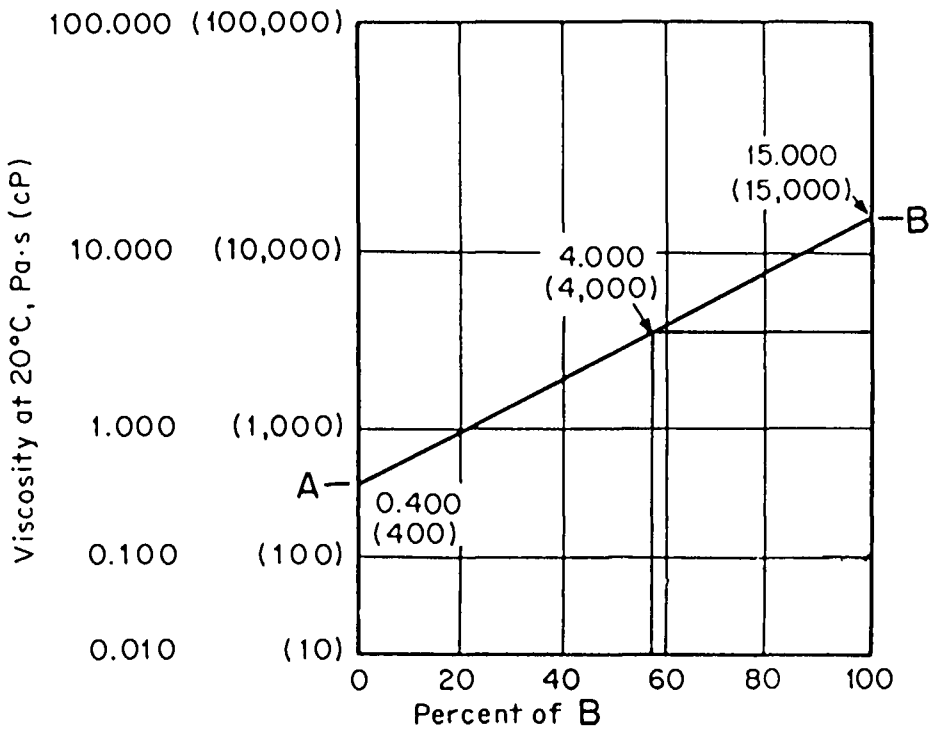


Figure 4. Blending chart for the determination of the viscosity a mixture of two cellulose ethers (MC or HPMC). See text for further details.

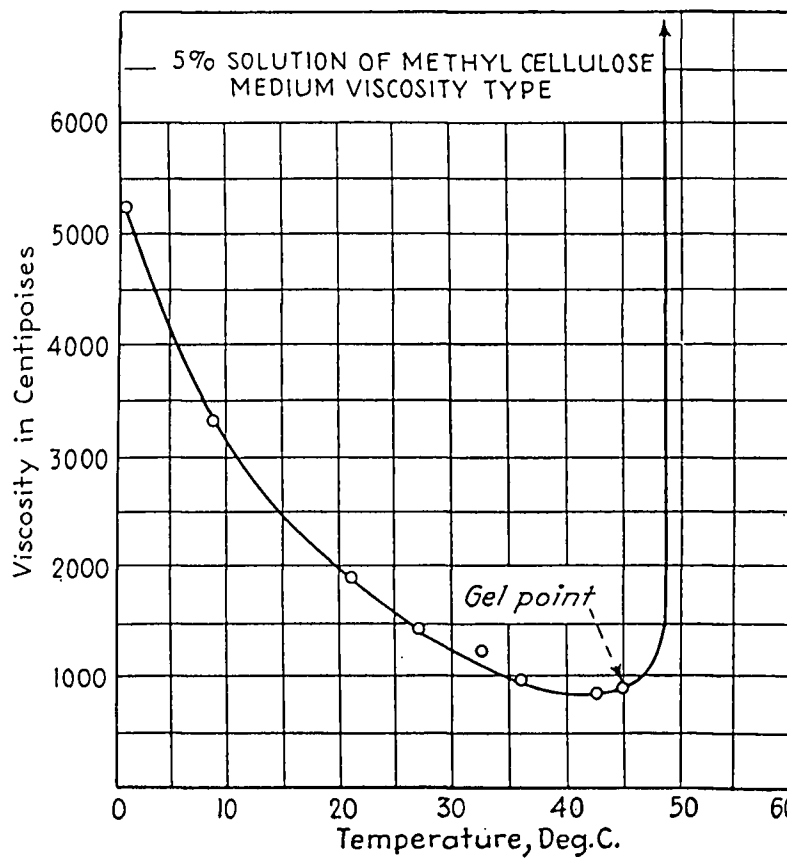


Figure 5. Effect of temperature on the viscosity of an aqueous methylcellulose solution.

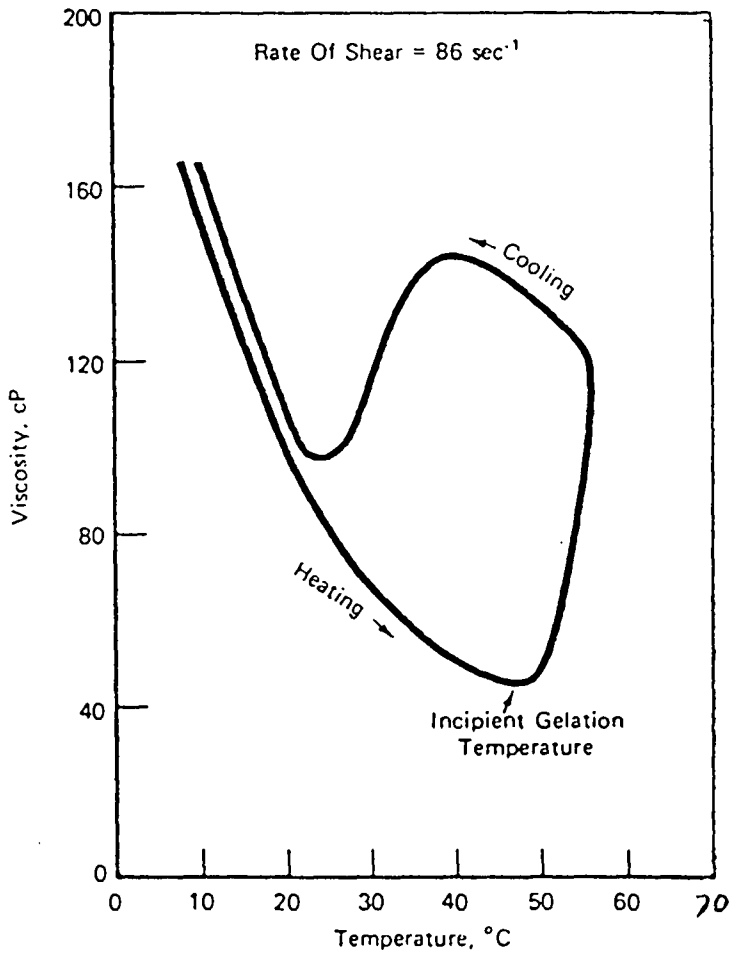


Figure 6. Thermal gelation of a 2% aqueous methylcellulose solution with a viscosity of 100 cP at 20°C. The heating rate was 0.25°C/min.

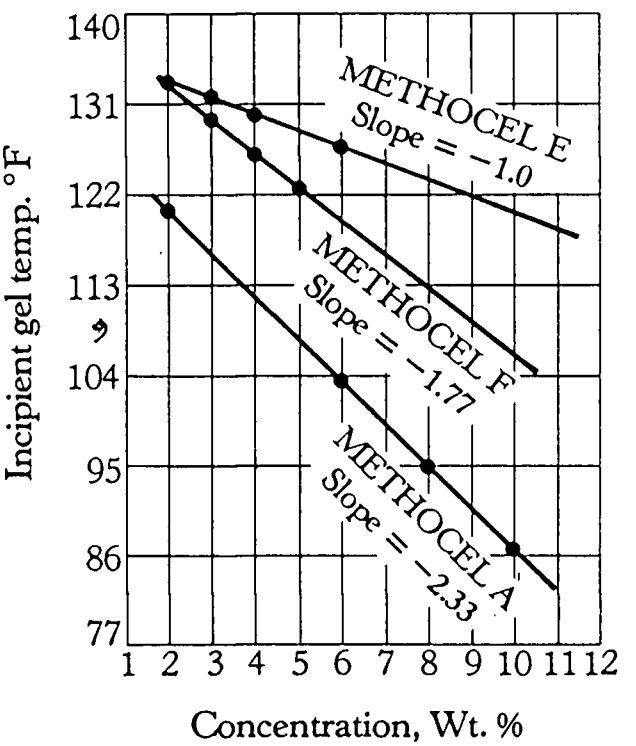


Figure 7. Effect of concentration on the gelation temperature of various commercial cellulose ethers.

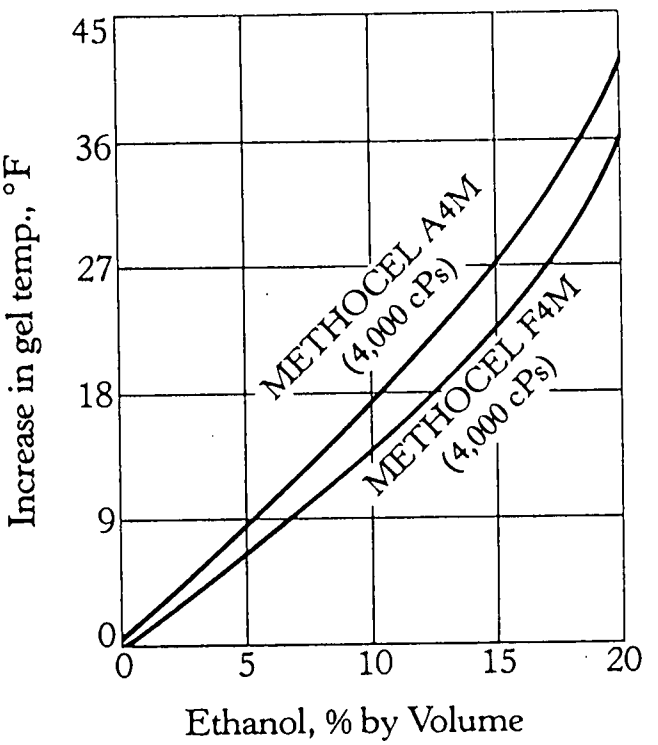


Figure 8. Effect of ethanol on the gelation temperature of 2% aqueous methylcellulose (Methocel A4M) and hydroxypropyl methylcellulose (Methocel F4M) solutions.

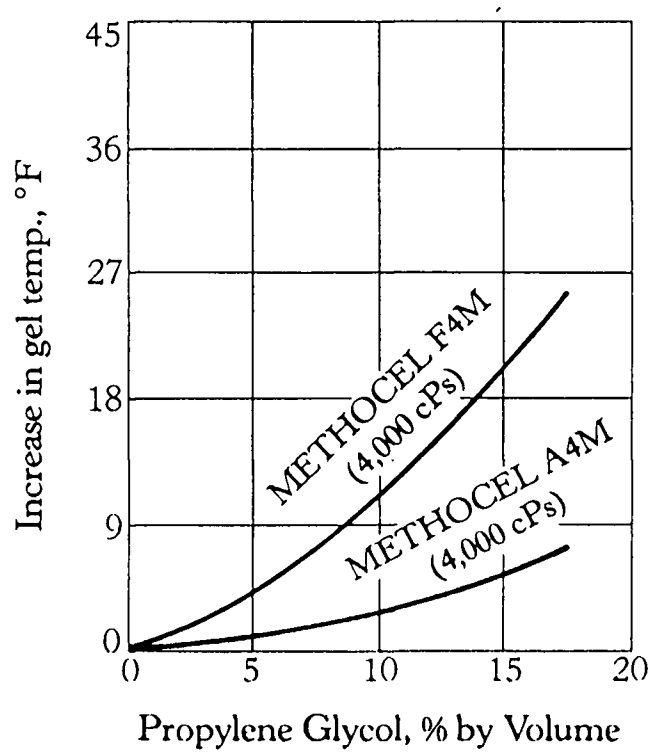


Figure 9. Effect of propylene glycol on the gelation temperature of 2% aqueous methylcellulose (Methocel A4M) and hydroxypropyl methylcellulose (Methocel F4M) solutions.

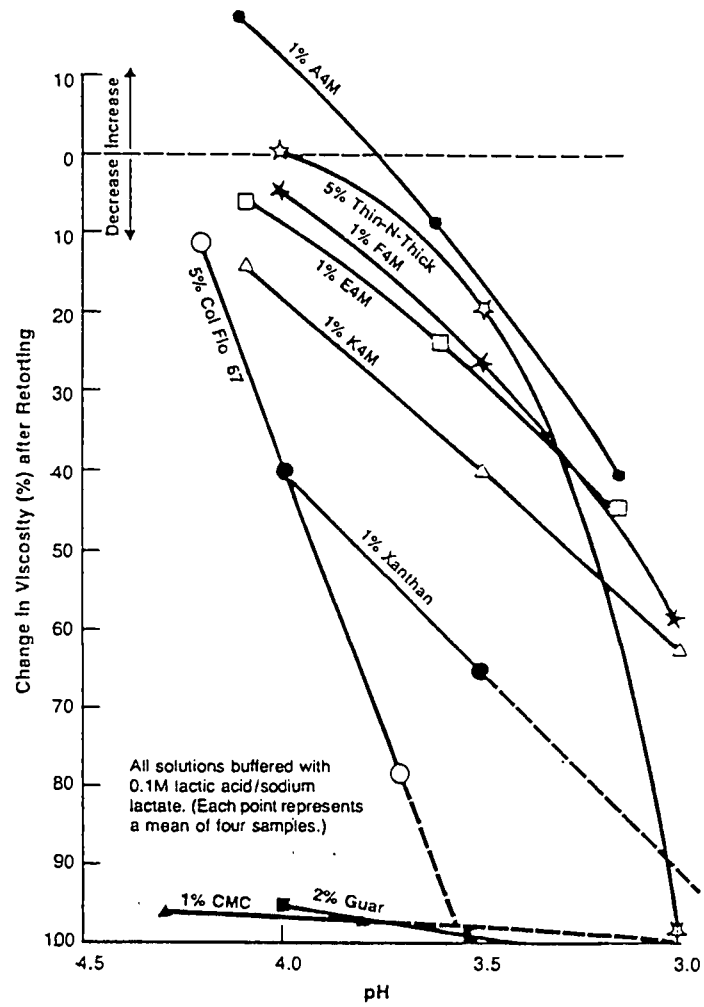


Figure 10. Effect of low pH and retorting temperature on the viscosity of various commercial thickening agents (A4M = methylcellulose, Thin-N-Thick and ColFlo 67 = starch, F4M , E4M and K4M = hydroxypropyl methylcellulose, CMC = carboxymethyl cellulose).

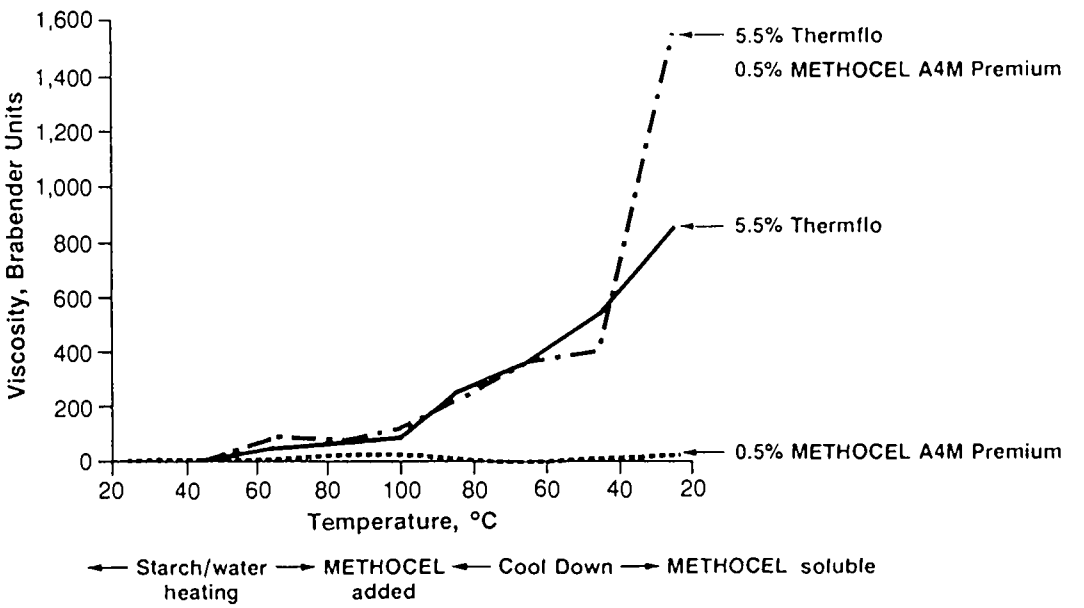


Figure 11. Effect of thermal processing on the viscosity of commercial thickening agents (Thermflo = starch, Methocel A4M Premium = methylcellulose).

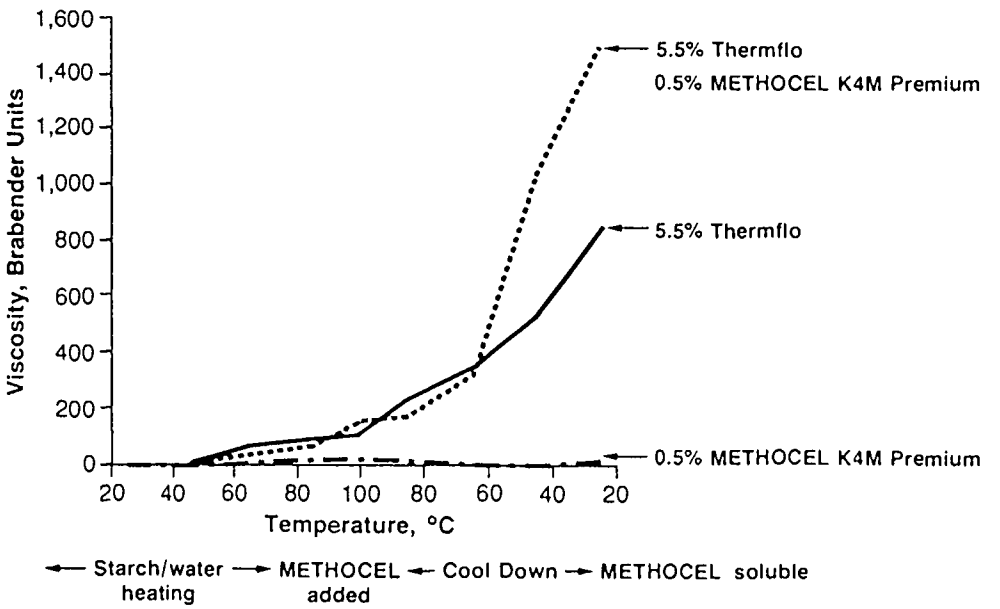
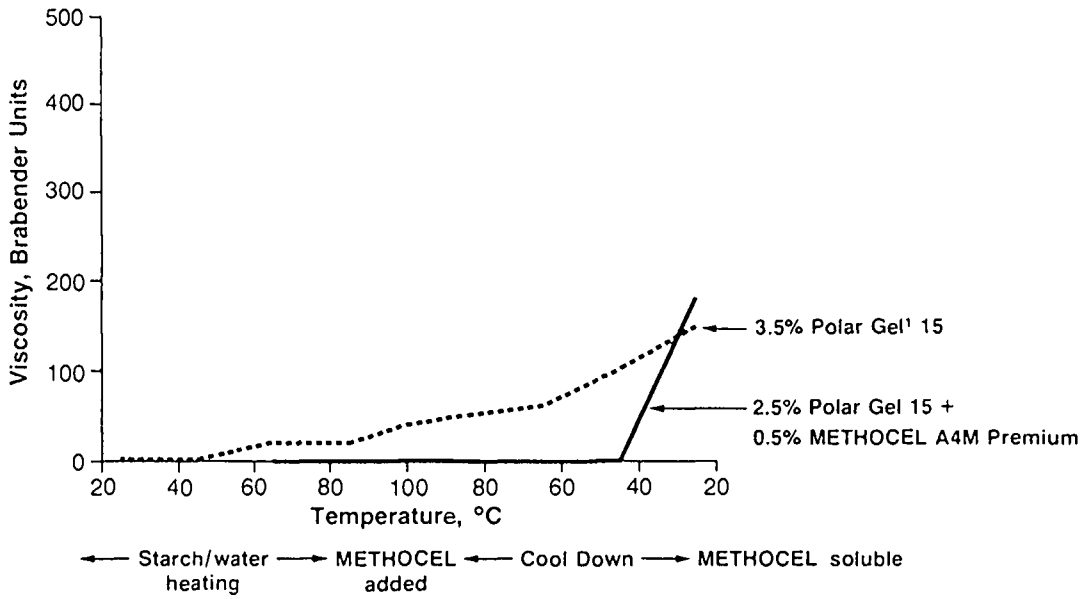


Figure 12. Effect of thermal processing on the viscosity of commercial thickening agents (Thermflo = starch, Methocel K4M Premium = hydroxypropyl methylcellulose).



[1] Modified waxy maize starch supplied by American Maize Products Co.

Figure 13. Effect of thermal processing on the viscosity of commercial thickening agents (Polar gel = starch, Methocel A4M Premium = methylcellulose).

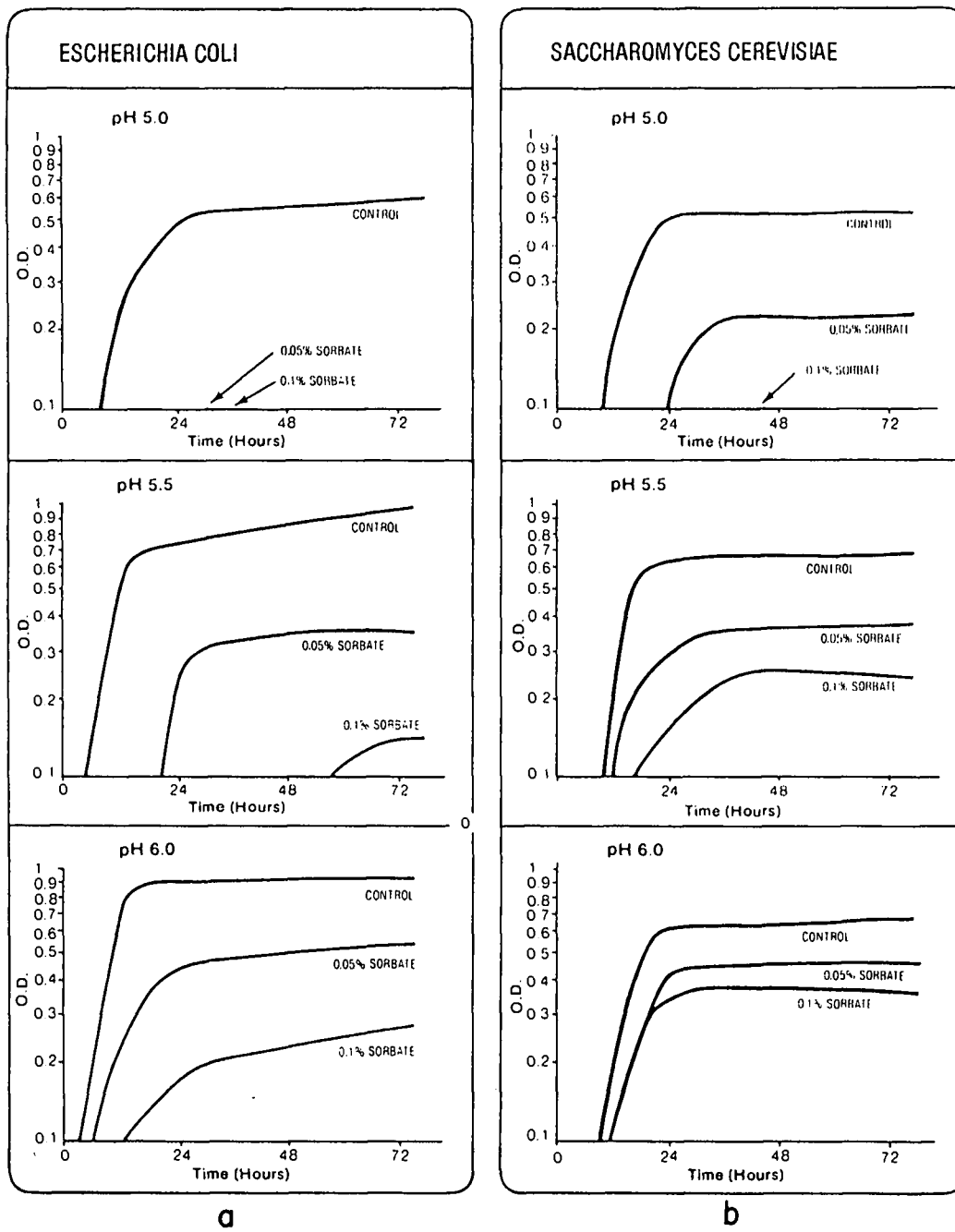


Figure 14. Effect of pH on the antimicrobial effectiveness of K-sorbate
a. *Escherichia coli*
b. *Saccharomyces cerevisiae*

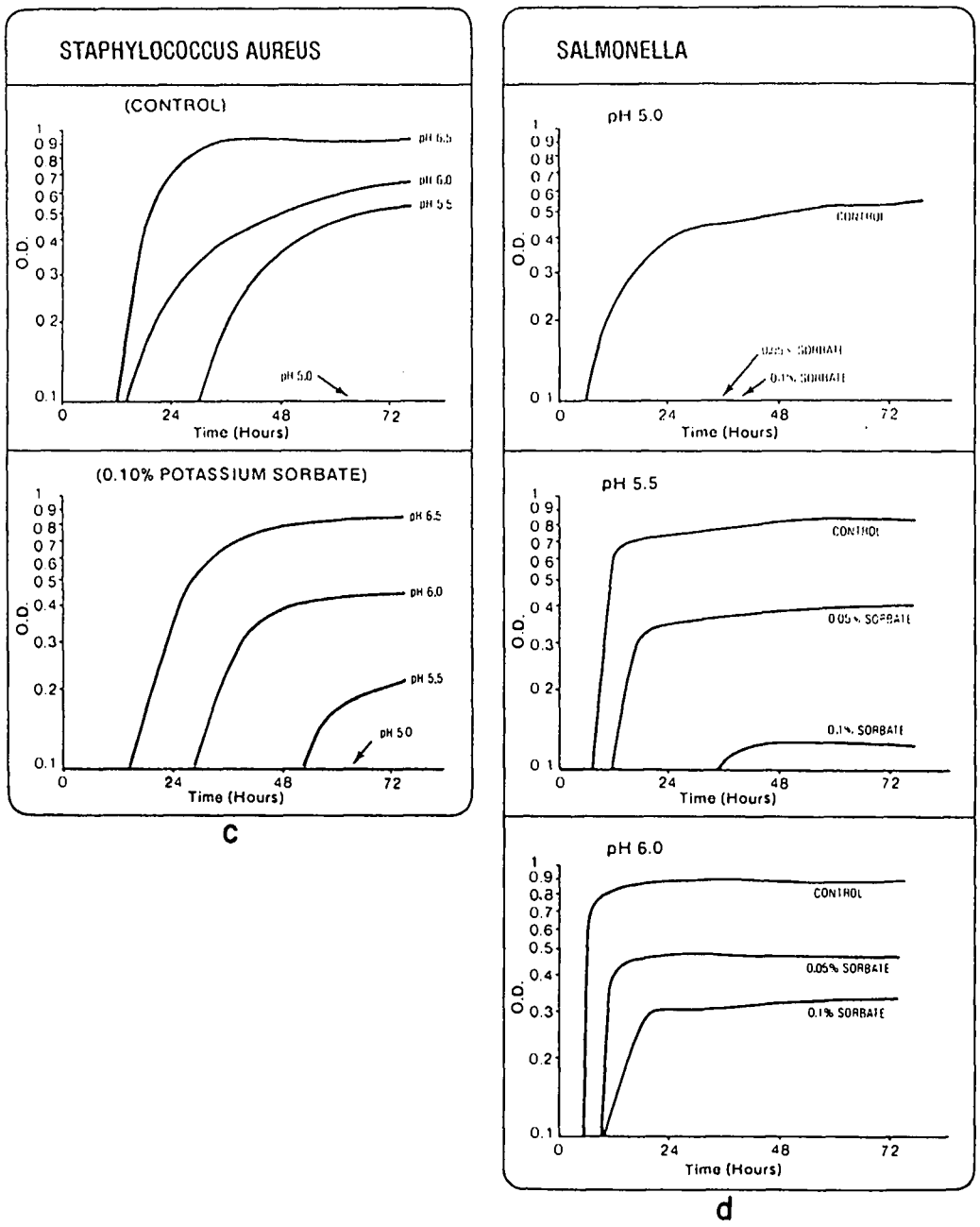


Figure 14. Effect of pH on the antimicrobial effectiveness of K-sorbate (continued).
c. *Staphylococcus aureus*
d. *Salmonella*

Figure 15. Determination of the mechanism of inhibition of lipohilic acids used as antimicrobial agents. See text for further details.

a. *Bacillus subtilis growth inhibition*

b. *L-serine transport inhibition in B. subtilis* vesicles.

⊖———⊖, linoleic acid (C_{18}, n=2)

⊖- - - - -⊖, oleic acid (C_{18}, n=1)

•———•, capric acid (C_{10})

■- - - - -■, caprylic acid (C_8)

Δ———Δ, caproic acid (C_6)

Δ- - - - -Δ, sorbic acid (C_6, n=2)

■———■, butyric acid (C_4)

O- - - - -O, propionic acid (C_3)

□- - - - -□, crotonic acid (C_4, n=1)

∇———∇, acetic acid (C_2)

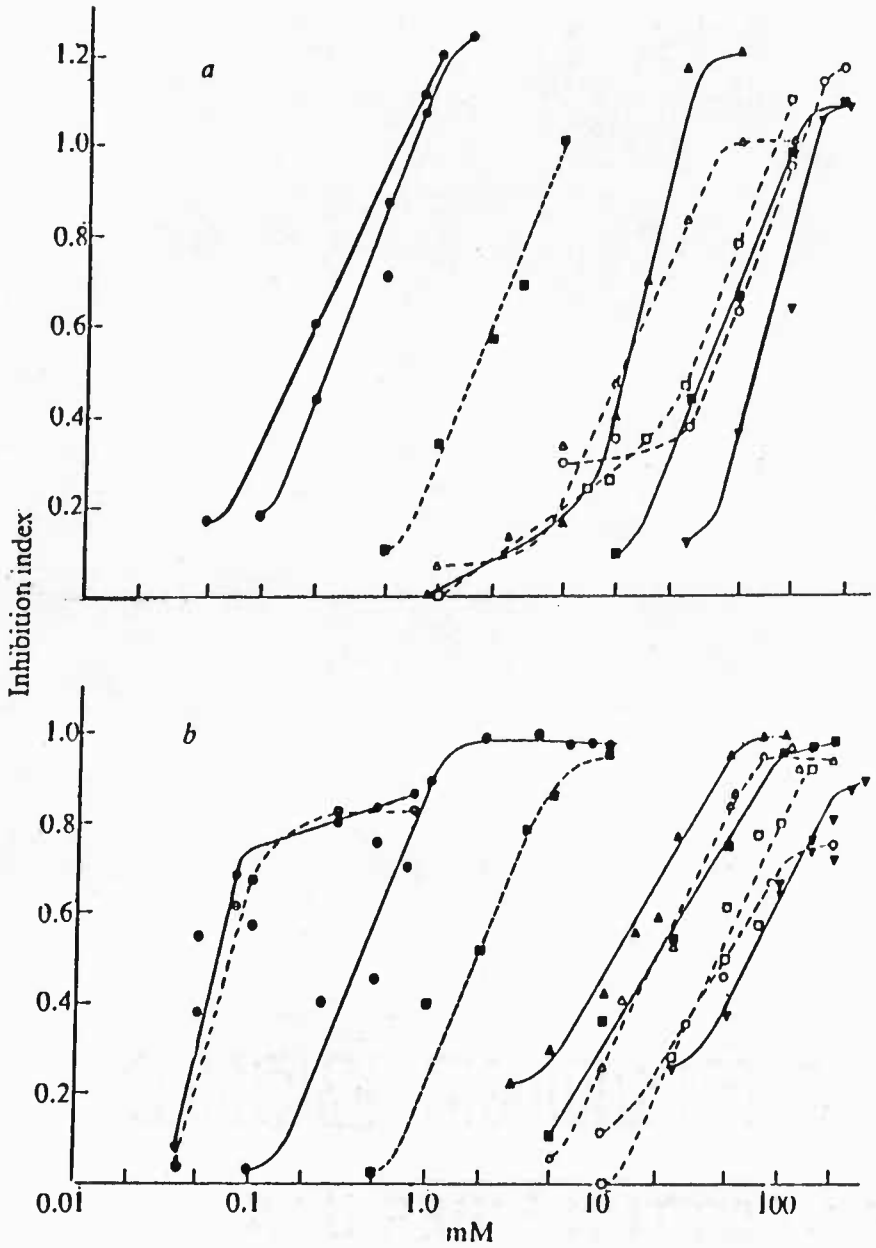


Figure 15

POTASSIUM SORBATE PERMEABILITY OF POLYSACCHARIDE FILMS: CHITOSAN, METHYLCELLULOSE AND HYDROXYPROPYL METHYLCELLULOSE

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Running Head: Potassium sorbate permeability

Potassium sorbate permeability of polysaccharide films: chitosan, methylcellulose and hydroxypropyl methylcellulose. Fakhrieh Vojdani and J. Antonio Torres, Department of Food Science and Technology, Oregon State University, Corvallis, Oregon 97331.

ABSTRACT

Edible coatings controlling preservative migration from surface to food bulk could inhibit surface microbial growth which is often the main cause of spoilage for many food products. In this paper we examine the potassium sorbate permeability behavior of chitosan, methyl cellulose and hydroxypropyl methyl cellulose based films. To gain an understanding of the permeation process permeability determinations were done at 5, 24, 32 and 40°C. Two theoretical models were found to agree well with the experimental data. Methyl cellulose was the most promising diffusion barrier with a permeability constant of 3.4 and 1.4×10^{-8} (mg/sec cm^2)(cm)/(mg/ml) at 24 and 5°C, respectively. Electron microscopy was used to examine the morphological characteristics of these films and showed they have no visible pores or channels at magnifications up to 10,000.

INTRODUCTION

The quality and stability of foods is often affected by diffusion phenomena. The progress of chemical reactions depend on the reactants having sufficient mobility at the molecular level to move to the reaction site (Duckworth, 1981; Simatos *et al.*, 1981). The control and reduction of moisture, gas or other solutes transfers from the environment into the food as well as their exchange between different regions of an heterogeneous food can be a main factor in the stability of a food product. In some cases it may be desirable to coat foods or food elements with an edible film or layer containing a high concentration of a given food additive. Recent work conducted by Fennema and coworkers (Kester and Fennema, 1986; Kamper and Fennema, 1985, 1984a,b) has centered on the control of moisture migration between regions with different water activities using modified cellulose based films.

A particularly interesting situation is the control of surface microbial growth which is often the main cause of spoilage for many refrigerated food products (Maxcy, 1981; Anderson *et. al.*, 1980; Gill, 1979; Vitkov, 1974, 1973). For example, fresh broilers in retail outlets have an initial concentration of 10^4 to 10^5 microorganisms/cm^2 and can be stored only for a few days at 3-5^oC and still maintain their freshness (Cunningham, 1979; Robach, 1979a). In the case of intermediate moisture foods (IMF), surface condensations caused

by temperature fluctuations result in temporary and local increases in surface water activity (a_w) leading to microbial spoilage (Torres *et al.*, 1985a,b; Torres, 1987).

To cope with surface microbial problems, food processors have used preservatives as a surface treatment. The use of potassium sorbate dips have been shown to reduce the total number of viable bacteria at refrigeration and temperature abuse conditions (Lueck, 1984; Robach and Sofos, 1982; Holley, 1981; D'Aubert *et al.*, 1980; Cunningham, 1979; Robach and Ivey, 1978). However, the shelf life extension achieved by such treatment is limited. Eventually microorganisms overcome the sorbate induced bacteriostasis due to diffusion of the preservative into the bulk of the food. Diffusion results in preservative concentration reduction on the surface where microbial spoilage is occurring (Torres, 1987; Greer, 1981). A tenfold reduction in sorbic acid diffusion rate (D) have been obtained by adjusting the a_w of the solution embedding a model food system (Guilbert *et al.*, 1985). Lowering the a_w of the model system from 1.0 to 0.88 using 40% w/w glycerol or 16% w/w salt reduced the apparent diffusivity at room temperature from 6.7 to 2.0 $x10^{-6}$ cm^2/sec. At 70% glycerol the a_w was 0.64 and D was $5x10^{-7}$ cm^2/sec.

The diffusion of sorbic acid in zein films has also been measured and found to be in the order of $3\text{-}7x10^{-9}$ cm^2/sec, i.e. about a 300 fold decrease as compared to the agar model with $a_w = 1.0$. The diffusion barrier properties of zein films

were confirmed in microbial tests using a model food system with a_w = 0.88 coated with zein and *Staphylococcus aureus* as the challenge microorganism (Torres *et al.*, 1985a; Torres and Karel, 1985).

In this paper we investigate the use of methylcellulose, hydroxypropyl methylcellulose, a mixture of both, and chitosan as coatings to retard sorbic acid diffusion from food surface into food bulk. Polysaccharides were chosen for their ability to form strong and clear films using relatively low price ingredients as compared to proteins such as zein. These films were characterized by electron microscopy and by measuring K-sorbate permeability in films soaked in aqueous glycerol to simulate lower a_w conditions. For comparison purposes permeability determinations we included chitosan films soaked in pure water. The use of edible coatings for this and other purposes has been recently reviewed by Guilbert (1986).

MATERIALS AND METHODS

Reagents

Methylcellulose (MC, Methocel A 15-LV, Premium) and hydroxypropyl methylcellulose (HPMC, Methocel F50, Premium) were obtained from Dow Chemical Co., Midland, MI. Chitosan (Lot No. 5112A) was obtained from Bioshell Inc., Albany, OR. Other chemicals used were glacial acetic acid, sodium hydroxide and glycerol from J.T. Baker Chemical (Phillisbury, NJ). Potassium sorbate (K-sorbate) was provided by Monsanto Co. (St. Louis, MO). Ethanol (95%, reagent) was obtained from Oregon State University Chemical stores.

Preparation of films

a. Chitosan films

0.5 g chitosan powder was dissolved in 100 ml of a 1.5% v/v acetic acid aqueous solution using continuous mechanical stirring. To avoid chitosan agglomeration chitosan was then slowly added. A clear mixture was achieved after three hours of continuous mixing which was then filtered through a medium porosity fritted disk Buchner type filtration funnel using a slight vacuum.

After allowing the solution to rest for 30 minutes, about 25 g were poured into 100x15 mm disposable Petri dishes. A film was formed after 7 hours drying in an oven at 45-47^{o}C. When the film had cooled down to room temperature it was removed from

the Petri dish and immersed in 25 ml 1 N sodium hydroxide for one hour. Finally the film was washed four times with 250 ml distilled water to remove excess reagents and then soaked in 50% v/v glycerol solution for 15-30 minutes (CHI/G films). In the case of permeability determinations in pure water this last step was not necessary (CHI/W films).

b. Cellulose ether films

5 g of hydroxypropyl methylcellulose (HPMC), methyl cellulose (MC) or a 3:1 mixture of HPMC and MC were suspended in 30 ml ethanol. The latter mixture had been reported to provide optimum retention of micronutrients entrapped in fortified rice coatings (Peil *et al.*, 1982).

While stirring the suspension with a mechanical agitator 70 ml water was then added. After 20 minutes mixing the solution was allowed to rest for 30 minutes to remove entrapped air. 10 g solution was then poured into the same Petri dish and dried at room temperature for 24-48 hours. The film was then removed from the dish and soaked for 1 hour in 50% v/v glycerol solution before measuring its thickness.

Film Thickness Measurement

The thickness of the films soaked in aqueous glycerol or water was measured using a top mounted Best Test Indicator (EDP No. 45987, Brown and Sharp Mfg. Co., N. Kingston, RI). The reported thickness values are the average of at least 20

measurements. Films were mounted on the permeability cell immediately after thickness measurement.

Permeability Test

Permeability values were determined using a cell similar to the one described by Torres (1987). It consisted of two mechanically agitated chambers separated by the film to be tested. The upper chamber contained a pure 50% (v/v) glycerol solution or pure water. The lower chamber contained the same solution with 2.5% w/v potassium sorbate. The cell was placed in an oven at 40 or 32°C, left at room temperature (24°C) or placed in a walk-in refrigerator at 5°C. All determinations were done at least in triplicates.

When mounted on the permeability cell the top side (air drying side) of the film faced the high K-sorbate concentration. Aqueous glycerol was used as the solvent to reduce a_w and to serve as a film plasticizer. Samples were taken from the upper chamber and the K-sorbate concentration was measured spectrophotometrically at 255 nm.

Films were inspected before and after every test to assure that results were not affected by cracks or other type of visually detectable failures. It should be noted that the permeability test is not a gentle experimental procedure and that the films are subjected to the mechanical abuse of stirrers and compression between the two permeability cell chambers.

Determination of permeability coefficients

Permeability coefficients (K) were calculated as described by Torres *et al.* (1985a). K-sorbate determinations were used to obtain plots of total amount of preservative transferred through the film as a function of time. After a time lag (L), a linear relationship is obtained. The slope of this curve is the steady state rate of K-sorbate transfer through the film (Rogers, 1985; Crank, 1976).

$$K = Fl/c \qquad (1)$$

F = ammount of K-sorbate permeated per unit time

l = thickness of the film

c = concentration in the high K-sorbate concentration chamber

These determinations were confirmed by using the following expression (Rogers, 1985; Crank, 1976):

$$L = l^2/6K \qquad (2)$$

L is obtained as an intercept on the time axis by extrapolation of the steady state rate of K-sorbate transfer through the film.

Effect of temperature on permeability

The permeability phenomena is a combination of two types of physical processes. First, there are sorption and desorption processes on both sides of the membrane which depend on the solubility of the diffusing molecule in the film (Karel, 1975). In addition, we have the diffusion of the permeate in the film. In most cases, the latter process is the controlling step and explains why permeability rates follow the Arrhenius activation energy model (Karel, 1975; Rha, 1975; Colton *et al.*, 1971; McElhaney *et al.*, 1970).

$$K = K_o \exp(-E_a/RT) \qquad (3)$$

where:

K_o = frequency constant

E_a = activation energy, Kcal/g-mole

R = universal gas constant

T = absolute temperature

Another approach to estimate the effect of temperature on permeability rate is by use of the following expression:

$$K\,\mu/T = \psi \qquad (4)$$

where:

K = permeability value

μ = solvent viscosity

T = absolute temperature

ψ = a constant

This expression is based on the Stokes-Einstein equation for the diffusion of a molecule in a medium of known viscosity (Guilbert *et al.*, 1985). This equation should be used with caution when the solution viscosity is high. At high viscosity this equation overestimates the lowering effect of temperature reductions on the diffusion constant (Perry and Green, 1984). This is not the case of water and the aqueous glycerol solution and temperatures used in this study.

Electron microscopy studies

Electron microscopy was used to evaluate film structure. Of particular interest were film uniformity and detection of pores. It was also used to confirm film thickness measurements.

The specimens were mounted on aluminum plancets using Avery Spot-O-Glue. The film was sectioned with a sharp razor blade and coated with approximately 100-200A^{o} of 60:40 gold-pladium in a Varian VE-10 vacuum evaporator at a vacuum of $1x10^{-5}$ torr. The microscopic examination was made using an AMRAY 1000A SEM operated at 20KV at the Electron Microscope Facility, Oregon State University. Images were recorded on Polaroid type 55 positive/negative 4x5 format film.

Statistical analysis

The statistical analysis of data was done on an IBM Personal Computer using SAS® (Anonymous, 1985).

RESULTS AND DISCUSSION

Film casting

All films formed were strong and flexible which facilitated the permeability measurements. This observation suggests that they would not fail during distribution if used as edible coatings. It should be noted that although all toxicological tests on chitosan have been positive it has not been petitioned for human consumption to the U.S. Food and Drug Administration.

As shown in Table 12, the thickness variation for films with the same composition was less than 10%. Thickness ranged from 0.02 for chitosan films to 0.12 mm for methylcellulose ether films.

Permeability determinations

Permeability determinations were done at a $a_w \approx 0.77$ (50 % aqueous glycerol). To examine the effect of higher a_w chitosan permeability was also determined at $a_w \approx 1.00$ (pure water).

Plots of total amount of K-sorbate diffused per unit area of film as a function of time at 5, 24, 32 and 40^{o}C have been summarized in Fig.16. These permeability curves followed the expected relationship with time. After a certain time period needed for the establishment of equilibrium conditions we had straight lines indicating a constant permeation rate F. As expected the rate of permeation decreases with temperature. The

slope and the thickness of the film for every individual run was measured and used with Eq. 1 to calculate individual permeability coefficients. The lowest permeability rate values were obtained for MC.

Average permeability values for experiments using aqueous glycerol were 8.6, 8.3, 3.4 and 5.8×10^{-8} at 24°C and 3.6, 3.7, 1.4 and 2.4×10^{-8} (mg/sec cm^2)(cm)/(mg/ml) at 5°C for chitosan (Fig. 16b), HPMC (Fig. 16c), MC (Fig. 16d) and the HPMC+MC mixture (Fig. 16e), respectively. As described by Torres (1987, Table 14.2) it is possible to roughly estimate the effect of these films on increased surface microbial stability. For example, if one uses 0.5 mm films, the surface protection using these films on an IM food can be predicted to last 1.5, 1.5, 4 and 2 months at 24°C and 3.5, 3.5, 8.5 and 5 months at 5°C at 5°C, respectively.

As shown in Figs. 16a and 16b and in Table 12 (K values) an important parameter to be considered is the hydration status of the film which would depend upon the food a_w. When determinations are done in pure water at 24 and 5°C the values were an order of magnitude higher than in aqueous glycerol. The values were 6.3 and 4×10^{-7} (mg/sec cm^2)(cm)/(mg/ml) which would reduce the surface protection period from 1.5 and 3.5 months to 6 and 9 days at 24 and 5°C, respectively. These estimations should be confirmed using specific food systems and challenging microorganisms. These studies would emphasize the importance of the food a_w.

Effect of temperature on permeability

Permeability values measured at 5, 24, 32 and 40°C (Fig. 16) were used to obtain Arrhenius plots and to determine an activation energy for the overall permeation process. The lack of breaking points in the Arrhenius plots (Fig. 17) indicates that no morphological changes occur within these films in the 5 to 40°C temperature range. A statistical analysis showed that there is no significant difference (α=.05) between the slopes for CHI, MC, HPMC and HPMC+MC films when the permeability was measured using a glycerol solution. The difference between the chitosan film in water versus all films in aqueous glycerol was found to be highly significant. As shown in Table 13, a 45% reduction in activation energy results when the K-sorbate permeation rate was determined using water instead of aqueous glycerol. The observation that the permeability values follow the Arrhenius model and that the activation energy is affected by the solvent embedding the film suggests that the diffusion process in the film occurs through the aqueous phase. Consequently, the performance of edible coatings controlling surface preservative concentration will depend strongly on the aqueous phase of the coated food.

Eq. 4 was used to estimate ψ values. As seen in Table 12 ψ was only slightly affected by temperature. No significant differences (α=.05) were observed for CHI in water and MC in aqueous glycerol. Most of the significant

differences for the other film/solvent combinations were observed only at 5°C.

ψ was normalized by dividing the value for a film-temperature combination by the average ψ for all temperatures for a specific film. As shown in Fig. 18 these values show very little dependence with temperature and for practical estimation purposes ψ can be assumed to be a constant. This observation suggests again that diffusion occurs in the aqueous glycerol phase since the Stokes-Einstein equation was derived for diffusion in a liquid medium.

Electron microscopy studies

The morphological characteristics of MC, HPMC, PMC and HPMC+MC films after soaking in aqueous glycerol were observed by electron microscopy. As shown in Fig. 19 these films were homogeneous and of uniform thickness. Examination of the film surface at the maximum magnification possible without film damage, 10,000x (Fig. 20), showed an absence of pores or other type of defects. The same lack of features were observed in examinations of film cross-sections. This means that if pores or channels exist they should be less than 0.1 μm.

CONCLUSIONS

This study confirms that it is possible to develop coatings that will enhance surface microbial stability (Torres, 1987) and thus could facilitate the development of refrigerated meat and other products with longer shelf life.

Based on the observation that the diffusion process occurs through the solvent phase future papers will examine the effect on potassium sorbate permeation by reducing the hydrophilicity of polysaccharide based coatings (Vojdani and Torres, 1987a,b).

Table 12.

EFFECT OF TEMPERATURE ON THE
POTASSIUM SORBATE PERMEABILITY THROUGH POLYSACCHARIDE FILMS

Film	40°C			32°C			24°C			5°C		
	K[a] x10^8	l[b]	ψ x10^11	K x10^8	l	ψ x10^11	K x10^8	l	ψ x10^11	K x10^8	l	ψ x10^11
CHI/G												
	13.7	21	285[q]	9.9	21	283[q]	8.6	24	320[pq]	3.6	20	355[p]
	17.1	25		11.4	23		8.5	24		3.5	27	
	13.7	20		11.4	23		8.8	23		3.6	22	
	Average ψ = (311 ± 34)x10^-11, P=.0088											
HPMC												
	13.9	98	269[r]	10.6	95	275[r]	8.1	98	308[q]	3.6	96	367[p]
	13.8	92		11.1	90		8.5	120		3.8	92	
	14.4	110		10.2	100		8.3	95		3.6	95	
	Average ψ = (305 ± 45)x10^-11, P=.0001											
MC												
	6.2	99	137[p]	5.0	113	131[p]	3.2	95	125[p]	1.4	140	144[p]
	6.5	100		5.7	112		3.3	103		1.4	110	
	7.5	104		4.6	90		3.6	120		1.5	120	
	Average ψ = (134 ± 8)x10^-11, P=.2228											
HPMC+MC												
	10.0	95	198[q]	8.5	98	214[q]	5.9	90	216[q]	2.6	114	244[p]
	10.5	110		8.1	110		5.6	95		2.2	115	
	10.4	100		8.2	104		6.0	85		2.5	111	
	Average ψ = (218 ± 19)x10^-11, P=.0072											
CHI/W[d]												
	95.7	28	200[p]	77.7	28	208[p]	62.5	26	200[p]	39.0	25	208[p]
	83.7	24		75.1	26		63.7	24		39.9	29	
	82.2	23		76.8	27		61.8	25		40.0	26	
	Average ψ = (204 ± 5)x10^-11, P=.5579											

[a] Permeability, (mg/sec cm^2)(cm)/(mg/ml)
[b] Film thickness, μm
[c] ψ = Kμ/T
[d] See text for definition of other terms
p,q,r ψ values for individual films with the same letter are not significantly different (α=.05)

Table 13.

ACTIVATION ENERGY (E_a) OF K-SORBATE PERMEATION

Film	E_a, cal/g-mole
CHI/G	6980
HPMC	6620
MC	7710
HPMC+MC	7270
Average	7150 ± 460
CHI/W	3950

See text for definition of terms

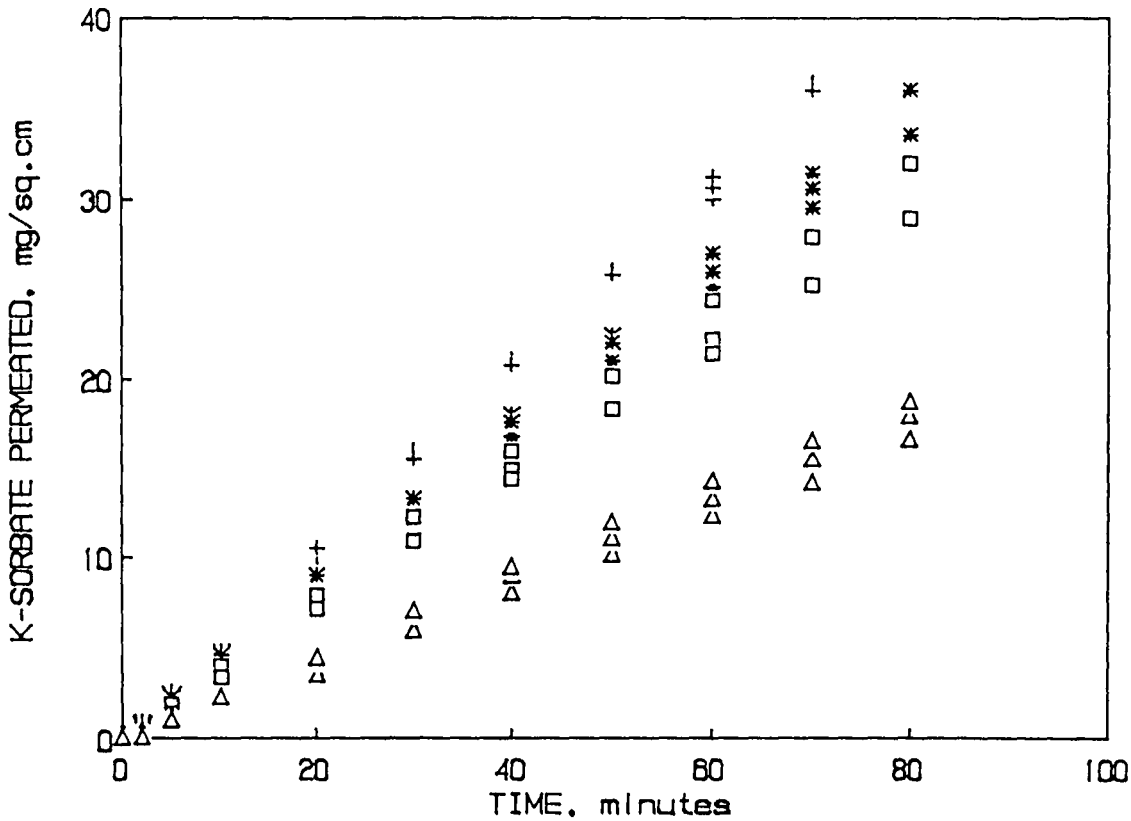

Figure 16. Effect of temperature on K-sorbate permeability.
a. CHI at $a_w \approx 1.00$
+, 40°C; *, 32°C; □, 24°C; △, 5°C

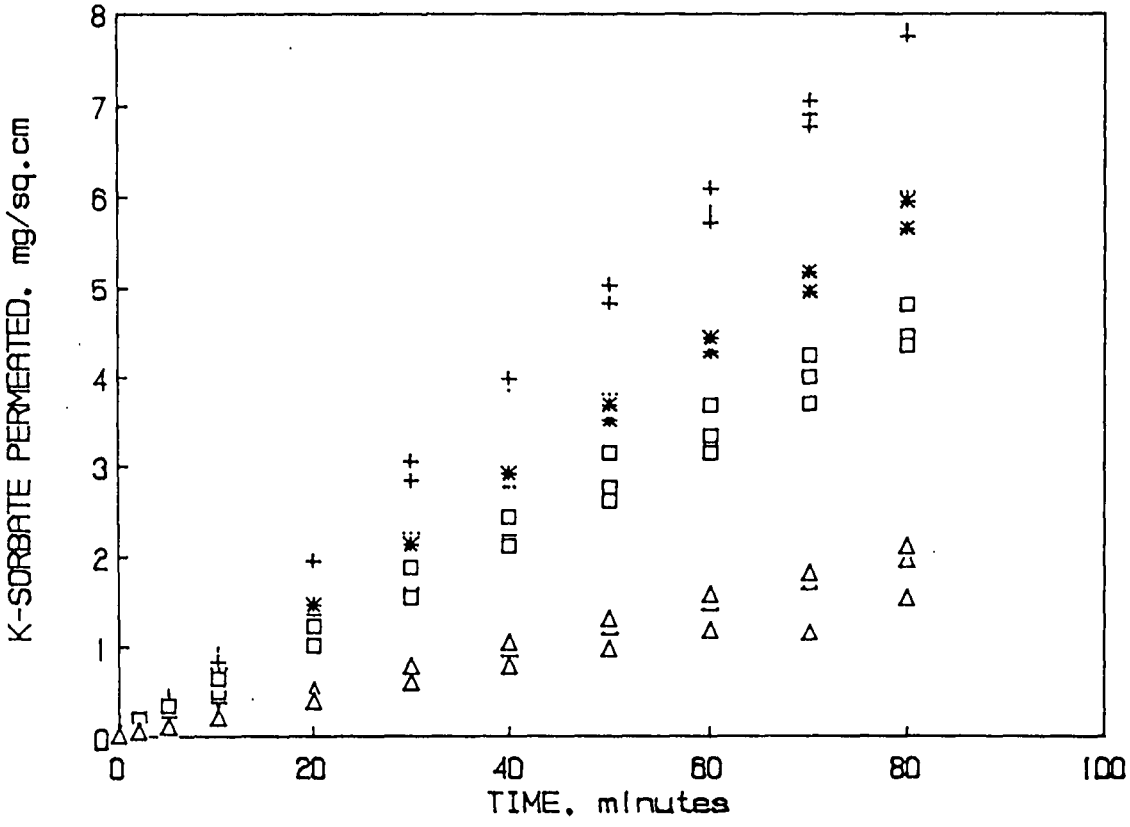

Figure 16. Effect of temperature on K-sorbate permeability (continued).
b. CHI at $a_w \approx 0.77$
+, 40°C; *, 32°C; □, 24°C; △, 5°C

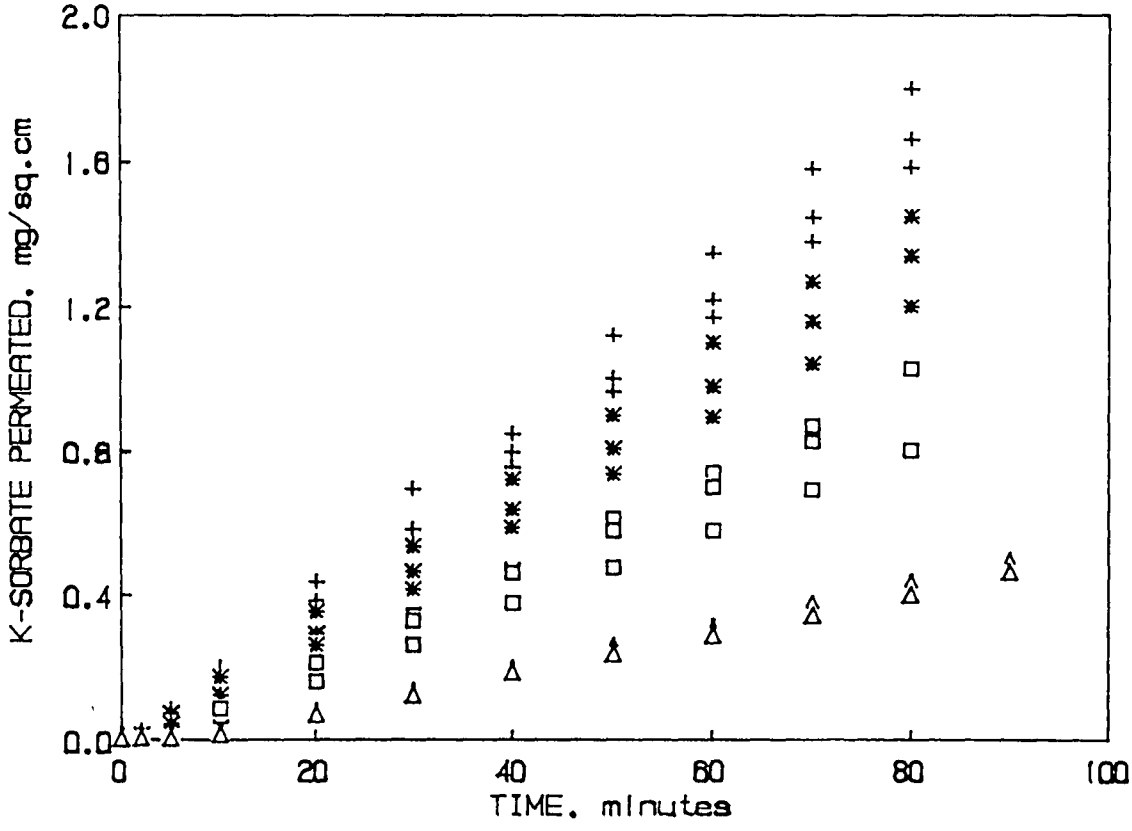

Figure 16. Effect of temperature on K-sorbate permeability (continued).
c. HPMC at $a_w \approx 0.77$
+, 40°C; *, 32°C; □, 24°C; △, 5°C

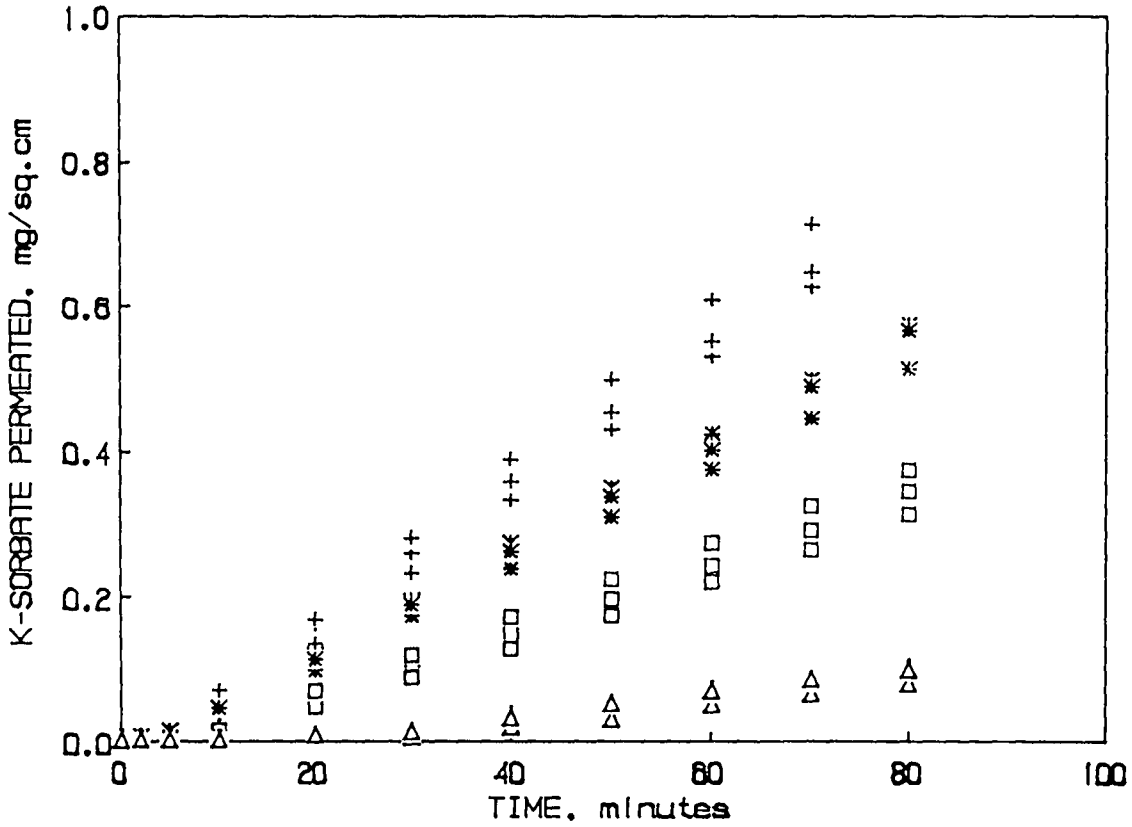

Figure 16. Effect of temperature on K-sorbate permeability (continued).
d. MC at $a_w \approx 0.77$
+, 40°C; *, 32°C; □, 24°C; △, 5°C

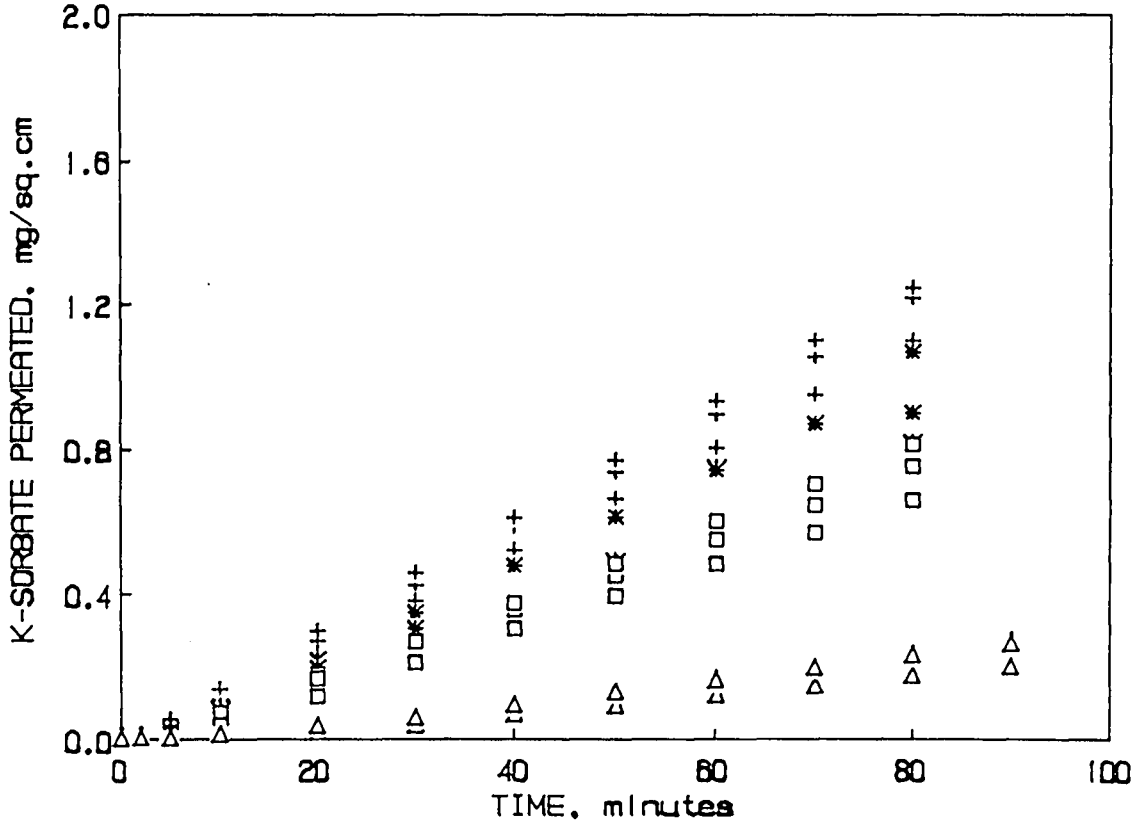

Figure 16. Effect of temperature on K-sorbate permeability (continued).
e. HPMC + MC at $a_w \approx 0.77$
+, 40°C; *, 32°C; □, 24°C; △, 5°C

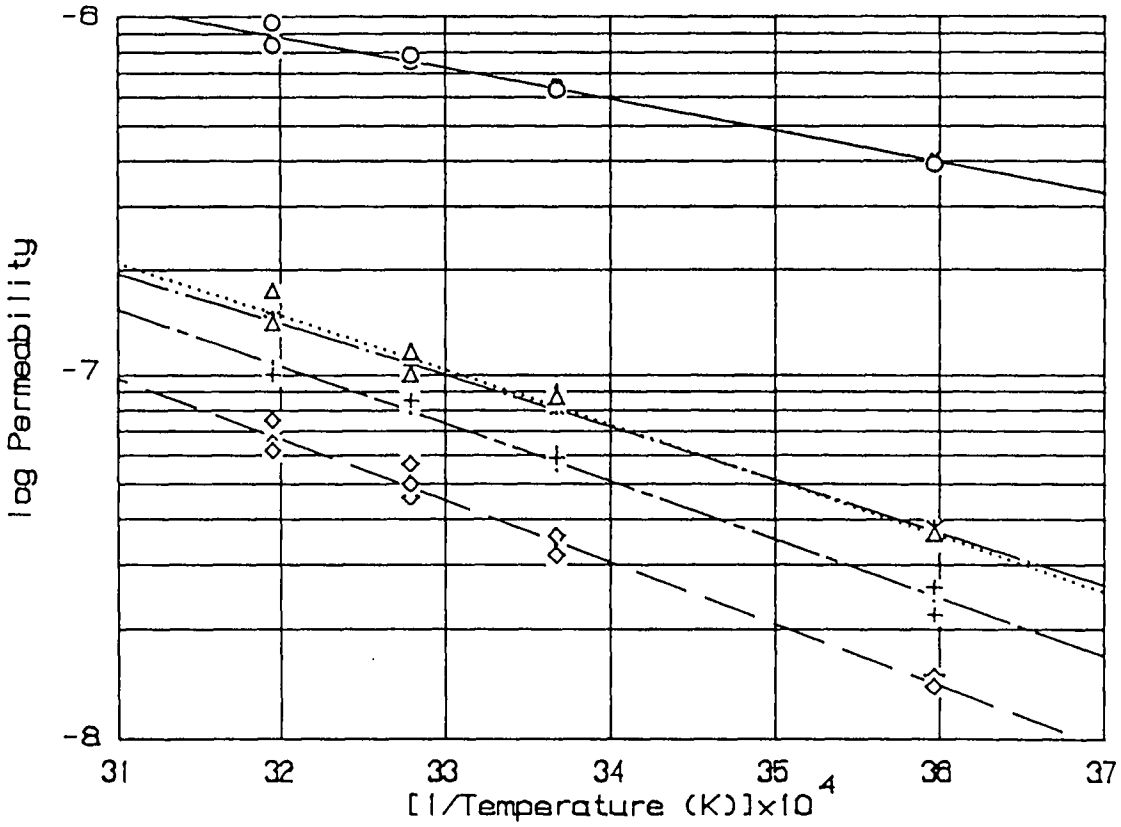

Figure 17. Arrhenius plots for permeability of K-sorbate through edible coating films.
○——○, CHI at high a_w
△·····△, CHI at low a_w
——·——, HPMC at low a_w
+—— — ——+, HPMC+MC at low a_w
◇—— ——◇, MC at low a_w

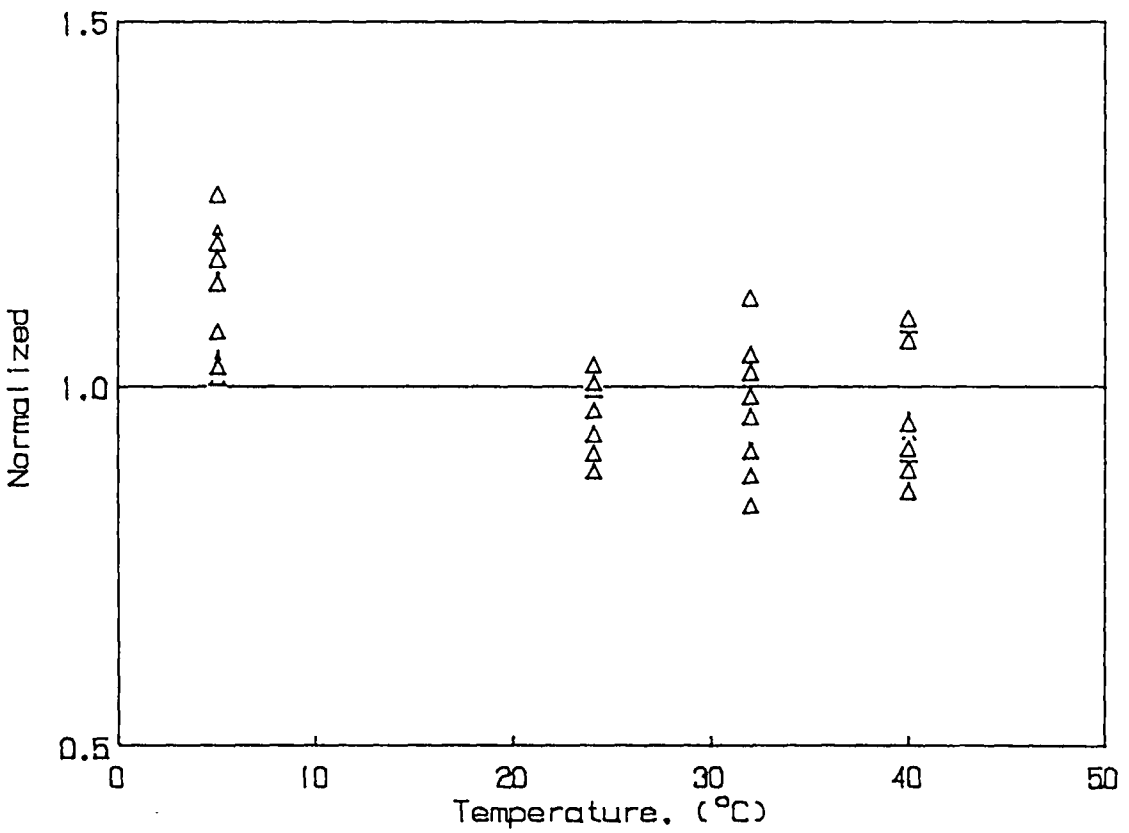

Figure 18. Normalized ψ for all edible coating films. See text for further details.

Figure 19. Electron microscopy photomicrographs of cross-sections of edible coating films (bar = 20 μm).
a. MC
b. HPMC
c. HPMC + MC
d. CHI

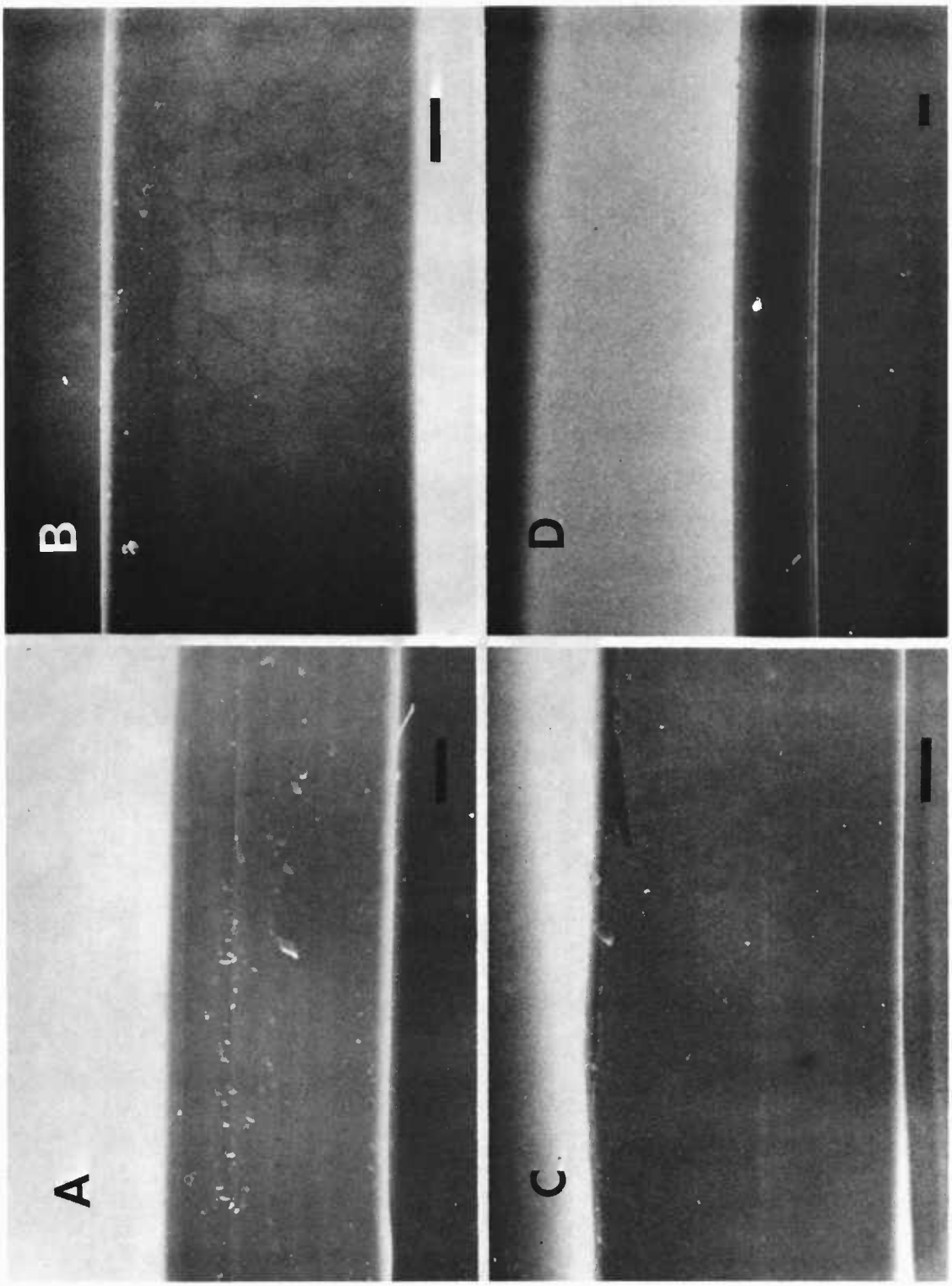

Figure 19

Figure 20. Electron microscopy photomicrographs of edible coating film surfaces (bar = 1 μm).
a. MC
b. HPMC
c. HPMC + MC
d. CHI

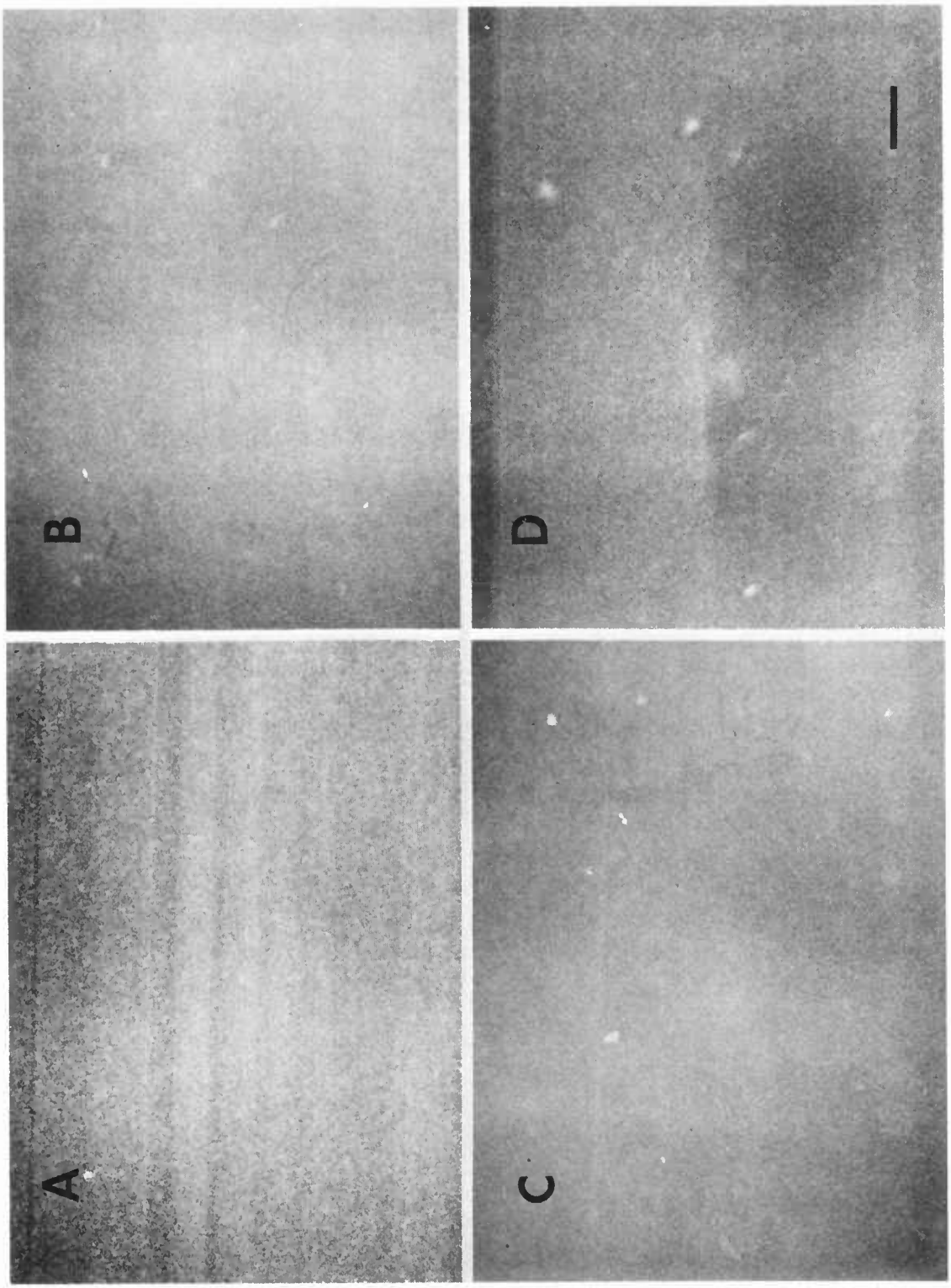

Figure 20

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POTASSIUM SORBATE PERMEABILITY OF METHYLCELLULOSE AND HYDROXYPROPYL METHYLCELLULOSE FILMS: EFFECT OF FATTY ACIDS

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Running Head: Potassium sorbate permeability

Potassium sorbate permeability of methylcellulose and hydroxypropyl methylcellulose films: effect of fatty acids. Fakhrieh Vojdani and J. Antonio Torres, Department of Food Science and Technology, Oregon State University, Corvallis, Oregon 97331.

ABSTRACT

Surface microbial stability is a major determinant of the shelf life of refrigerated meat products. Surface microbial growth has also been noticed in intermediate moisture foods exposed to temperature fluctuations. The solution to this problem is to apply edible coatings that control the diffusion of antimicrobial agents applied on the food surface into the food bulk. Films with such properties were identified using a permeability cell. We found that methyl- and hydroxypropyl methyl cellulose mixed with lauric, palmitic, stearic and arachidic acid lowered significantly potassium sorbate permeation rate as compared to cellulose ether films containing no fatty acids.

Permeability determinations at 5, 24, 32 and 40°C showed excellent agreement with the Arrhenius activation energy model for the permeation process.

INTRODUCTION

Surface microbial growth is often the main cause of spoilage for many refrigerated food products (Maxcy, 1981; Anderson *et. al.*, 1980; Olson *et al.*, 1980; Gill, 1979; Vitkov, 1974, 1973). For example, fresh broilers in retail outlets have an initial concentration of 10^4 to 10^5 microorganisms/cm^2 and can be stored only for a few days at 3-5^{o}C and still maintain their freshness (Cunningham, 1979; Robach, 1979a).

In recent years there has been an increased interest in the control of food spoilage by reducing water activity (a_w) to a safe level. The low a_w and preservatives used in modern intermediate moisture food (IMF) technology retards or stops the growth of bacteria, molds and yeasts (Bone, 1987; Richard-Molard and Lesage, 1986). However, IMF technology has serious sensory acceptability problems associated with the high solute concentrations required to reduce a_w. An alternative is refrigerated IMF technology, a combination that requires lower solute concentrations (Torres, 1987).

IMF products are generally packaged in moisture proof materials and therefore not affected by external relative humidity conditions. However, their stability is affected by changing storage temperatures (Torres, 1987). In packaged IMF's subjected to fluctuating temperatures, water migration within the package can possibly occur by a sorption mechanism (Singh *et al.*, 1984). Redistribution of water and surface

condensations cause localized increases in surface a_w. It is on these surface regions where microbial growth can occur even when bulk a_w is below a safe level.

Based on the above considerations, there is a need to develop new processes that specifically enhance surface microbial stability. These treatments should be FDA acceptable, i.e. safe and effective and include only food grade materials. Surface conditions should inhibit or at least reduce microbial growth rate to such an extent, that product shelf life is extended significantly (Torres and Karel, 1985; Torres *et al.*, 1985a,b).

To cope with surface microbial problems, food processors have used preservatives as a surface treatment. The use of potassium sorbate dips have been shown to reduce the total number of viable bacteria at refrigeration and temperature abuse conditions (Lueck, 1984; Robach and Sofos, 1981; D'Aubert *et al.*, 1980; Cunningham, 1979; Robach and Ivey, 1977). Moreover, sensory analysis studies have indicated that it is not possible to distinguish between chicken parts treated with the preservative and those dipped in distilled water (Cunningham, 1979). It should be noted that the sensory response will depend upon the specific food involved (Bodyfelt, 1986, 1981).

However, the shelf life extension achieved by such treatment is limited. Eventually microorganisms overcome the sorbate induced bacteriostasis due to diffusion of the preservative into the bulk of the food. Diffusion results in preservative

concentration reduction on the surface where microbial spoilage is occurring (Torres, 1987; Greer, 1981).

Recently, the use of edible films as a gas, moisture or solute barrier has been proposed to protect food products. Polysaccharide based films such as cellulose, modified cellulose and starch; proteins such as zein, collagen, gelatin, ovalbumin and serum albumin; plant and microbial polysaccharides such as agar, carrageenan, alginate, pectin, dextran, gum ghatti, scleroglucan, pullulan, curdlan; and waxes and lipid derivatives have all been considered for food applications (Guilbert, 1986; Anders, 1984; Guilbert *et al.*, 1983; Hannigan, 1983; Peil *et al.*, 1981).

For instance, the diffusion barrier properties of zein films were confirmed in microbial tests using a model food system and *Staphylococcus aureus* as the challenge microorganism. In this study, a cheese analog model was coated with zein, sprayed with sorbic acid and then inoculated with *S. aureus* S-6. Samples stored at 30°C and exposed to relative humidity cycles showed almost a tenfold increase in microbial stability as compared to uncoated controls. A reduced preventative diffusion due to the barrier properties of zein films was identified as the mechanism for stability improvement (Torres *et al.*, 1985a; Torres and Karel, 1985). Unfortunately, zein, a corn protein is expensive and has limited commercial production. The film is very water-resistant but has an objectionable flavor (Guilbert, 1986).

Films prepared using a blend of various macromolecules, polysaccharides, proteins and lipids have been predicted to have cooperative functionalities i.e., they may take advantage of the individual properties of each constituent and lead to films with the desired barrier properties (Kester and Fennema, 1986). For example, the polysaccharide may impart structural cohesion and serve as a structural matrix. A protein may give rise to a very tight structure by inter- or intramolecular folding, and a lipid adds a hydrorepulsive character. A naturally occurring example of these multicomponent systems are the glycoproteins, lipoproteins and glycolipids present in cellular membranes (McElhaney *et al.*, 1970).

Bilayer films composed of cellulose ether and lipids, a blend of palmitic and stearic acid (Kester and Fennema, 1986; Kamper and Fennema, 1985, 1984a,b) showed a significant decrease in moisture permeability.

Edible films composed of hydroxypropyl methylcellulose as a support for, or as part of an emulsion mixture with various kinds of lipids, have shown excellent resistance to water vapor permeability. Films containing stearic acid or solid lipids, such as beeswax, paraffin, hydrogenated palm oil, yielded permeabilities of 0.2 (g mil)/(day^{-1} m^2 mm Hg) or less which is lower than the value for low density polyethylene (Kamper and Fennema, 1984a).

This paper investigates the possibility of using these and other similar films to control the diffusion of surface applied

potassium sorbate. The barrier properties of these films are expected to maintain a high concentration of potassium sorbate on the food surface for as long as possible (Torres, 1987). Potassium sorbate was chosen for its wide range of bacteriostatic and mycostatic properties. Other advantages include its consumption safety and its tasteless and odorless properties even at high concentration.

Potassium sorbate is effective in most foods in a concentration range of 0.05 to 0.3% by weight (Huhtanen *et al.*, 1983; Shaw *et al.*, 1983; Elliott *et al.*, 1982; Kathleen and Pierson, 1982; Robach and Sofos, 1982; LaRocco and Martin *et al.*, 1981; Restaino *et al.*, 1981; Yousef and Marth, 1981; Sofos *et al.*, 1980; To and Robach, 1980; Robach, 1979b). However, the higher the sorbate application level, the longer will be the period of time of microbial inhibition (Gray *et al.*, 1984). As noted by Torres and Karel (1985) high surface concentrations can be bactericidal.

Most research on permeability has been directed to the control of moisture migration problems. Little information is available on the diffusion of other molecules, particularly food preservatives. Therefore, this study was designed to examine specifically the permeability and diffusion properties of sorbates in edible films of methylcellulose and hydroxypropyl methylcellulose emulsified with lauric, palmitic, stearic and arachidic acid.

MATERIALS AND METHODS

Reagents

Methylcellulose (MC, Methocel A 15-LV, Premium) and hydroxypropyl methylcellulose (HPMC, Methocel F50, Premium) were obtained from Dow Chemical Co., Midland, MI. Polyethylene glycol 400 (PEG), lauric acid (C12, 99-100%), palmitic acid (C16, 99%), stearic acid (C18, 99%), arachidic acid (C_20, 99%), oleic acid (C18:1, cis, 99%) and elaidic acid (C18:1, trans, 99%) were obtained from Sigma Chemical Co., St. Louis, MO. Other chemicals used were glycerol (J.T. Baker Chemical, Phillisbury, NJ), and potassium sorbate (K-sorbate, Monsanto Co., St. Louis, MI). Ethanol (95%, reagent) was obtained from OSU Chemical stores.

Preparation of films

Film preparation methods were adapted from those described by Kamper and Fennema (1984a,b). 4.5 g of cellulose ether (MC or HPMC) was placed in a 400 ml beaker and mixed with 50 ml of hot water (ca. 90°C) and stirred until all particles were thoroughly melted and a uniform suspension was obtained. While continuously stirring we added 100 ml ethanol and the fatty acids at MC (or HPMC):lipid ratios of 45:1, 45:5, 45:10, 45:15, and 45:20. Finally, and still while stirring and heating, 0.5 g PEG were also added. Heating rate was adjusted so that at the end of the 15 minutes total preparation time, the solution

temperature would be about 84-85°C. Heating facilitated the incorporation of the fatty acid into the solution.

Entrapped air constituted a serious experimental problem. To overcome this difficulty the solution was subjected to a reduced pressure for two minutes.

Film compositions with the desired MC (or HPMC) fatty acid ratio were spread thinly and uniformly while still hot on about 4-5 plates 20x20 cm using a thin layer chromatography (TLC) applicator. A warm film spreader and warm plate were used to facilitate film formation. The spreader was set to 0.75-0.85 mm and then slowly and steadily pulled across the plates. The plates were left at room temperature for four minutes to allow for lipid orientation within the wet film and then placed in an oven at 80-85°C for 15 minutes.

After drying and cooling, the films were removed from the plates very carefully, placed in plastic bags, stored at room temperature and used within 3-4 days.

Permeability test

Permeability values were determined using a cell similar to the one described by Torres (1987). It consisted of two mechanically agitated chambers separated by the film to be tested. The upper chamber contained a pure 50% (v/v) glycerol solution with a $a_w \approx 0.77$. The lower chamber contained the same solution with 2.5% w/v potassium sorbate. The cell was placed in an oven at 40 or 32°C, left at room temperature

(24°C) or placed in a walk-in refrigerator at 5°C.

Films to be tested were soaked in aqueous 50% (v/v) glycerol for at least one hour. When mounted on the permeability cell, the lipid side of the film faced the high K-sorbate concentration. Aqueous glycerol was used as the solvent to reduce a_w and to serve as a film plasticizer. Samples were taken from the upper chamber and the K-sorbate concentration was measured spectrophotometrically at 255nm.

Film thickness measurement

Dried films were soaked in glycerol-water 50% v/v for approximately one hour and then placed on a surface plate. The thickness of the wet film was measured using a top mounted Best Test Indicator (EDP No. 45987, Brown and Sharp Mfg. Co., N. Kingston, RI). The reported thickness values are the average of at least 20 measurements.

Determination of permeability coefficients

Permeability coefficients (K) were calculated as described by Torres *et al.* (1985a). K-sorbate determinations were used to obtain plots of total amount of preservative transferred through the film as a function of time. After a time lag (L), a linear relationship is obtained. The slope of this curve is the steady state rate of K-sorbate transfer through the film (Comyn, 1985; Rogers, 1985; Crank, 1976).

$$K = Fl/c \quad (1)$$

F = amount of K-sorbate permeated per unit time

l = thickness of the film

c = concentration in the high K-sorbate concentration chamber.

These determinations were confirmed by using the following expression (Rogers, 1985; Crank, 1976):

$$L = l^2/6K \quad (2)$$

L is obtained as an intercept on the time axis by extrapolation of the steady state rate of K-sorbate transfer through the film.

Statistical analysis

The statistical analysis of data was done on an IBM Personal Computer using SAS® (Anonymous, 1985).

RESULTS AND DISCUSSION

Table 14 summarizes all experimental conditions used in this study. Permeability values were obtained at 24°C for MC and HPMC based films containing lauric, palmitic, stearic or arachidic acid at five different concentrations. To determine the effect of temperature MC and HPMC band films at a cellulose ether (CE): fatty acid ratio of 45:15 were also tested at 5, 32 and 40°C. Pure MC and HPMC controls at 5, 24, 32 and 40°C were also included. All determinations (72) were done at least in duplicates.

Film casting

The concentration of 3% cellulose ether in aqueous alcohol resulted in a suitable viscosity for film casting in agreement with the results reported by Kamper and Fennema (1984a). The beneficial effects of polyethylene glycol were also noted.

We should note that serious film preparation difficulties were observed at a cellulose ether:fatty acid ratio of 45:20, particularly for the longer fatty acid chain lengths. The usual film preparation difficulties included fatty acid losses, removal from the glass plate, distortion, cracking and pinhole formation during oven drying. Although the thickness variation between individual films was large (Table 15), the variation within an individual film was less than 10%. Most films ranged from 20 to 30 μm thickness, although a few films had to be

cast thicker, 40 to 50 μm, to overcome sticking to the glass plates.

Films were inspected before and after every test to assure that results were not affected by cracks or other type of visually detectable failures. It should be noted that the permeability test is not a gentle experimental procedure and that the films are subjected to the mechanical abuse of stirrers and the compression between the two permeability cell chambers.

Permeability estimations

Plots of total amount of K-sorbate diffused per unit area of film as a function of time at 24 ^{o}C have been summarized in Figs. 21 (HPMC-based films) and 22 (MC-based films). These graphs show a significant effect of fatty acid type and fatty acid concentration on permeability rate. The general trend is towards lower permeation rates as the fatty acid concentration increases. There is also a consistent difference between individual replicates. This difference is caused by the slight thickness variation between individual films.

The slopes of the linear portion of these graphs and the thickness of each individual film were used to estimate K-sorbate permeability constants. As shown in Figs. 21 and 22 the permeability curve followed the expected relationship with time. After a certain time period needed for the establishment of equilibrium conditions we observed a linear relation between K-sorbate diffused and time. The linear relationship was not

perfect in the case of lauric acid which might indicate film changes during testing. Such changes could include losses of lauric acid into the solution, structural changes in the film due to the low melting point of this fatty acid (45°C, Sigma Chemical Co.) and sorption and desorption of the diffusing molecule. The latter ones are unlikely since they would have affected the other films too. Lauric acid films were also more flexible and therefore more prone to be stretched out during testing. Stretching will decrease film thickness and increase surface area. This could also explain the poorer linearity of lauric acid permeability plots.

Duplicate average permeability values ranging from 4.6×10^{-8} and 8.2×10^{-8} (mg/sec cm^2)(cm)/(mg/ml) for the MC and HPMC controls, respectively, to 4.0×10^{-9} and 6.0×10^{-9} (mg/sec cm^2)(cm)/(mg/ml) for MC:C12 = 45:20 and HPMC:C12 = 45:20, respectively, have been summarized in Table 15. The values for the composite films are comparable to those obtained for zein by Torres *et al.* (1985a). The ability of zein films to control microbial growth has been demonstrated for the case of intermediate moisture foods surface inoculated with *Staphylococcus aureus* S-6. Reduction of sorbic acid diffusion rate from surface into food bulk resulted in significant shelf life extension as shown in storage studies under abuse testing conditions (Torres and Karel, 1985).

These permeability values should also be compared with published apparent diffusion values for sorbic acid in food

systems. In an intermediate moisture agar model system Guilbert *et al.* (1983) reported a value of $2.0x10^{-6}$ cm^2/sec. Torres *et al.* (1985a) found that in the case of an intermediate moisture cheese analog the value was $1.0x10^{-6}$ cm^2/sec. Although permeability and diffusion are not exactly the same phenomena, the values are in most cases, comparable if they are reported in consistent units. Therefore, we can estimate that the mass transfer process in CE:fatty acid films is about three orders of magnitude lower than in typical food systems.

Effect of fatty acid concentration and chain length

In general, Table 15 indicates that permeability decreased as the concentration of the fatty acid was increased. As noted before some film casting difficulties were observed in films with a CE:fatty acid ratio=45:20. These film casting difficulties interrupted the tendency towards lower permeability values as the fatty acid concentration was increased. For example, the MC:C18 films with ratios of 45:1, 45:5, 45:10, 45:15 and 45:20 had the following permeability values: 42, 35, 12, 8.9 and 10.5 $x10^{-9}$ $(mg/sec\ cm^2)(cm)/(mg/ml)$. Commercial users should therefore explore the highest fatty acid concentration possible with industrially available equipment. Higher concentrations are, however, limited by the need for MC and HPMC to provide structural support to these films.

Table 15 indicates also that permeability decreased as the fatty acid chain length increased. At all fatty acid concentrations the lowest permeability values are obtained for arachidic acid. This is not true for the CE:fatty acid=45:20 ratio for the reasons already discussed. This observation is consistent with published data on the permeability of synthetic lecithin liposomes. De Gier *et al.* (1968) found that increasing the lecithin fatty acid chain length decreased the permeability of glycerol and erythritol through these artificial membranes. Demel *et al.* (1968) showed that the glucose permeability of liposomes is also influenced by the fatty acid substituents present in lecithin.

Effect of fatty acid double bonds

McElhany *et al.* (1970) showed that the permeability of liposomes and intact cells of *Mycoplasma laidlawii* B could be systematically and dramatically altered by the geometrical configuration and the number of double bonds present in the fatty acid component of a membrane. Their experiments using synthetic liposomes showed that the rate of glycerol permeation increased in the order elaidic < oleic < linoleic. Table 16 shows that our results are consistent with their observations and would therefore discourage the incorporation of unsaturated fatty acids to cellulose ether based films.

Effect of temperature

The permeability phenomena is a combination of two types of physical processes. First, there are sorption and desorption processes on both sides of the membrane which depend on the solubility of the diffusing molecule in the film (Karel, 1975). In addition, we have the diffusion of the permeate in the film. In most cases, the latter process is the controlling step and explains why permeability rates follow the Arrhenius activation energy model (Karel, 1975; Rha, 1975; McElhaney *et al.*, 1970).

$$K = K_o \exp(-E_a/RT) \quad (3)$$

where:

K_o = frequency constant

E_a = activation energy, Kcal/g-mole

R = universal gas constant

T = absolute temperature

Permeability values measured at 5, 24, 32 and 40°C for HPMC and MC based films were used to obtain Arrhenius plots as shown in Figs. 23 and 24, respectively. The CE:fatty acid ratio was 45:15. These graphs revealed the expected linear relationships and permitted the calculation of an activation energy for the overall permeation process. The lack of breaking points in the Arrhenius plots indicate that no morphological changes occur within these films in the 5 to 40°C temperature

range. Activation energy values for CE:FA films ranged from 7.36 to 7.84 Kcal/g-mole, which are consistent with published values. Sweet and Zull (1969) found that the activation energy for diffusion of glucose from phospholipid micelles was 10 to 11 Kcal/g-mole.

As shown in Table 17, no significant differences can be seen between the activation energies for all films including controls. This suggests that there is a common phenomena underlying the permeation process. The activation energy can be interpreted as reflecting the molecular interaction between the diffusant and the diffusing medium (Rha, 1975). Therefore, a common activation energy value suggests that the diffusion process occurs in the glycerol solution present in all films. The incorporation of fatty acids does not change the fact that the diffusion occurs in the solvent phase, i.e. in a 50% (v/v) aqueous glycerol solution. Most likely the fatty acids change film hydration and this possibility should be examined in future studies. It is also likely that the fatty acids interact with the cellulose ether molecules. Such behavior has been observed in other polysaccharide-fatty acid interactions. Whittam *et al.* (1986) reported that X-ray diffraction studies suggests that fatty acids could be entrapped within the helical amylose chain in a similar form to the blue amylose-iodine complex.

Another approach to estimate the effect of temperature on permeability rate is by use of the following expression:

$$K\ \mu/T = \alpha \qquad (4)$$

where:

K = permeability value

μ = solvent viscosity

T = absolute temperature

α = a constant

This expression is based on the Stokes-Einstein equation for the diffusion of a molecule in a medium of known viscosity (Guilbert *et al.*, 1985). It should be noted that it should be used with caution when the solution viscosity is high. At high viscosity this equation overestimates the lowering effect of temperature reductions on the diffusion constant (Perry and Green, 1984). This is not the case of the aqueous glycerol solution and temperatures used in this study. Viscosity values were obtained from the literature (Perry and Green, 1984; Newman, 1968).

As seen in Table 18 this expression is also valid if permeability values are used instead and suggests again that diffusion occurs in the aqueous glycerol phase. We obtained α values that were a function of the cellulose ether and the fatty acid type, but were not affected by temperature.

Using Eq. 4 we estimated that the permeability at 5°C of a MC:palmitic acid=45:20 ratio film is approximately 1.5×10^{-9} (mg/sec cm^2)(cm)/(mg/ml).

CONCLUSIONS

As described by Torres (1987, Table 14.2) it is possible to roughly estimate the effect that CE:FA films with the permeability values measured in this study might have on increased surface microbial stability. For example, if one uses a MC:palmitic acid=45:20 film on a food stored at 24°C, K = 4.9×10^{-9} $(mg/cm^2)(cm)/(mg/ml)$ (Table 15), the surface protection can be predicted to last 30 days for a 0.1 mm film and 120 days for a 0.2 mm thick film. If the item is stored under refrigeration (5 °C) these values become 82 and 328 days, respectively (Torres, 1987; Vojdani and Torres, 1987b). These estimations should be confirmed using a specific food system and challenging microorganism. An advantage of MC and HPMC based films is that they could be washed out by the consumer to eliminate the potassium sorbate or other preservative remaining on the surface as well as the coating itself.

The influence of the solubility in the film and the hydrodynamic volume of the diffusing solute should also be considered when selecting a diffusion barrier film. In our experimental work we considered only potassium sorbate. Other preservatives will give lower or higher permeability values depending upon their specific properties.

Another important parameter to be considered in future studies is the hydration status of the film. In our model

system studies we used a 50% (v/v) aqueous glycerol solution. This represents the case of an intermediate a_w food. Other model systems need to be examined.

The excellent agreement between the experimental data and the Arrhenius activation energy model indicates that it should be relatively simple to conduct storage stability studies under changing storage temperature conditions.

This study confirms that it is possible to develop coatings that will enhance surface microbial stability (Torres, 1987; Vojdani and Torres, 1987) and thus facilitate the development of refrigerated meat products with longer shelf life.

TABLE 14.

EXPERIMENTAL TESTING CONDITIONS

Cellulose ether (CE)	Fatty acid (FA)	CE:FA ratio	Testing[a] temperature, °C
MC	lauric	45: 1	5
HPMC	palmitic	45: 5	24
	stearic	45:10	32
	arachidic	45:15	40
	control[b]	45:20	

[a] Only for a CE:FA=45:15 ratio
[b] No fatty acid

TABLE 15.

EFFECT OF FILM COMPOSITION ON
POTASSIUM SORBATE PERMEABILITY AT 24°C

	Lauric		Palmitic		Stearic		Arachidic	
	K[a] $X10^{-9}$	l[b]	K $X10^{-9}$	l	K $X10^{-9}$	l	K $X10^{-9}$	l
A. MC								
45:0[c]	44	28						
	47	29						
45:1	64	24	45	30	41	33	21	30
	63	28	44	25	42	34	24	34
45:5	53	26	37	29	36	34	15	26
	48	30	34	31	35	33	13	24
45:10	43	26	14	22	13	31	8.0	30
	41	28	14	27	11	33	7.4	31
45:15	27	34	12	34	9.4	38	4.9	42
	30	28	12	40	8.4	42	4.8	49
45:20	22	30	4.4	40	8.4	40	---	---
	21	32	5.4	36	12	30	---	---
B. HPMC								
45:0	87	29						
	78	25						
45:1	86	21	65	26	58	24	51	21
	84	21	66	29	54	21	43	22
45:5	68	21	46	28	40	27	32	20
	72	22	45	24	39	24	32	22
45:10	56	20	31	25	24	20	17	27
	50	24	31	28	26	23	19	25
45:15	43	28	20	33	16	23	9.5	29
	48	30	21	37	15	22	9.2	36
45:20	28	25	6.9	24	25	25	----	----
	28	22	5.4	27	24	30	----	----

[a] Permeability coefficient, (mg/sec cm^2) (cm)/(mg/ml)
[b] Film thickness, μm
[c] Controls with no fatty acid

TABLE 16.

EFFECT OF DOUBLE BONDS ON
POTASSIUM SORBATE PERMEABILITY AT 24°C

Film[a]	$K^{b}_{x10^{-9}}$	1^{c}
MC:C18	13	31
(stearic)	11	33
MC:C18:1, trans	31	35
(elaidic)	33	41
MC:C18:1, cis	36	32
(oleic)	37	29

[a] All films have a CE:FA=45:15 ratio
[b] Permeability, (mg/sec am^2) (cm)/(mg/ml)
[c] Film thickness, μm

TABLE 17.

ACTIVATION ENERGY (E_a) OF K-SORBATE PERMEATION

Film	E_a, Kcal/g-mole
MC:C12	7.71
MC:C16	7.84
MC:C18	7.49
MC:C20	7.63
MC:FA, average	7.67±0.15
HPMC:C12	7.42
HPMC:C16	7.63
HPMC:C18	7.40
HPMC:C20	7.36
HPMC:FA, average	7.56±0.12
MC, control	7.44
HPMC, control	7.36

TABLE 18.

EFFECT OF TEMPERATURE ON POTASSIUM SORBATE PERMEABILITY

T, °C	Lauric		Palmitic		Stearic		Arachidic	
	K^a $\times 10^{-9}$	ψ^b $\times 10^{-11}$	K $\times 10^{-9}$	ψ $\times 10^{-11}$	K $\times 10^{-9}$	ψ $\times 10^{-11}$	K $\times 10^{-9}$	ψ $\times 10^{-11}$
A. MC								
40	55	105	22	43	14	27	9.1	18
	51	98	21	41	17	32	9.7	19
32	43	111	17	45	12	31	7.3	19
	41	107	14	36	12	32	7.3	19
24	30	111	12	45	9.4	35	4.8	18
	27	100	12	44	8.4	31	4.9	18
5	9.9	100	4.8	48	3.2	32	2.1	21
	12	122	4.0	40	3.6	36	2.0	20
Average		107±8		43±4		32±3		19±1
B. HPMC								
40	86	165	37	70	28	53	17	33
	80	153	37	70	31	59	16	31
32	67	173	27	71	20	51	12	31
	59	152	24	62	23	59	13	34
24	44	161	21	79	15	54	9.5	35
	49	180	19	70	16	60	9.2	34
5	19	194	8.1	81	6.5	66	3.5	35
	17	172	6.9	70	5.8	58	4.0	40
Average		169±14		72±6		58±5		34±3

[a] Permeability coefficient, (mg/sec cm^2) (cm)/(mg/ml).

[b] $\alpha = K\mu/T$

where:

μ = solvent viscosity in centipoise

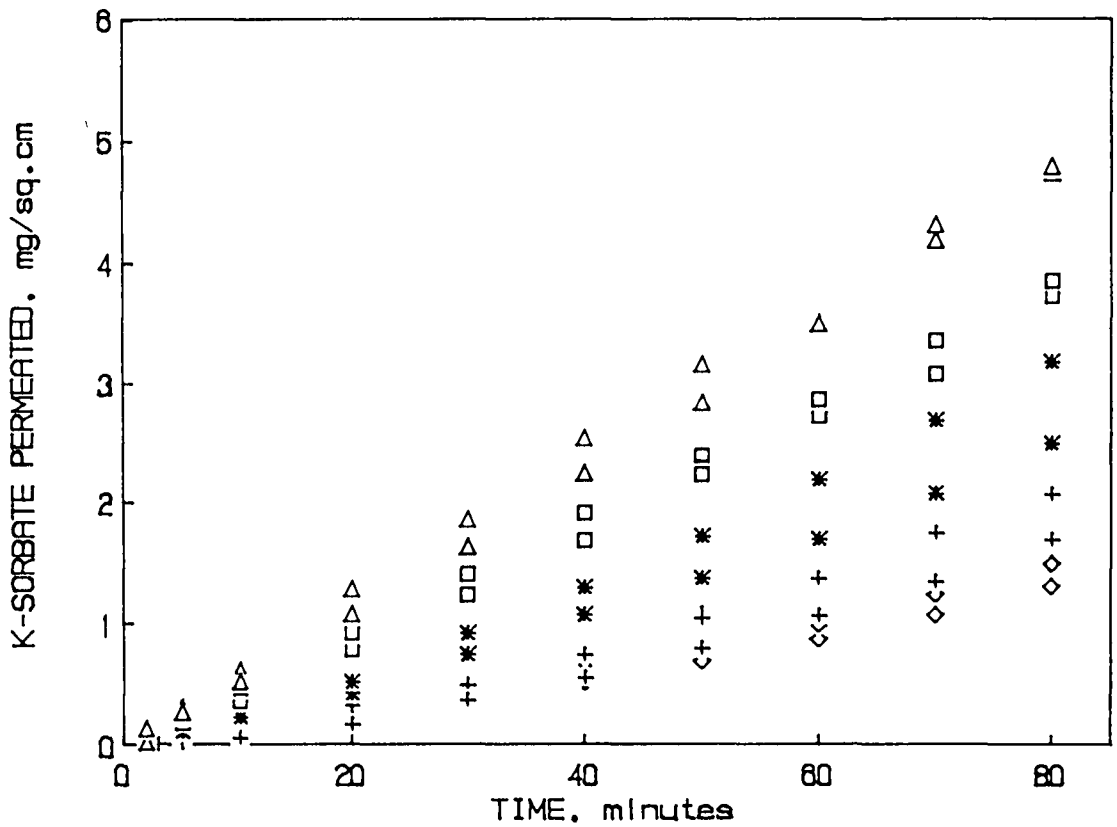

Figure 21. Effect of fatty acid concentration on K-sorbate permeability at 24°C through HPMC-based films.
a. lauric acid
△, 45:1; □, 45:5; *, 45:10; +, 45:15; ◊, 45:20

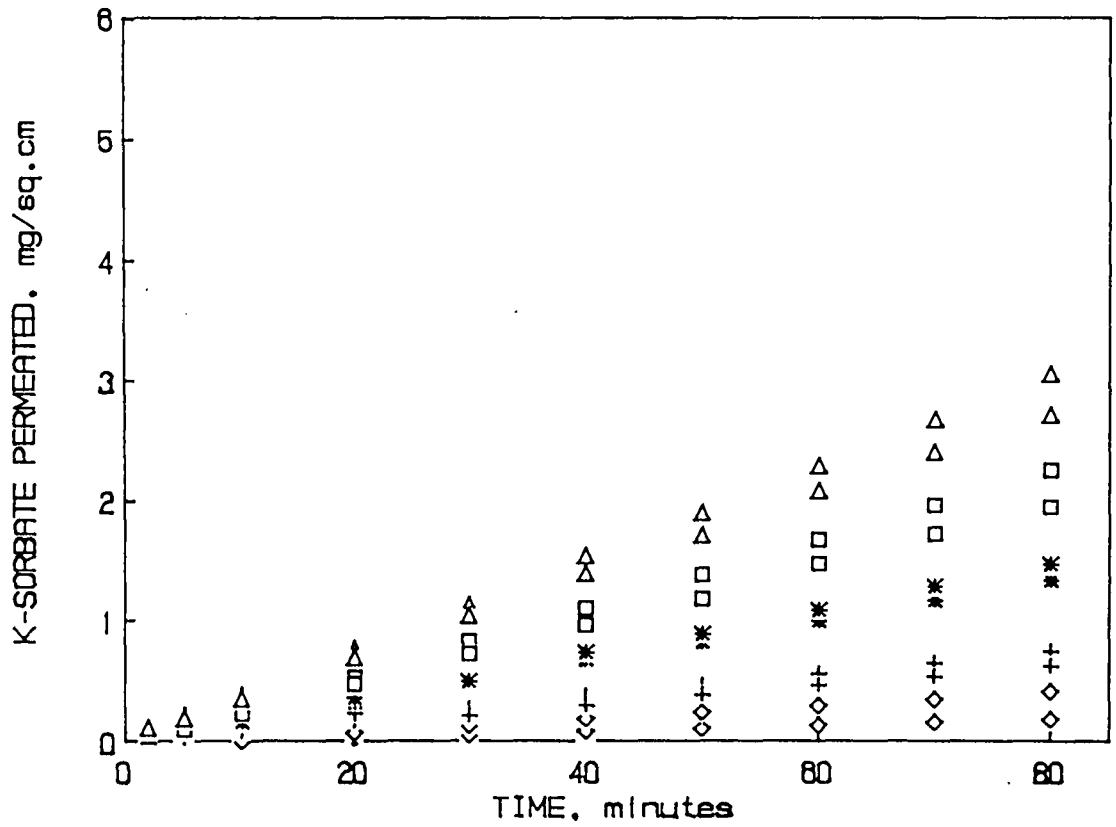

Figure 21. Effect of fatty acid concentration on K-sorbate permeability at 24°C through HPMC-based films (continued).
b. palmitic acid
△, 45:1; □, 45:5; *, 45:10; +, 45:15; ◇, 45:20

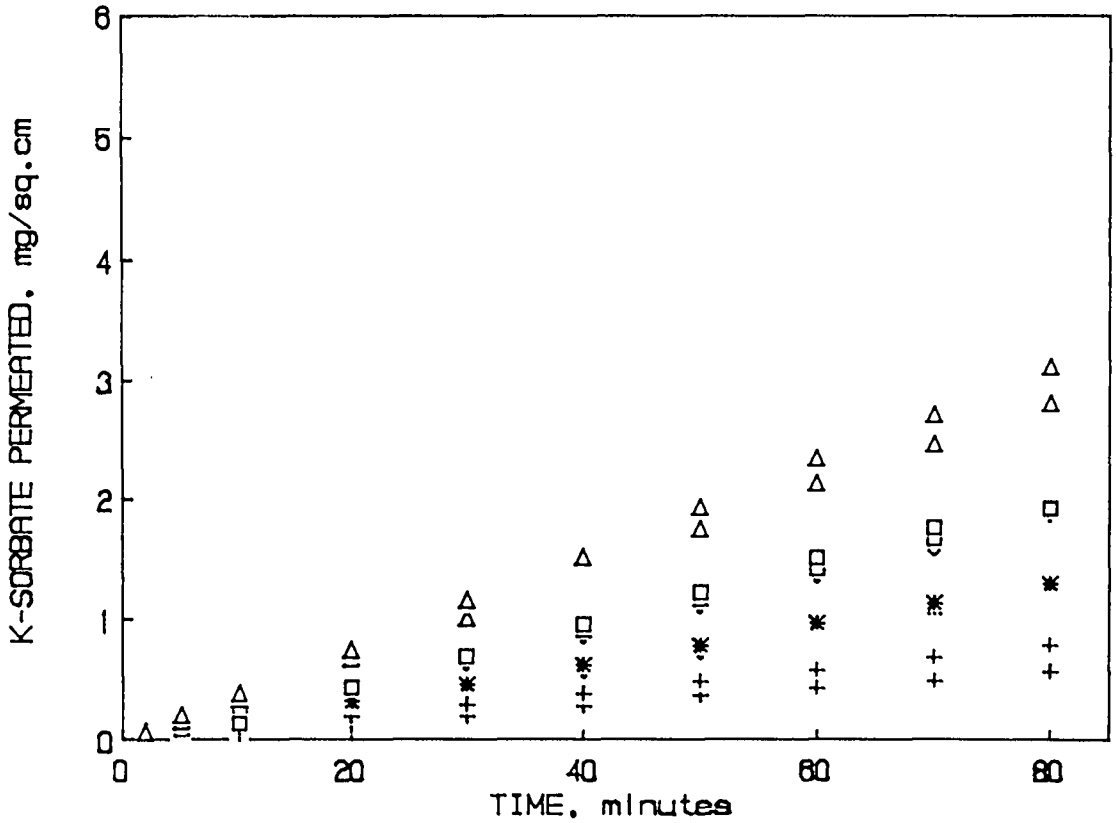

Figure 21. Effect of fatty acid concentration on K-sorbate permeability at 24°C through HPMC-based films (continued).
c. stearic acid
△, 45:1; □, 45:5; *, 45:10; +, 45:15; ◇, 45:20

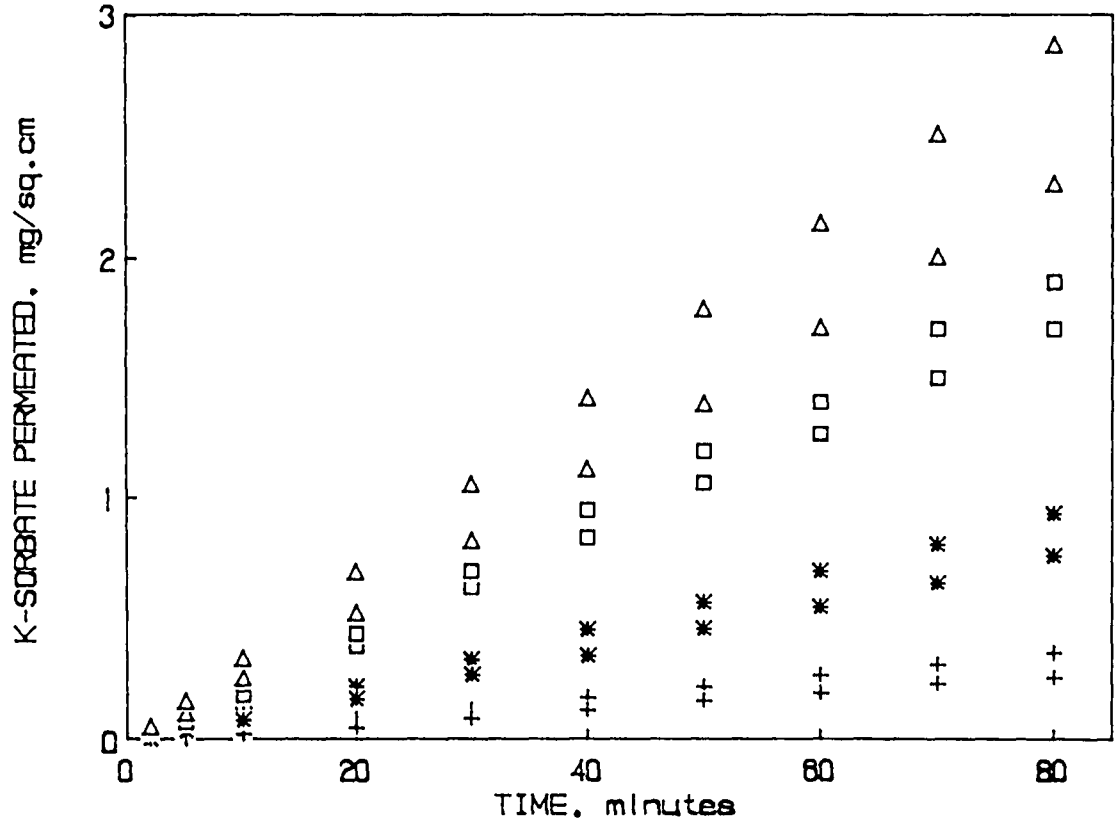

Figure 21. Effect of fatty acid concentration on K-sorbate permeability at 24°C through HPMC-based films (continued).
d. arachidic acid
△, 45:1; □, 45:5; *, 45:10; +, 45:15; ◊, 45:20

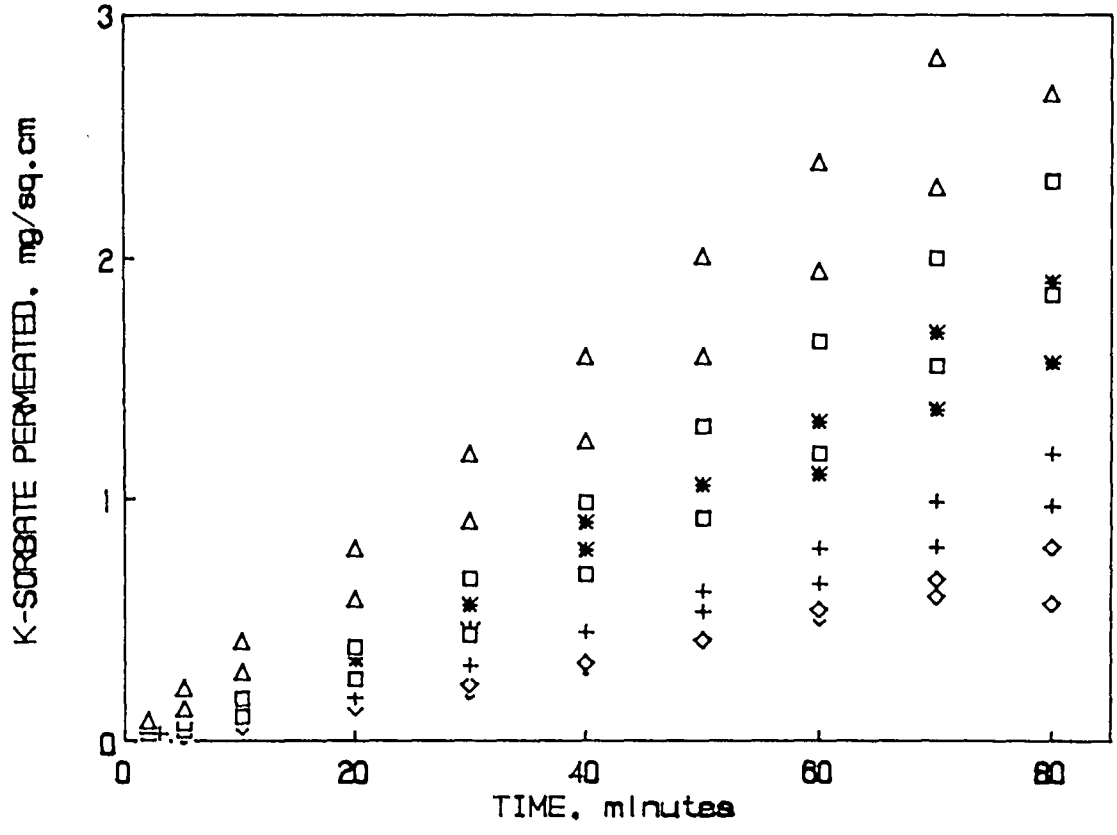

Figure 22. Effect of fatty acid concentration on K-sorbate permeability at 24°C through MC-based films.
a. lauric acid
△, 45:1; □, 45:5; *, 45:10; +, 45:15; ◇, 45:20

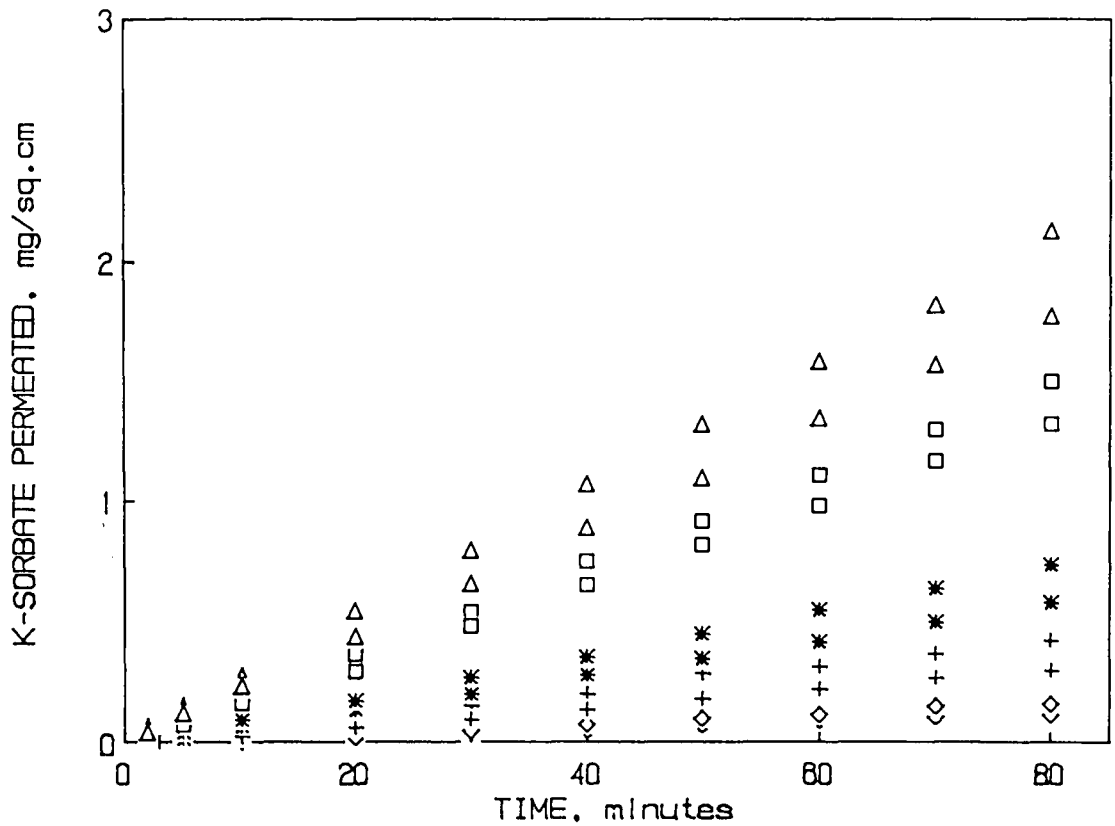

Figure 22. Effect of fatty acid concentration on K-sorbate permeability at 24°C through MC-based films (continued).
b. palmitic acid
△, 45:1; □, 45:5; *, 45:10; +, 45:15; ◇, 45:20

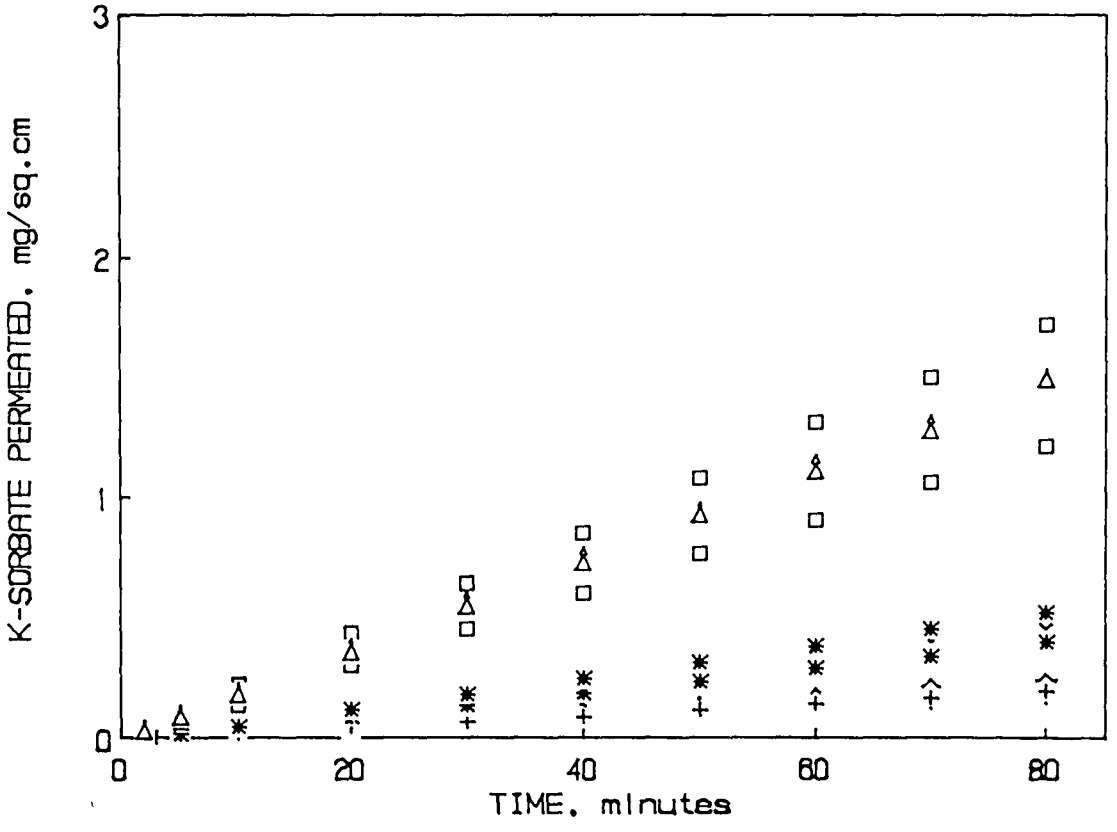

Figure 22. Effect of fatty acid concentration on K-sorbate permeability at 24°C through MC-based films (continued).
c. stearic acid
△, 45:1; □, 45:5; *, 45:10; +, 45:15; ◊, 45:20

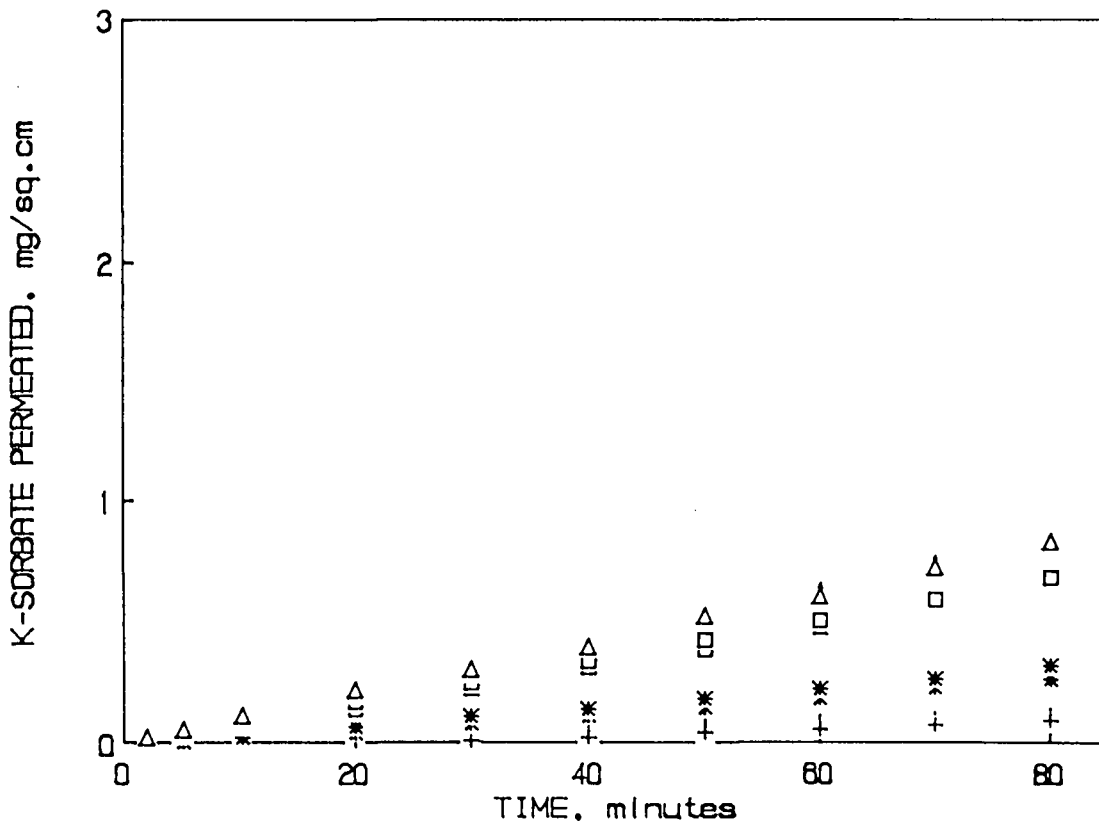

Figure 22. Effect of fatty acid concentration on K-sorbate permeability at 24°C through MC-based films (continued).
d. arachidic acid
Δ, 45:1; □, 45:5; *, 45:10; +, 45:15; ◊, 45:20

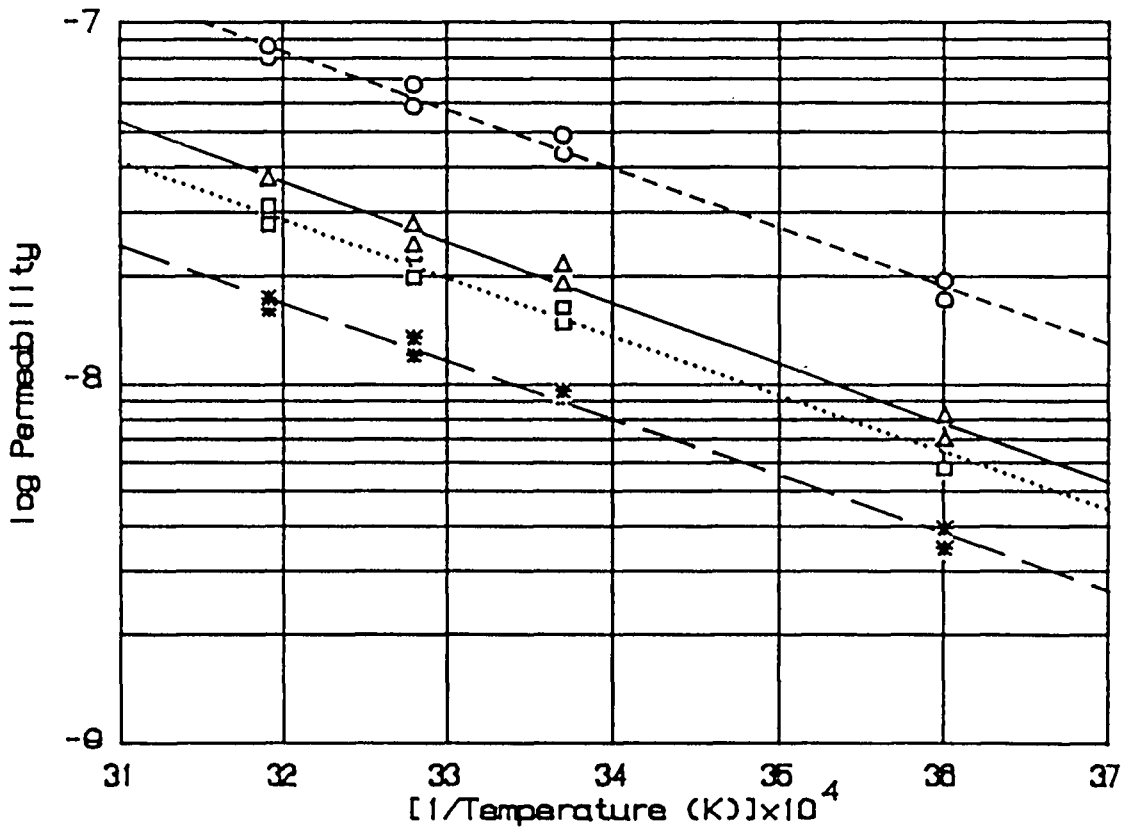

Figure 23. Arrhenius plots for permeability of K-sorbate through HPMC-based films.
O, lauric acid; Δ, palmitic acid; □, stearic acid; *, arachidic acid.

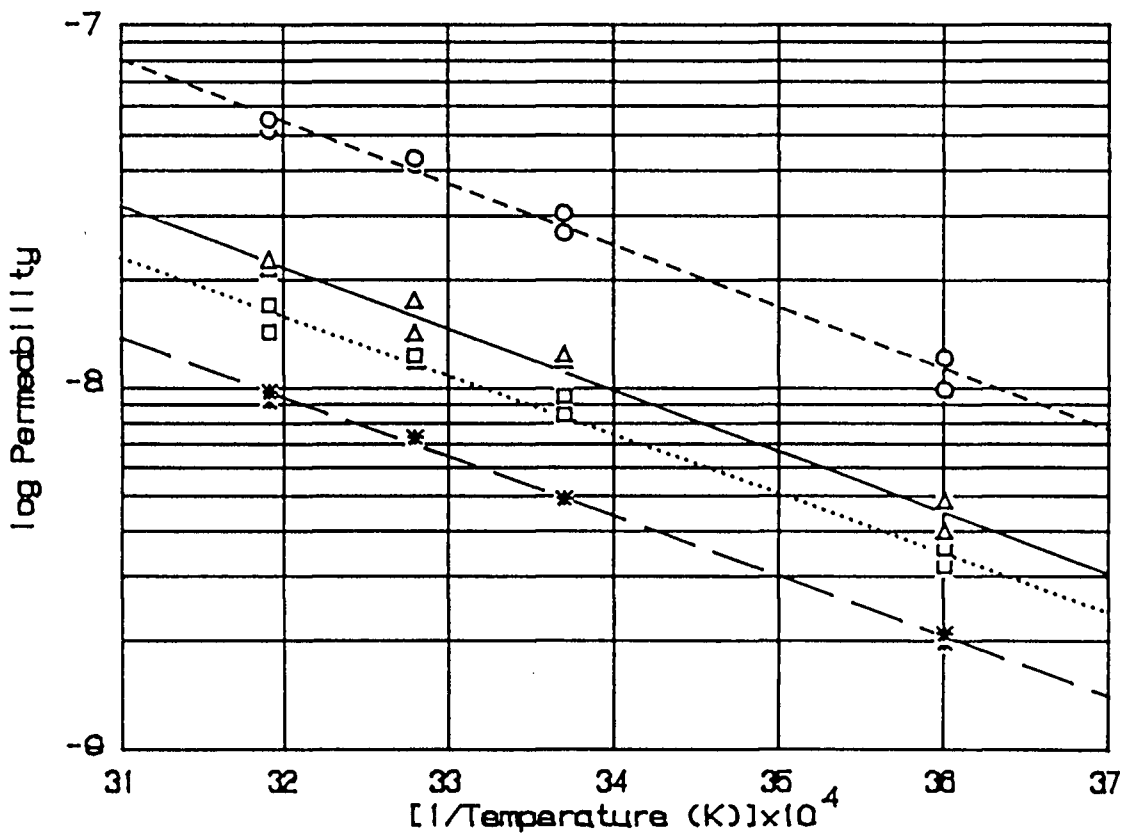

Figure 24. Arrhenius plots for permeability of K-sorbate through MC-based films.
○, lauric acid; △, palmitic acid;
□, stearic acid; *, arachidic acid.

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POTASSIUM SORBATE PERMEABILITY OF EDIBLE CELLULOSE ETHER MULTI-LAYER FILMS

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Running Head: Potassium sorbate permeability

Potassium sorbate permeability of edible cellulose ether multi-layer films. Fakhrieh Vojdani and J. Antonio Torres, Department of Food Science and Technology, Oregon State University, Corvallis, Oregon 97331.

ABSTRACT

Spoilage by microorganisms growing on food surfaces is the shelf life limiting factor for many refrigerated food products. For example, fish and fresh broilers can be stored refrigerated only for a few days before product deterioration caused mostly by surface microbial growth. Similar problems affect intermediate moisture food products when storage temperature is not constant but fluctuates. As shown in previous publications from our laboratory this shelf life limitation can be overcome by edible coatings lowering the diffusion rate into the food of antimicrobial agents applied on food surfaces. A permeability cell has been used to evaluate films with potassium sorbate barrier properties. In this paper we have examined the effect of casting technique and formulation on the permeability rate of methyl- and hydroxypropyl methyl cellulose based films. Film components studied included lauric, palmitic, stearic, and arachidic acid; hydrogenated palm oil and white beeswax. Permeability constant determinations ranging from 10^{-9} to 10^{-11} (mg/sec cm^2)(cm)/(mg/ml) indicate that surface

resistance to microbial growth during product commercialization could be enhanced significantly. Scanning electron microscopy provided insight on the structure of these films.

INTRODUCTION

Surface microbial stability is a major determinant of the shelf life of many food products including refrigerated meats and intermediate moisture foods (IMF) (Vojdani and Torres, 1987a,b). During storage and commercialization of refrigerated meats, beef, poultry and seafood, nearly all microbial growth occurs on the surface and gram-negative bacteria are predominant (Anonymous, 1983; Leistner *et al.*, 1981; Dainty *et al.*, 1975; Ingram and Dainty, 1971).

In the case of IMF's, surface condensations caused by temperature fluctuations result in temporary and local increases in surface water activity (a^w). Rapid microbial growth is possible in these localized regions (Torres, 1987; Torres *et al.*, 1985a,b).

The solution to surface microbial stability problems investigated in this and previous papers is to combine an edible impermeable coating and an antimicrobial agent such as potassium sorbate. The concentration of the surface applied preservative is kept high by selecting an edible coating that retards preservative diffusion from food surface into food bulk (Torres, 1987; Torres *et al.*, 1985a,b). The use of edible coatings for this and other purposes has been recently reviewed by Guilbert (1986).

In a previous study (Vojdani and Torres, 1987a), the potassium sorbate barrier properties of films composed of a

mixture of fatty acids (FA) and cellulose derivatives, methyl cellulose (MC) and hydroxypropyl methylcellulose (HPMC), were determined using a permeability cell (Torres, 1987). Edible films with similar compositions have been studied by Fennema and coworkers (Kester and Fennema, 1986; Kamper and Fennema, 1985, 1984a,b) who have demonstrated their water vapor migration barrier properties. Methylcellulose films with palmitic, stearic and arachidic acid with a MC:FA=45:15 ratio showed the following K-sorbate permeability values at 24°C: 1.2×10^{-8}, 8.9×10^{-9} and 4.9×10^{-9} (mg/sec cm^2)(cm)/(mg/ml), respectively. These values are comparable with those measured for zein films. Microbial challenge tests have shown that zein films controlling surface K-sorbate concentration provide a tenfold increase in surface microbial stability (Torres and Karel, 1985).

Permeability determinations were also done at other temperatures. At 5°C, the same films had the following permeabilities: 4.4×10^{-9}, 3.4×10^{-9} and 2.1×10^{-9} (mg/sec cm^2)(cm)/(mg/ml), respectively.

In this paper we determine the effect on permeability values as a result of film casting technique modifications. We have also examined films containing hydrogenated palm oil and white beeswax. Beeswax is known to provide excellent moisture transfer control (Watters and Brekke, 1961). Electron microscopy techniques were used to examine the microscopic characteristic of these and other films previously reported (Vojdani and Torres, 1987b).

MATERIALS AND METHODS

Reagents

Methylcellulose (MC, Methocel A 15-LV, Premium) and hydroxypropyl methylcellulose (HPMC, Methocel F50, Premium) were obtained from Dow Chemical Co., Midland, MI. Polyethylene glycol 400 (PEG), lauric acid (C12, 99-100%), palmitic acid (C16, 99%), stearic acid (C18, 99%), arachidic acid (C20, 99%) were obtained from Sigma Chemical Co., St. Louis, MO. A C16-C18 (1:1, w/w) blend was prepared in the laboratory. Other chemicals used were glycerol (J.T. Baker Chemical, Phillisbury, NJ), white beeswax (Eastman Kodak Co., Rochester, NY), hydrogenated palm oil (Durkee 27, Durkee SCM, Corp., Cleveland, OH) and potassium sorbate (K-sorbate, Monsanto Co., St. Louis, MI). Ethanol (95%, reagent) was obtained from OSU Chemical stores.

Film Preparation

a. Double layer films

The same technique previously described for single layer films (Vojdani and Torres, 1987b; Kamper and Fennema, 1984a) was used for the preparation of double layer films. The only difference between the first and the second layer was that the base layer contained no fatty acids. The second layer was cast with a MC or HPMC:fatty acid ratio of 45:20. The first layer was allowed to dry before applying the second layer which was

done without changing the thickness setting (0.75 mm) of the thin layer chromatography spreader.

The objective of this experiment was to determine whether improved films could be obtained by reducing the influence of the glass plate on film casting.

b. **Coated films**

A base layer containing no fatty acids was prepared as described by Vojdani and Torres (1987b), and then dried and peeled off from the plate. It was then very carefully replaced on the glass plate making sure that no air bubbles had formed between the plate and the film. A hot solution of lipids or edible wax was then spread on the base layers using the same warm spreader and a thickness of 0.25 mm. This application method differed from Kamper and Fennema (1984a) who painted the second layer onto the film.

c. **Embedded films**

In this method the lipid layer was secured between two layers of HPMC. The top and bottom HPMC layers provided protection against cracking during testing.

The purpose of this group of experiments and the previous one was to evaluate the contribution of pure lipids to the reduction of K-sorbate migration from the surface into the food bulk.

Film thickness measurement

The thickness of films prepared in this study was measured as described by Vojdani and Torres (1987a,b).

Determination of the permeability of multi-layer films

A multi-layer film composed of three sheets of thickness l_1, l_2 and l_3, and permeabilities P_1, P_2 and P_3, respectively, placed in series has under steady state conditions an effective permeability, P_{123}, given by (Ashley, 1985; Crank, 1976; Karel, 1975):

$$l_{123}/P_{123} = l_1/P_1 + l_2/P_2 + l_3/P_3 \qquad (1)$$

where:

P_{123} = permeability of the composite film

$l_{123}=l_1+l_2+l_3$

In the case of double layer films, and assuming that the two layers are of equal thickness, the equivalent formula for double layer is:

$$2/P_{12} = 1/P_1 + 1/P_2 \qquad (2)$$

Estimations of enhanced surface microbial stability

The enhanced surface microbial stability period can be estimated using the equation derived by Torres *et al.* (1985a):

$$t = l^2/(86400\pi f^2 P) \qquad (3)$$

where:

t = stability period, (days)

l = coating thickness, (cm)

P = permeability coefficient (mg/sec cm^2)(cm)/(mg/ml)

f = an dimensionless factor (=0.05)

f represents the reduction in surface concentration caused by diffusion into the food bulk. It should be noted that this expression gives only rough estimations that should be confirmed by microbial challenge tests (Torres and Karel, 1985).

Electron microscopy studies

Electron microscopy was used to evaluate film structure and the distribution of fatty acids in them. Of particular interest were film uniformity and detection of pores. It was also used to confirm film thickness measurements.

The specimens were mounted on aluminum plancets using Avery Spot-O-Glue. The film was sectioned with a sharp razor blade and coated with approximately 100-200A^o of 60:40 gold-pladium in a Varian VE-10 vacuum evaporator at a vacuum of $1x10^{-5}$ torr. The microscopic examination was made using an AMRAY 1000A SEM operated at 20KV at the Electron Microscope Facility, Oregon State University. Images were recorded on Polaroid type 55

positive/negative 4x5 format film. Samples were photographed at effective tilt angles of +30 to +60^{o} towards the detector with respect to the incident electron beam.

Statistical analysis

The statistical analysis of data was done on an IBM Personal Computer using SAS® (Anonymous, 1985).

RESULTS AND DISCUSSION

Selected values obtained from a previous study on the permeability of single layer films have been summarized in Table 19 (Vojdani and Torres, 1987b) and include MC and HPMC:FA ratios of 45:15 and 45:20. In the case of the 45:15 ratio we include data at 5 and 24°C. We should also note that experimental difficulties were found in the casting on glass plates of films with a MC and HPMC:FA=45:20 ratio (Vojdani and Torres, 1987b).

Permeability studies

a. Double layer films

Table 20 summarizes our determinations of the permeability values of films cast with the first layer containing no fatty acid while the second layer contains various fatty acids at a MC or HPMC:FA=45:20 ratio. The first layer was cast with the thin layer applicator set at 750 μm which when dry had a thickness of approximately 20 μm. The second layer was cast on the surface of this dry layer without changing the applicator setting. This procedure allowed us to assume that the thickness of each layer was approximately the same. Eq. (2) was then used to estimate the permeability of the second layer (K_2) using double layer values (K_{1+2}).

The trend previously reported (Vojdani and Torres, 1987b), longer chain fatty acids yielding better films, is not quite

evident, particularly for HPMC:FA films. The lowest permeability value was observed for MC:palmitic acid films. The reasons for the better values obtained for palmitic acid are not clear and require further studies.

Estimated values (K_2, Table 20) are lower than the equivalent values obtained for single layer films (Table 19, 45:20 data at 24°C). This was due to the single layer film casting difficulties encountered with higher fatty acid concentrations which were not noticed in double layer films. Single layer films containing arachidic acid could not even be prepared with a 45:20 ratio (Table 14, Vojdani and Torres, 1987b). Therefore, a more relevant comparison between single and double layer films is to compare K_2 values at a 45:20 ratio (Table 20) with K values obtained at a 45:15 ratio (Table 19). This comparison shows an improvement for films cast on top of a MC or HPMC base as compared to those cast on glass. This suggests that future studies should examine the properties of coatings on food model surfaces.

Using the approach described by Torres (1987, Table 14.2) it is possible to estimate the effect that double layer films with the permeability values measured in this study might have on increased microbial stability. For example, if one assumes that it is possible to cast films on a food with the same permeability value (K_2) as reported in Table 20 the surface protection can be predicted to last 2 months for a 0.1 mm film

and 7 months for a 0.2 mm thick MC:palmitic acid=45:20 ratio film.

b. Coated films

MC and HPMC films were coated with hydrogenated palm oil and a palmitic-stearic mixture (50% w/w) but resulted in brittle surfaces that could not be tested in the permeability cell. On the other hand, HPMC films coated with beeswax resulted in excellent films with extremely low permeability values. Values reported in Table 21 should only be considered an upper boundary estimate. The permeability cell method became an inconvenient procedure for coatings with permeability values in the order of 10^{-11} (mg/sec cm^2)(cm)/(mg/ml). Moreover, predicted surface protection for such low permeability values indicate that product deterioration would occur by a spoilage mechanism other than surface microbial growth.

At lower temperatures the permeability can be expected to be even lower. Permeability values at other temperatures can be estimated using an Arrhenius model approach. An alternative is the use of the following expression whose validity for several films was demonstrated by Vojdani and Torres (1987a,b):

$$K\ \mu/T = \alpha \qquad (4)$$

where:

K = permeability value

μ = solvent viscosity

T = absolute temperature

α = a constant

Using this approach we estimated that the permeability at $5^{o}C$ of a HPMC film coated with beeswax to be ca. $1x10^{-11}$ (mg/sec cm^2)(cm)/(mg/ml), i.e. 2-3 orders of magnitude lower than the values obtained by Torres *et al.* (1985a) for zein films.

c. Embedded films

An attempt was made to determine the permeability of pure fatty acid films. A palmitic-stearic acid mixture (50% w/w) was embedded between two layers of HPMC. Samples of the first, the first two and all three layers were used to determine the approximate thickness of each layer needed to estimate the permeability coefficient of the pure fatty acid layer (Table 21). The average K_2 value was $2.6x10^{-9}$ (mg/sec cm^2)(cm)/(mg/ml) and should be compared with the higher values obtained for single layer films containing these fatty acids (Table 19).

Kamper and Fennema (1984a) reported that HPMC films coated with hydrogenated palm oil had good moisture barrier properties. Unfortunately, embedded films using hydrogenated palm oil as the middle layer cracked easily and could not be

tested in our permeability cell.

Electron microscopy studies

Morphological differences between individual film compositions and casting techniques were examined by electron microscopy (Figs. 25-27). Fig. 25 shows the presence of fatty acid crystals on the film surface. As shown in Fig. 26 films were of uniform thickness and consistent with the values measured with a Best Test Indicator (Vojdani and Torres, 1987a,b). None of the films examined showed the presence of cracks or pores. The thickness differences between films with different composition was expected (Vojdani and Torres, 1987b). Cross sections of thicker films, typically beeswax coated and multiple layer films were more difficult to prepare and show some tearing during cutting. No major differences were seen between MC and HPMC based films, thus the following discussion will concentrate on the effect of the fatty acid component.

a. Single layer films

Fig. 25 shows top view photomicrographs of the surface of single layer films containing palmitic, stearic and arachidic acid at a MC or HPMC:FA=45:15 ratio. Lauric acid films are not shown because they could not withstand the electron beam.

Although single layer films were cast from an homogeneous mixture (Vojdani and Torres, 1987b) fatty acids tended to migrate to the surface and formed mostly vertical crystals. As

noted by Kamper and Fennema (1984a) the fatty acids orient themselves at the air-film solution interface during film formation.

Differences can also be seen between the fatty acid crystalline structures observed. Both palmitic and stearic acid formed on the film surface polygonal flat crystals; however, stearic acid crystals were larger. On the other hand, arachidic acid formed large rectangular, flat and ribbon-like crystals.

Surface spots with no crystals were observed in films with stearic acid, were more frequent in the case of arachidic acid but were not seen with palmitic acid. This observation seems to be consistent with the apparent fatty acid distribution differences within the film itself. The cross section shown in Fig. 26a suggests that in films with palmitic acid there is a bottom to top surface increase in the distribution of fatty acids which is less pronounced for stearic (Fig. 26c) and arachidic acid (Fig 26b). This suggests that palmitic acid had fewer difficulties in reaching the film surface as compared to stearic and arachidic acid. Further studies are required to confirm these preliminary observations.

b. Double layer films

No major differences were noted on the surface of double layer films. The most significant observation was that electron microscopy studies provided support to our assumption that each layer had the same thickness (Figs. 26b and 26d). Note also the

lack of features of the cellulose ether layer.

c. Coated films

Figs. 27a and 27b show a HPMC film coated with beeswax. In this case no crystalline structures were observed and the beeswax formed a very compact layer without voids. Kamper and Fennema (1984a) had observed that films coated with solid beeswax were very effective barriers to the transfer of water vapor, but that these films were extremely brittle. We found that our preparation method yielded films that were not only extremely good K-sorbate permeability barriers but that were also very flexible.

d. Embedded films

Fig. 27d shows a palmitic-stearic acid mixture embedded in between two layers of HPMC of approximately equal thickness. During casting some of the fatty acids leached out as shown by the presence of crystals on the film surface.

Fig. 27c confirms our previous observation that hydrogenated palm oil embedded in between HPMC layers formed poor films. This photomicrograph shows that individual layers tended to separate.

CONCLUSIONS

Permeability determinations summarized in Tables 19-21 are in the range of 10^{-9} to 10^{-11} (mg/sec cm^2)(cm)/(mg/ml). The lowest values corresponded to HPMC-beeswax double layer films. Single layer films containing fatty acids could be used to extend the shelf life of refrigerated products whose stability is limited by surface microbial growth. Estimations of the surface protection period possible by the use of double layer films with beeswax indicate that these films could significantly extend the shelf-life of intermediate moisture foods stored at room temperature conditions.

Scanning electron microscopy studies suggests a correlation between fatty acid distribution and permeability values. For example, single layer films containing palmitic acid have been found to have lower than expected permeability values which correlated with the better surface fatty acid coverage noted in photomicrographs of these films. In the case of double layer films using beeswax a uniform surface coverage was also noted.

Edible bilayer films of the kind reported here and in previous papers (Vojdani and Torres, 1987a,b) have the potential to control surface microbial spoilage. These films are flexible and strong and should be able to withstand mechanical stresses during food distribution. However, there is a need to determine the diffusion of sorbate in food model systems coated with these

films. The effectiveness of diffusion control should also be evaluated using microbial challenge tests.

TABLE 19.

EFFECT OF COMPOSITION ON THE PERMEABILITY[a] OF SINGLE LAYER FILMS

	$Kx10^9$ MC or HPMC:FA 45:15 5°C	$Kx10^9$ MC or HPMC:FA 45:15 24°C	$Kx10^9$ MC or HPMC:FA 45:20 24°C
A. MC			
Control		49	49
		47	42
Lauric	10	30	22
(C12:0)	12	27	22
Palmitic	4.8	12	4.4
(C16:0)	4.0	12	5.4
Stearic	3.2	9.4	12
(C18:0)	3.6	8.4	8.4
Arachidic	2.1	5.4	---
(C20:0)	2.0	4.9	---
B. HPMC			
Control		87	87
		78	78
Lauric	19	48	28
(C12:0)	17	43	28
Pamitic	8.1	20	6.1
(C16:0)	7.0	21	6.0
Stearic	5.8	15	24
(C18:0)	6.5	16	26
Arachidic	3.5	9.2	---
(C20:0)	3.9	9.5	---

a $(mg/sec\ cm^2)(cm)/(mg/ml)$

TABLE 20.

EFFECT OF COMPOSITION ON THE PERMEABILITY[a] OF DOUBLE LAYER FILMS AT 24°C

	K_{1+2}[b] x10^9	K_1[c] x10^9	K_2[d] x10^9	l_{1+2}[e] μm
A. MC				
Control	----	47		26
		44		28
Lauric	22		15	46
	22		15	48
Palmitic	4.4		2.3	54
	6.0		3.2	48
Stearic	11		6.0	54
	12		6.8	54
Arachidic	6.1		3.2	38
	6.3		3.4	38
B. HPMC				
Control	---	87		28
		78		26
Lauric	31		19	44
	29		18	42
Palmitic	25		15	38
	28		17	40
Stearic	30		18	46
	28		17	48
Arachidic	11		5.6	44
	12		6.6	42

a (mg/sec cm^2)(cm)/(mg/ml)
b Permeability of double layer film
c Permeability of base layer
d Permeability of top layer with a MC or HPMC:FA=45:20 ratio
e Thickness of double layer film, μm

TABLE 21.

EFFECT OF COMPOSITION AND TEMPERATURE
ON THE PERMEABILITY[a] OF VARIOUS MULTI-LAYER FILMS

	K_{x10^9}	K_2[b]	l_1[c]	l_{1+2}[d]	l_{1+2+1}[e]
HPMC+Beeswax					
at 24°C	0.045	0.034	30	133	-
	0.058	0.045	25	115	-
HPMC-C18,16-HPMC					
at 24°C	4.2	2.9	21	109	130
	3.4	2.2	22	100	122
at 5°C	1.9	1.2	23	105	128
	1.3	0.85	20	90	110

a (mg/sec cm^2)(cm)/(mg/ml)
b Permeability of the second layer, i.e. bees wax or the C18-C16 mixture
c Thickness of the base layer, μm
d Thickness of the base and second layer, μm
e Thickness of the three layer film, μm

Figure 25. Electron microscopy photomicrographs of the surface of single layer films (bar = 10 μm).
a. CE:palmitic acid=45:15
b. CE:stearic acid=45:15
c. CE:arachidic acid=45:15
See text for further details.

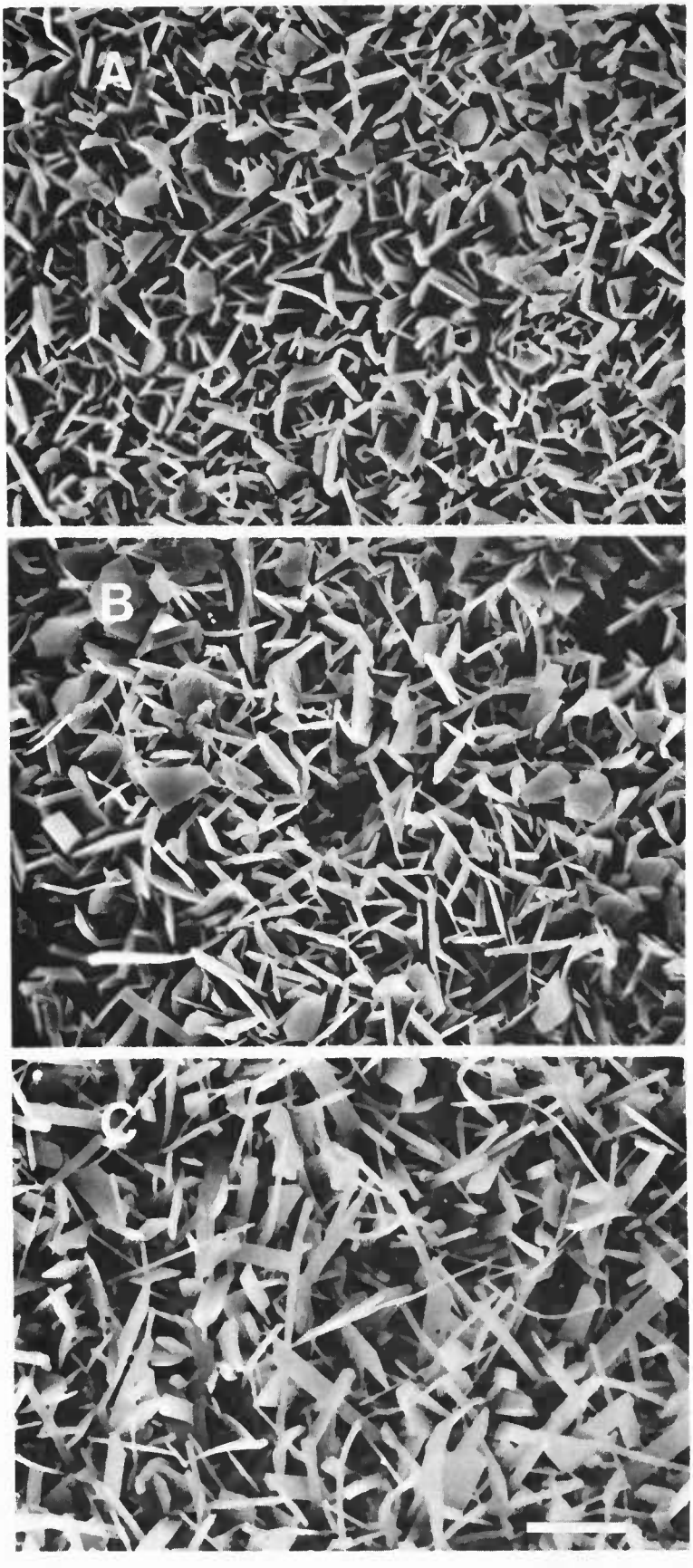

Figure 25

Figure 26. Electron microscopy photomicrographs of cross-sections of edible coating films (bar = 10 μm).
a. MC:palmitic acid=45:15, single layer film
b. HPMC:steraic acid=45:15, double layer film
c. HPMC:arachidic acid=45:15, single layer film
d. HPMC:palmitic acid=45:15, double layer film
Numbers 1,2,3 indicate layer application order.
See text for further details.

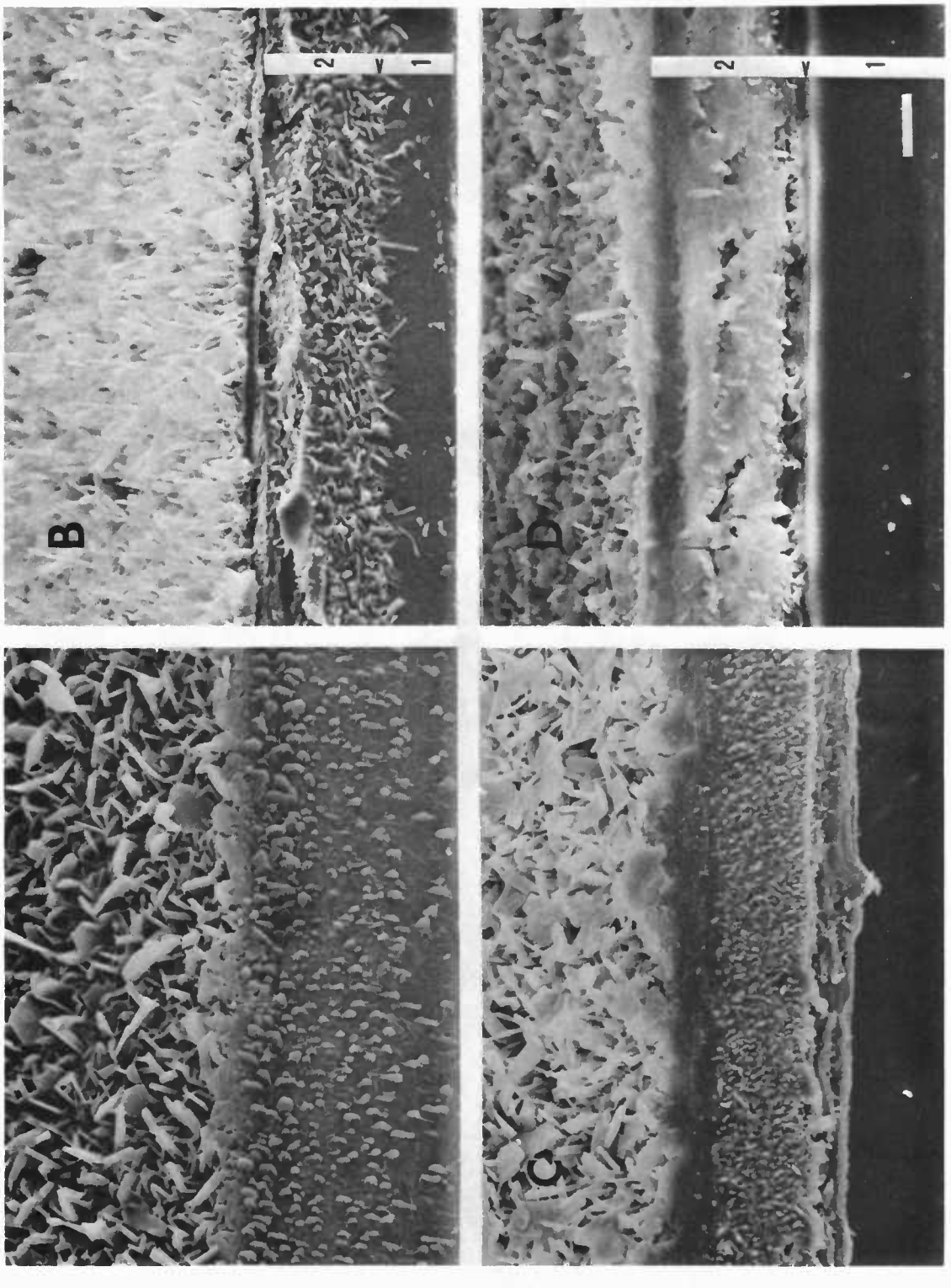

Figure 26

Figure 27. Electron microscopy photomicrographs of multi-layer films (bar = 10 μm).
a. surface of bees wax coated HPMC film
b. cross-section of bees wax coated HPMC film
c. cross-section of hydrogenated palm oil embedded between two HPMC layers
d. cross-section of a C18-C16 mixture embedded between two HPMC layers
Numbers 1,2,3 indicate layer application order.
See text for further details.

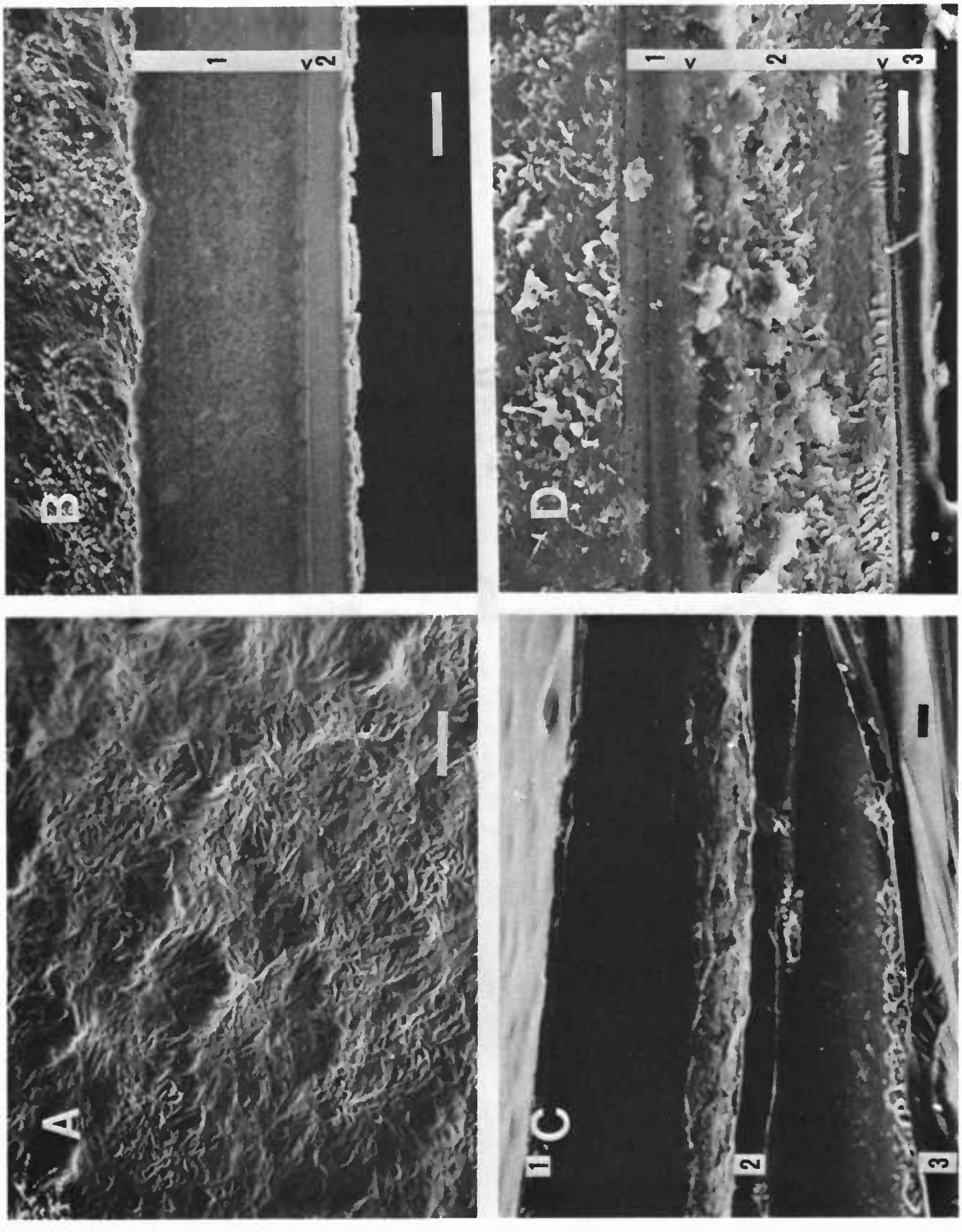

Figure 27

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SUMMARY AND CONCLUSIONS

This study confirms that it is possible to develop coatings that will enhance surface microbial stability and thus could facilitate the development of refrigerated meat and other products with surface microbial stability problems. Obviously, freezing a food product extends microbial shelf life, but it does so at a very high energy cost and loss of convenience to the consumer.

A mechanistic analysis of permeability values for potassium sorbate in polysaccharide films -chitosan, methylcellulose and hydroxypropyl methylcellulose- showed that their barrier properties are based on control of the diffusion process occurring in the solvent embedding the film. This observation suggested that potassium sorbate permeation rate could be reduced by increasing the hydrophobicity of methylcellulose and hydroxypropyl methylcellulose films. Permeability coefficients of methylcellulose and hydroxypropyl methylcellulose emulsified with lauric, palmitic, stearic and arachidic acid confirmed this prediction. Lower permeation rates were observed when the fatty acid concentration and the fatty acid chain length increased.

The effect of other hydrophobic additives, triglycerides and bees wax on the potassium sorbate permeability coefficient in methylcellulose and hydroxypropyl methylcellulose based films were also studied. The lowest permeability values were obtained

for a hydroxypropyl methylcellulose film coated with a bees wax layer.

An advantage of methylcellulose and hydroxypropyl methylcellulose based films is that they could be washed out by the consumer to eliminate the K-sorbate or other preservative remaining on the surface as well as the coating itself.

Surface protection predictions as described by Torres (1987, Table 14.2) were used to roughly estimate the effectiveness of these films and found that the surface microbial protection would have a highly significant commercial impact. The same calculations can be used to estimate the minimum coating thickness for a desired surface preservative concentration as a function of the coating preservative mass transfer properties and the desired shelf life for the food product. However, surface protection estimations should be confirmed using specific food system and challenging microorganism combinations.

The influence of the preservative solubility in the film and its hydrodynamic volume should also be considered when selecting a diffusion barrier film. In our experimental work we considered only K-sorbate. Other preservatives will give lower or higher permeability values depending upon their specific properties.

There is also a need to determine the diffusion of sorbate in food model systems coated with these films. The effectiveness of diffusion control should also be evaluated using microbial challenge tests.

Another important parameter to be considered in future studies is the hydration status of the film. In our model system studies we used water and a 50% (v/v) aqueous glycerol solution. These conditions represent a fresh, high moisture product and an intermediate a_w food, respectively. Other model systems need to be examined.

The excellent agreement between the experimental data and the Arrhenius activation energy model indicates that it should be relatively simple to conduct storage stability studies under changing storage temperature conditions and thus evaluate the effectiveness of these films under "real" conditions.

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