# EXPLORATION ON THE STABILITY OF INTERMEDIATE MOISTURE FOODS

by

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## ABSTRACT

Microbial stability of intermediate moisture foods (IMF's) has been found to be affected by unsteady state environmental conditions. Temperature fluctuations induce localized surface condensations resulting in potential surface outgrowth before moisture diffuses into the food product. Due to organoleptic, cost and/or safety restrictions it is not always possible to include safety margins by lowering water activity, or increasing preservative content in IMF formulations.

The solution explored in our current research has been to consider surface a separate region of the food and, develop

processes that enhance its growth inhibiting properties providing a much needed safety margin on the surface.

The first approach to enhanced surface resistance to microbial growth was to maintain, for as long as possible, an uneven preservative concentration: high on the surface and low in food bulk. We found that this was possible by the use of zein based coatings that reduced diffusion into food bulk of sorbic acid applied on the surface.

The effectiveness of this surface treatment was shown in *Staphylococcus aureus* S-6 surface challenge experiments under extreme testing conditions. Samples with bulk water activity ($a_w$) of 0.88, stored at 30C under constant 88% relative humidity (RH), remained stable for 16 or more days. Uncoated controls were stable for only 2 days. Samples with bulk $a_w$=0.85 exposed at 30C to cycles of 12 hours at 85% RH and 12 at 88% RH, remained stable for 28 or more days. Uncoated controls were stable for only 3 days.

Sorbic acid distribution studies showed that the mechanism for the improvement was diffusion control. Apparent diffusion coefficients for sorbic acid were 3-7 x $10^{-9}$ $cm^2$/sec in zein coatings and 1 x $10^{-6}$ $cm^2$/sec in the IMF model used in this study.

The second approach to microbial stability was the establishment of a pH difference between coated surface and food bulk.

Preliminary experimental work showed that samples with reduced surface pH remained free of microbial growth significantly longer than untreated controls. However, as soon as the pH difference disappeared rapid growth occurred. The increased resistance to microbial growth at reduced pH is associated with the increased bacteriostatic effectiveness of lipophilic acids such as sorbic acid. Since a surface pH reduction increases availability of the most effective form of the preservative, it results in significant microbial stability improvements.

It has been shown that it is possible to establish permanent and controlled pH differences between surface and bulk values. A negatively charged macromolecule can be immobilized in the form of a food surface coating component while other molecules, particularly electrolytes, can move freely. The immobilization of the macromolecule can be described best using a Donnan equilibrium model. This model allowed us to estimate pH differences between surface and food bulk. It also predicted that the key parameters were electrolyte concentration and the number of charged groups of the macromolecule.

To prove the validity of the $\Delta$pH concept we formulated an IMF model with low total electrolyte concentration (about 0.005M) and coated it with a deionized mixture of $\lambda$-carrageenan and agarose. Measured pH differential was in the 0.3 to 0.5 pH units. Such a reduction resulted in a 2.5 fold increase in the

surface availability of the active form of sorbic acid as compared to food bulk.

The effectiveness of this surface treatment was shown in *S.aureus* S-6 challenge tests at $a_w$= 0.88, RH = 88% and 30C. We found a stability period of about 20 days. It seems possible to obtain even longer periods. In our tests the loss of stability was most probably due to growth occurring in the sample bulk and not on the surface.

## ACKNOWLEDGEMENTS

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This thesis is dedicated to my children,

Marcia and Toni

for love, so intense and so immense, I thought it could only be found in impossible dreams; to my wife,

Anita

without her love, patience and hard work to support our little family, I could have never realized my dream; to my parents

Amario and Aida

from whom I learned how strong and beautiful family love can be.

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# 1. Introduction.

Foods with improved microbial stability can be fabricated by reducing their water activity ($a_w$). This parameter can be controlled by adjusting moisture content and by the presence of solutes. These products, so called intermediate moisture foods (IMF's), are microbiologically stable as long as:

$$a_w < a_w^* \qquad (1)$$

where:

$a_w = a_w(t, x, y, z)$

$= a_w$ at any time (t) and any location (x,y,z) within the IMF

$a_w^*$ = critical $a_w$ value

Above $a_w^*$, microbial growth is possible. Its value will depend upon parameters such as pH, concentration and type of preservative(s), expected microbial contamination, etc.

This stability condition may be temporarily lost due to changing environmental conditions. The purpose of this thesis is to analyze the resulting microbial stability problems.

## 1.1 Approach to IMF product development

The process of developing an IMF can be simplified if shelf life and organoleptic quality are represented as a function

of the various fabrication parameters, such as water content, use of solutes, pH, concentration of preservatives, heat processing conditions, and/or many of the other hurdles used to obtain the desired microbial stability.

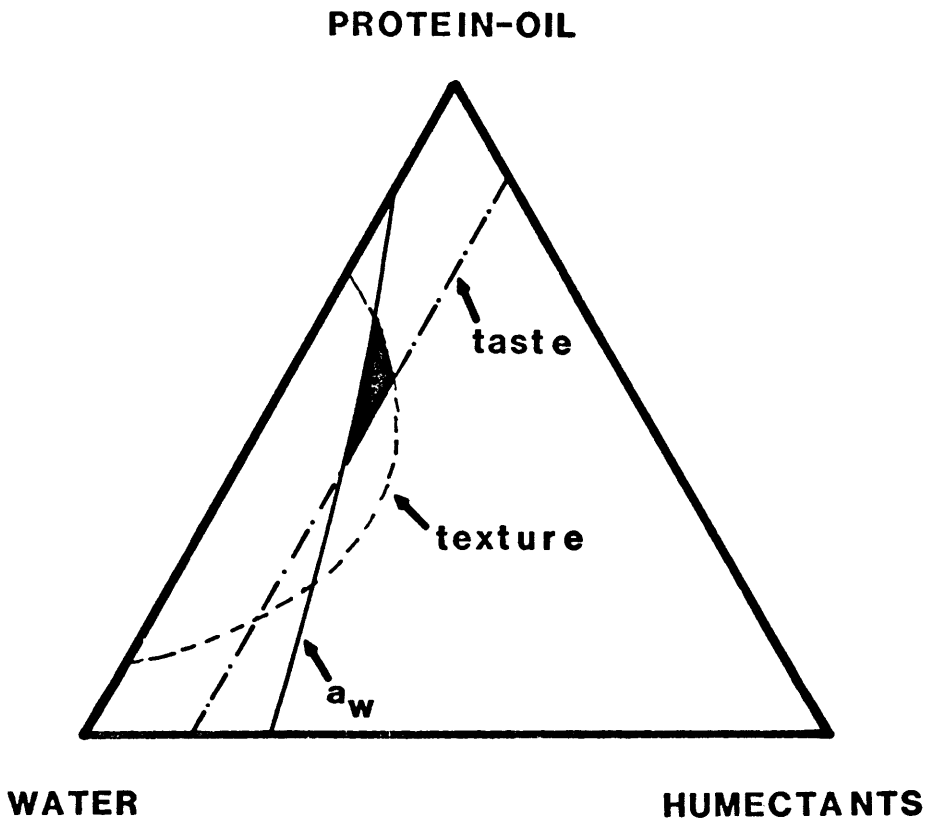
Figure 1 shows a specific example where this approach was used (Motoki, 1979). It corresponds to the development of the intermediate moisture cheese analog used as a model system in this thesis. A region of acceptable product formulations was defined by the identification of the following key factors:

a. an acceptable texture, a region identified through mechanical measurements using an Instron;
b. an acceptable taste, a region identified thru organoleptic tests; and
c. a minimum $a_w$, a region identified thru measurements with an electric hygrometer.

Figure 1 illustrates the product development difficulties surrounding IMF technology. The region of acceptable combinations, represented by the shaded area, is quite limited. Therefore, it is not possible to include considerations on possible and/or out-of-control situations that could impair their microbial stability.

Figure 1

DEVELOPMENT OF THE INTERMEDIATE MOISTURE CHEESE ANALOG USED AS A MODEL SYSTEM IN THIS THESIS



## 1.2 Effect of unsteady-state environmental conditions on microbial stability

Microbial stability of IMF's is also affected by unsteady-state environmental conditions. This possibility has been mostly ignored by researchers working in this field, who have tested them under conditions of constant temperature and humidity conditions (Bhatia and Mudahar, 1982; Erickson, 1982; Theron and Prior, 1980; Hanseman et al., 1980; Flora et al., 1979; Pavey, 1972; Anonymous, 1972).

Most IMF's are not affected by changing external environmental relative humidity conditions. These products are generally packaged in moisture proof materials. However, temperature fluctuations can result in extensive surface condensation problems.

### 1.2.1 Examples of surface condensation problems

Examples of unsteady state temperature situations abound in the production and commercialization of IMF's. The following ones are given only to emphasize the fact that these surface condensations will disrupt the delicate microbial stability balance, expressed as $a_w < a_w^*$. As a consequence, surface microbial growth is then often observed.

1.2.1.1 Temperature fluctuations during production and distribution

Let us consider an IMF ($a_w$ = 0.80), packed at T(1) = 100F and stored in a warehouse at T(2) = 70F. Furthermore, this product could be loaded into a truck at T(3) = 100F (hot summer day) or a railroad car at T(3') = 35F (winter day) to be unloaded into another warehouse at T(4) = 70F.

This type of situation was analyzed in Appendix A. We showed that while headspace humidity condensation will play only a minor role, moisture evaporation from warm food pieces can result in as much as 10 ml condensation for a 1 lb net weight package. Water drops on the package inside, acting as cold condensing surfaces and on the product itself, will provide localized spots with high $a_w$ levels. We estimated that condensations on food surfaces would allow microbial growth, i.e. $a_w > 0.85$, for several hours.

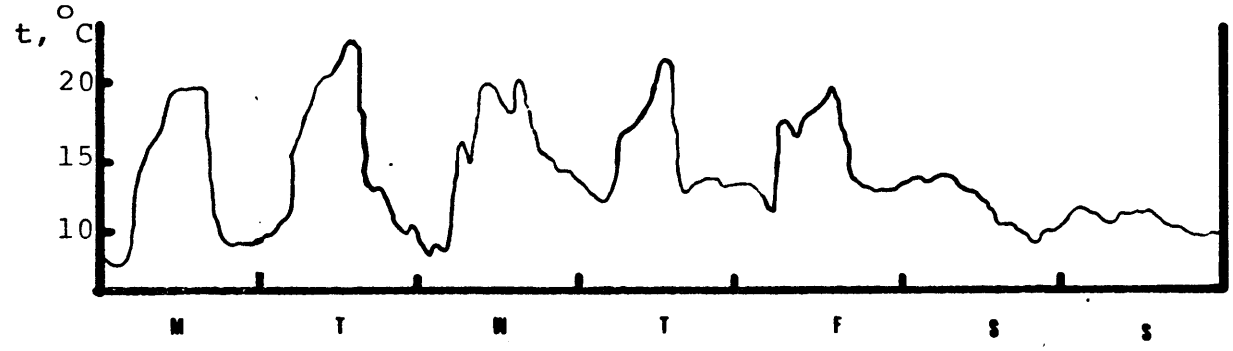
1.2.1.2 Temperature fluctuations inside a food warehouse

Grundke and Kuklov (1980) measured the temperature inside a commercial warehouse (Figure 2). Temperature fluctuations were especially severe during the winter season --to save energy a heating system is turned on and off. A similar situation could be expected in a consumer kitchen, where an air conditioning or heating system is turned off and on. The constant repetition of these temperature cycles could create

Figure 2

TEMPERATURE FLUCTUATIONS IN A FOOD WAREHOUSE


a. Week in summer




b. Week in winter

severe microbial stability problems due to the repetitive creation of surface conditions with high $a_w$.

1.2.1.3 Lack of temperature uniformity within a product

A typical example of this kind of situation is a refrigerated IMF sitting on a display shelf (Reid, 1976). The product is heated up by a light source while it is cooled down by a cold source. Let us assume a product with $a_w$ = 0.90 and that it is under the influence of a 2C temperature difference. As shown in Table 1 the vapor pressure on the warm side will be even higher than the saturation vapor pressure on the cool side. The vapor pressure differential will result in water being transferred from the warm to the cool side until equilibrium conditions are established.

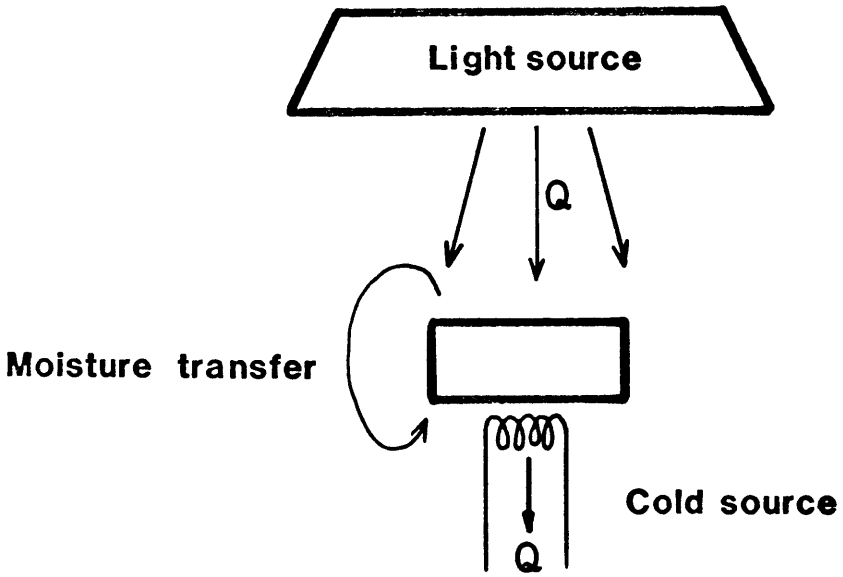
1.2.2 Research problem and solution proposed

The above examples have shown that localized surface $a_w$ conditions play an important role in the overall microbial stability of IMF products. Therefore, there is a need to develop treatments that can protect IMF surfaces against unstable temperature environments.

The solution explored in this thesis has been to consider surface a separate food region and, develop processes that enhance specifically its growth inhibiting capacity. This

Table 1

EFFECT ON VAPOR PRESSURE OF A WITHIN PRODUCT TEMPERATURE DIFFERENTIAL CAUSED BY THE HEAT ORIGINATED FROM AN ILLUMINATION SOURCE




| | Cold side (T) mm Hg | | Warm side (T + 2C) mm Hg | |
|---|---|---|---|---|
| T, C | $p_v^o$ | $p_v$ | $p_v^o$ | $p_v$ |
| 0 | 4.6 | 4.1 | 5.3 | 4.8 |
| 5 | 6.5 | 5.9 | 7.5 | 6.8 |

would provide a much needed safety margin on the surface, an option not available when considering the product as a bulk.

## 2. Aims of the project

The solution proposed to the microbial stability problem resulting from unstable temperature conditions is a surface layer with an improved capacity to resist microbial growth. Thus, if a growth allowing condition appears on the surface microenvironment, the surface properties will inhibit, or at least reduce, growth rate to such an extent, that $a_w$ equilibration will occur before high cell concentrations are reached.

### 2.1 General aims

Increase surface microbial growth resistance by the use of edible coatings with specific properties. Two different experimental approaches have been followed to satisfy this enhanced stability goal:

a. Approach 1

Maintain an unequal distribution of preservative, i.e. start with a higher (initial) concentration of preservative(s) on the surface and use a coating to maintain the concentration difference for as long as possible. This approach, called **"reduced preservative diffusion"**, required the selection of a coating capable of reducing preservative diffusion rate from food surface into food bulk.

b. Approach 2

Create a surface microenvironment where pH is lower than that of food bulk. Most food preservatives, particularly sorbic acid, are lipophilic acids whose effectiveness is pH dependent (Freese et al., 1973). Moreover, growth itself is strongly affected by pH (e.g. Leistner and Rodel, 1976). This approach called the **"reduced surface pH microenvironment"**, required the identification of the conditions under which a charged macromolecule could be used to maintain a stable reduced surface pH.

2.2 Specific aims

1. Analysis of unsteady state situations where surface condensations are significant and cause unexpected microbial stability problems.

2. Development and microbial stability testing of an IMF model where the surface modification effectiveness will be measured. Particularly, collect background information on microbial outgrowth as affected by pH, $a_w$ and K-sorbate concentration.

3. On the reduced preservative diffusion approach: (1) develop experimental approaches for the rapid identification of an appropriate coating composition; and, (2) develop mathematical expressions and analytical methods to study

K-sorbate distribution between surface and food bulk of treated and untreated samples.

4. On the reduced surface microenvironment pH approach: (1) develop a model and derive an expression for the pH difference between food surface and food bulk as a function of experimental conditions; (2) use this expression as a guideline for the selection of coating material(s) and application conditions; and, (3) execute microbial stability tests and determine stability improvements to evaluate the effectiveness of the surface modification procedures.

# 3. Literature survey and theoretical background

Modern IMF's are an example of highly engineered food products. Their success or failure depends on a clear understanding on their limitations.

## 3.1 Water activity

### 3.1.1 The concept of water activity

The concept of water activity occupies a key role in the formulation of IMF. Scott (1957) showed that there was a correlation between $a_w$ and microbial growth. The value $a_w$ is thermodynamically defined as the ratio at a given temperature of the fugacity, f, of water in some given state, and its fugacity f in a state which for convenience, has been chosen as reference state (Reid, 1976). In the case of aqueous solutions the most convenient reference state is pure water. Fortunately, fugacity can be substituted by vapor pressure.

Since $a_w$ is defined at a fixed temperature it is a function of temperature. Fortunately, in most cases it is not a strong function of it (van den Berg and Bruin, 1978; Reid, 1976). In some cases this may not be true as has been reported by many authors (Mazza and LeMaguer, 1978; Troller and Christian, 1978; Iglesias and Chirife, 1976a,b; Iglesias *et al.*, 1976; Rasekh *et al.*, 1971; Saravacos and Stinchfield, 1966; Heldman *et al.*, 1965 and Fenton, 1941). However, for practical purposes we can assume

that $a_w$ is independent of temperature and will be the assumption used in this project.

### 3.1.2 Equations for the prediction of $a_w$

A very large number of equations has been derived to predict the $a_w$ of foods (Chirife, 1980; van den Berg and Bruin, 1978). None has been able to describe the whole range, nor to accommodate hysteresis phenomena nor the effect of temperature. This is due to the many ways in which water interacts with food components.

The theoretical estimation of $a_w$ have included solutions theory based on the free energy model of Gibbs; theories about physical sorption if a surface can be distinguished (i.e. the structure formed by the common food polymers: proteins and carbohydrates); or a combination of both if the particular system requires so. In applying these theories, it has been found that physical sorption theories work better at low $a_w$, (e.g. the classical B.E.T equation) and solution theories are more succesful at higher $a_w$ (Chirife and Ferro Fontan, 1980; Benmergui et al., 1979; Ferro Fontan et al., 1979a,b; Chirife et al., 1979). An excellent review on the theoretical prediction of $a_w$ was presented by van den Berg and Bruin (1978).

The subject of empirical and semi-emipirical equations has been extensively covered by the group of Chirife in Argentina. His excellent review on fitting equations to water

sorption isotherms should be considered a primary reference on this field and was used extensively in this project (Iglesias and Chirife, 1982, Chirife and Iglesias, 1978; Boquet et al., 1978). Other published work of this group include the determination of the parameters for the B.E.T. (Iglesias and Chirife, 1976c) and the Halsey equation (Iglesias and Chirife, 1976b; Iglesias et al., 1976a); comparison of the Halsey and Henderson equations (Iglesias and Chirife, 1976c); the application of the concept of local isotherms (Iglesias and Chirife, 1976d); and the application of the Smith equation for the high $a_w$ region (Chirife et al., 1979). They also studied the determination of $a_w$ for mixtures of dehydrated food products in the low $a_w$ range using the B.E.T. equation (Iglesias et al., 1979); and evaluated the precision of Ross equation to predict $a_w$ of mixtures in the IM range (Chirife, 1978). This equation has been derived to calculate the $a_w$ of a given system with known composition. Therefore the formulation of an IMF is a trial-and-error process.

Recently an equation has been proposed to estimate the final moisture content of complex systems, given the desired $a_w$ and also the moisture content for the individual components at the desired $a_w$ value (Lang and Steinberg, 1980). This would eliminate the trial-and-error process associated with Ross' equation. Therefore, it was decided to test the accuracy claimed by the authors, using ten products from the literature and the model system used in this study. Appendix B summarizes our calculations. We were unable to predict equilibrium moisture

content with the accuracy reported by the authors. It should be recognized that good agreement (5% error) was found when the equation was applied to our model system (product 11).

## 3.2 Microbiological aspects of IMF's

### 3.2.1 Microbiology of IMF's and the 'hurdle' concept

IMF's range in $a_w$ from 0.7 to 0.9 and in water content from 20 to 50%. These foods may be susceptible to mold growth, enzymatic degradation, or non enzymatic browning unless appropriate preventive measures are taken. Since they do not fall under the scope of this project, changes not related to microbial growth will be ignored.

The value $a_w$ is only one of the environmental factors with which the IMF technologists can control growth of microorganisms. Their growth and survival will be influenced by many other factors such as temperature, pH, gas composition of the environment, and the presence of inhibitory substances, such as preservatives. Moreover, it has been shown that when other factors are less than optimal, the inhibitory effects of $a_w$ are enhanced (e.g. Troller and Christian, 1978).

Food preservatives are used mainly as fungistatic agents, although they do have some bacteriostatic activity. Those most commonly in use today are lipophilic acids. Generally they inhibit growth of microorganisms without killing them. As shown in Table 2 their activity results from the undissociated

form. Therefore, they are not active at high pH (Eklund, 1983; Freese et al., 1973). Based on their $pK_a$ the undissociated percentage has been calculated as a function of pH as shown in Table 3. However, acidification is usually organoleptically undesirable. This incompatibility between enhanced microbial stability and organoleptic quality is typical of IMF technology limitations.

Table 2

EFFECTIVENESS RATIO BETWEEN THE DISSOCIATED ($K_2$) AND UNDISSOCIATED ($K_1$) FORM OF SORBIC ACID [1]

| Organism | $K_2/K_1$ |
|---|---|
| Bacillus subtilis | 70 (0.96 [2]) |
| Bacillus cereus | 15 (0.44) |
| Escherichia coli | 100 (0.96) |
| Pseudomonas aeruginosa | 15 (0.97) |
| Staphylococcus aureus | 600 (0.89) |
| Candida albicans | 10 (0.84) |

[1] Eklund, 1983.

[2] Fraction of total variance explained by analyzing data model.

Table 3

EFFECT OF pH ON THE % UNDISSOCIATED FORM OF LIPOPHILIC ACIDS USED AS PRESERVATIVES

| Compound | $pK_a$ [1] | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| benzoate | 4.2 | 94 | 61 | 14 | 2 | .2 |
| sorbate | 4.8 | 98 | 86 | 39 | 6 | .6 |
| propionate | 4.9 | 99 | 88 | 43 | 7 | .8 |
| parabens | 8.5 | 99 | 99 | 99 | 99 | 97.0 |

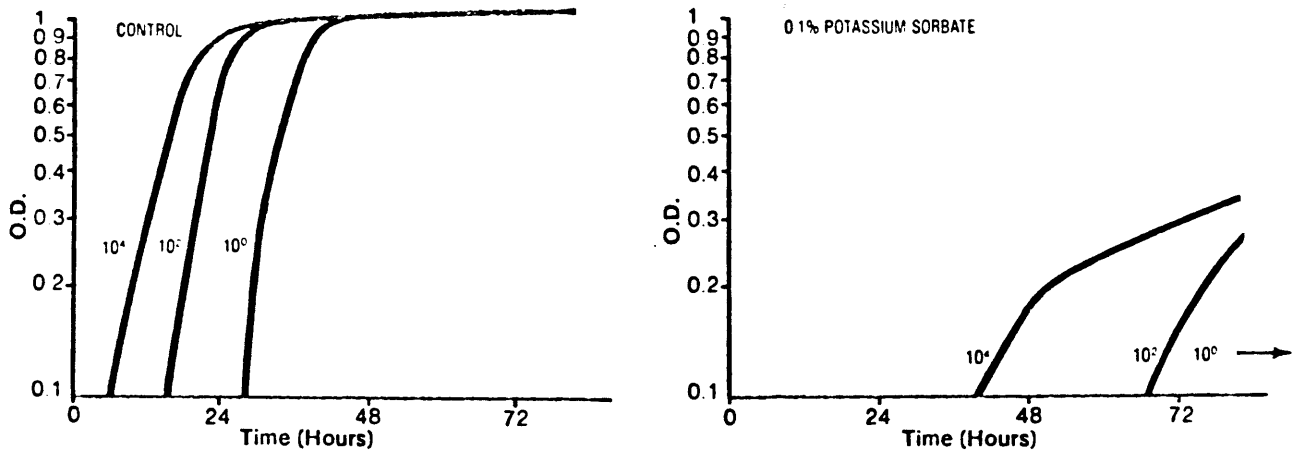
[1] Freese et al., 1973; Sauer, 1977.

A measure of the significance of good sanitation is illustrated by Figure 3 which shows the growth of *Saccharomyces cerevisiae*, *Escherichia coli* and *Bacillus coagulans* at pH 5.5 and 30C. The lower the initial contamination, the longer the sorbate protection (Anonymous 1978a). In the case of IMF's it would be preferable to decrease microbial load before $a_w$ is reduced. Heat resistance of microorganisms increases as $a_w$ is lowered (Corry, 1974, 1975, 1976).

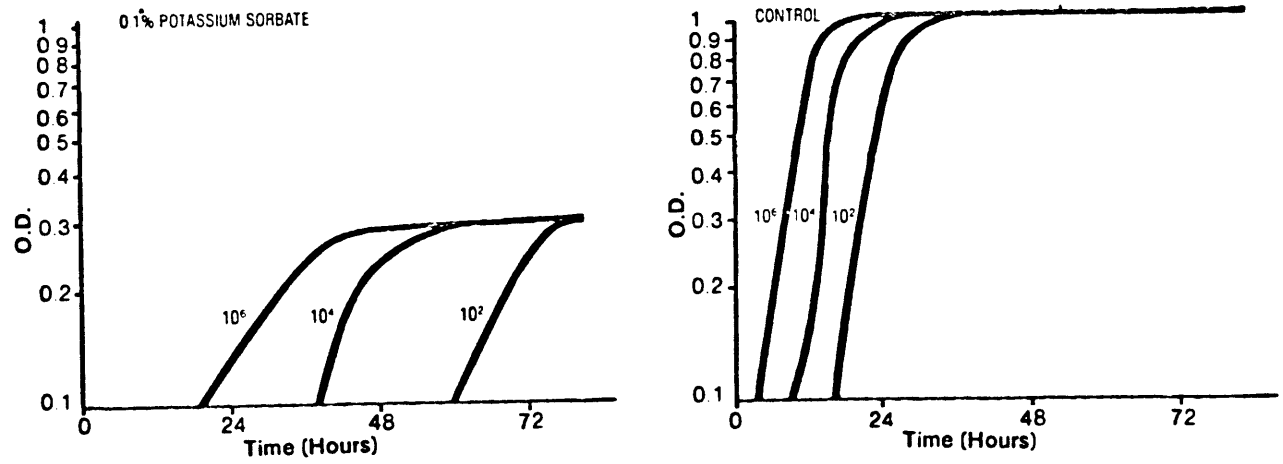
Table 4 summarizes the hurdles available to the IMF technologist. We will add another factor to this list: a

Figure 3

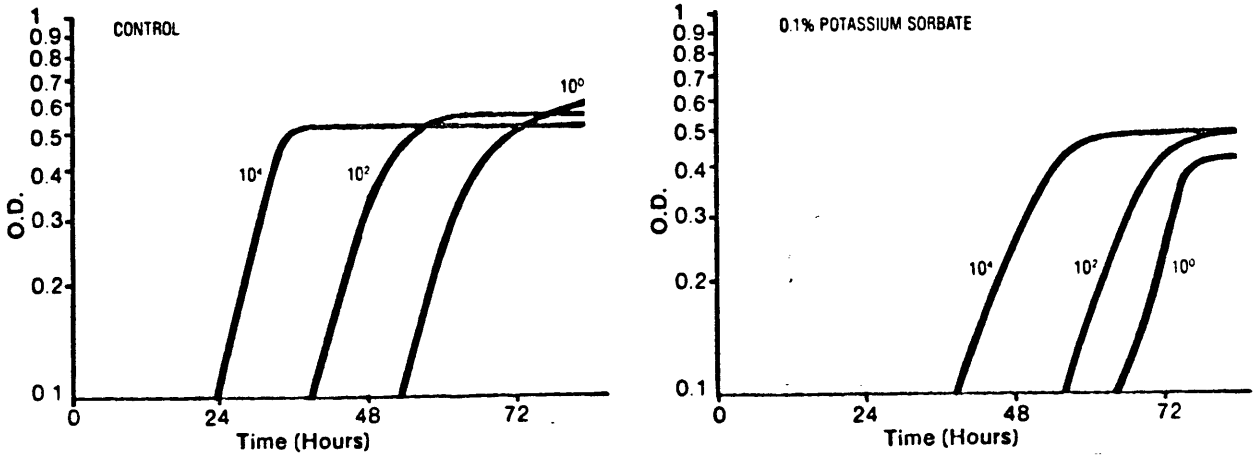
A MEASURE OF THE SIGNIFICANCE OF GOOD SANITATION




a. *Saccharomyces cerevisiae*, inoculum levels as indicated




b. *Escherichia coli*, inoculum levels as indicated




c. *Bacillus coagulans*, inoculum levels as indicated

coating with enhanced resistance to microbial growth. From the above presented facts it can be concluded that the application of a coating with a higher concentration of preservatives, and/or a pH lower than the optimal for bacterial growth and therefore closer to the range where the preservatives are most effective, would constitute a major contribution to the stability of IMF's. Evidently, such a coating would only enhance the stability of the surface, but we have already shown that there is a need to overprotect the surface.

### 3.2.2 Surface microbial load

In a previous section the idea of IMF ingredients heat pretreatment was suggested to reduce microbial load. However, subsequent handling can cause recontamination. Even though some products are cooked or pasteurized, slicing and packaging gives

Table 4

HURDLES USED IN IMF [1,2]

| Hurdle | Bacteria | Yeasts | Molds |
|---|---|---|---|
| $a_w$ | + | + - | + - |
| pH | + - | + - | + - |
| redox potential ($E_h$) | + - | + - | + - |
| storage temp. [3] | + - | - | - |
| thermal process | + | + | + |
| preservatives [4] | + - | + - | + - |
| competing microflora | + - | + - | + - |

[1] Adapted from Leistner and Rodel (1976)
[2] + = Inhibition
+ - = Some inhibition
- = No inhibition
[3] See also Farrel and Upton (1978), Theron and Prior (1980)
[4] See also Raccach et al. (1979)

ample opportunity for recontamination and shelf life usually ends as a result of microbial growth (Stiles and Ng., 1979).

Another problem of surface contamination is that viable counts can be highly variable (Anderson et al., 1980; Gill, 1979). This is particularly important in IMF's. We have already

shown that bacteriostatic barriers can be overcome by a large number of cells.

We should also consider the problem of *Staphylococcus aureus*, whose minimal $a_w$ requirement depends on oxygen concentration. Under anaerobic conditions its minimal $a_w$ is 0.91 while under aerobic conditions it is 0.86 (Scott, 1953). Therefore, the surface of foods, where oxygen could be more readily available is the region we should be more concerned with potential outgrowth of this ubiquitous organism (Pawsey and Davies, 1976; Lubieniecki-von Schelhorn, 1975).

Therefore, we see in surface contamination another reason to overprotect IMF surfaces.

Microbial challenge studies on the surface require knowledge of cell concentrations at the surface. In the case of meats it has been found that about $10^6$ cells/cm$^2$ is the level at which spoilage odors can be detected (Gill and Newton, 1980; Newton and Rigg, 1979). Gill and Newton (1980) found that growth on adipose fresh meat ceased at $10^8$ cells/cm$^2$. Other authors reported that no definite changes occur in the meat until surface bacterial count exceeded $10^8$ cells/cm$^2$ (Ingram and Dainty, 1971). Results by Sauter et al. (1979) showed that growth on lamb carcasses depended strongly on the fat layer thickness, which supports the generally accepted observation, that bacterial growth on food surfaces is limited by the diffusion of fermentable substrates from within the food. Therefore, it is

advisable to specifically determine the surface growth potential of our particular model system.

## 3.3 Preservative applications on the surface of foods

Preservatives are applied by a variety of methods selected on the basis of processing convenience and type of product. They can be either distributed evenly by direct addition into the mixture, concentrated on the surface by dipping, spraying or dusting, or by incorporation into the wrapping material when this is in relatively close contact with the product. Examples will be given only on food surface applications.

### 3.3.1 Application examples

#### 3.3.1.1 Dairy products

Solid cheeses are sprayed or dipped. After treatment, they are allowed to dry thoroughly to avoid imperfect heat sealing when wrapped. As shown in Table 5, K-sorbate in water solutions ranging from 20 to 40% is most commonly used (Anonymous, 1978a). More specific details on procedures recommended by Pfizer Chemicals in accordance to Federal Standards of Identity regulations can be found elsewhere (e.g. Anonymous, 1976b).

Table 5

RECOMMENDED METHODS FOR SURFACE TREATMENT
OF CHEESE AND CHEESE PRODUCTS

| Product | Solution concentration %w/v | Solids deposited [2] %w/w | Directions |
|---|---|---|---|
| Cheddar Colby Monterrey Jack Blue, etc. | 20-40 | .10-.30 | Dry sprayed or dipped before wrapping to avoid poor seals. |
| Muenster Edam, Gouda | 20 | .05-.10 | Because of bland flavor, .1% deposition should not be exceeded. |
| Provolone Pasta filata Caciocavallo Siciliano | 20-40 | .10-.30 | Preservative treatment should follow 24-hour hold period after brine dip. 4-7 days before wax dipping or film packaging. |
| Mozzarella | 20-40 | .10-.20 | Processing varies. No specific treatment recommended. |
| Swiss, Gruyere, Emmentaler | 20-40 | .10-.30 | Not dipped because "eye-flooding". Important to spray eyes. |

[1] Anonymous, 1978a.
[2] Concentration of dip, exposure time and cheese surface volume ratios pieces will determine amount picked up. Solution should be changed regularly to prevent contamination by resistant microorganisms.

Propionic acid and sodium and calcium propionate too, are also effective in preventing mold growth on cheese surfaces, but high concentrations lead to bitterness (Kosikowski, 1977, p.552) which is not a problem with sorbates (Anonymous, 1978b).

3.3.1.2 Bakery products

Bakery products are sterile as they come out of the oven, except for some bacteria which survive the high temperatures. Mold spores present in the air then contact the surface of the product during cooling, packaging, and sometimes during distribution and consumer storage (Anonymous, 1977a). Therefore addition of preservatives is desirable.

Calcium propionate and even less active preservatives have been used in an attempt to minimize the inhibiting effect of preservatives on yeast fermentation and, therefore on the leavening action. Sorbic acid and potassium sorbate, used widely in chemically leavened snack cakes, are considered too active to be added to yeast raised doughs. A solution to this problem has been proposed by Monsanto (Anonymous, 1977b). The product is baked or griddled in the usual way, but immediately after the oven or after depanning, the product is covered evenly on all surfaces with a sorbate solution. The water evaporates in seconds from the hot surface, leaving a protective shield of sorbate. Tests on English muffins were quite encouraging as shown in Table 6 (Anonymous, 1978b). Similar tests on variety

Table 6

ENGLISH MUFFINS, DAYS OF MOLD FREE SHELF-LIFE OF COATED AND UNCOATED SAMPLES [1]

| Muffins with: | days in commercial bakery |
|---|---|
| no preservative | 5 |
| 0.5% Ca propionate in dough | 7 |
| 1.0% Ca propionate in dough | 9 |
| 0.1% Sorbate in dough and 0.1% as a surface spray | 12 |
| and 0.2% as a surface spray | 26 |

[1] Ref.: Anonymous, 1978b

bread, hamburger buns, rolls and tortillas were also positive (Anonymous, 1977a).

3.3.1.3 Dried fruits

Dried fruits are often moisturized to attain desirable texture for consumer appeal. Potassium sorbate has been used in these high moisture dried fruits to protect them against mold and yeast spoilage (Nury et al., 1960). In another study the sorbic

acid gradient was also measured (Bolin et al., 1980). Thirty four percent moisture prunes (skin:pulp = 37:73) dipped in a 1.7% potassium sorbate solution showed that after one day of dipping, the skin contained 708 ppm sorbic acid and fruit pulp 207. At the end of 30 weeks, skin contained 312 ppm and pulp only 204. This discrepancy, lack of final equilibration but rapid initial diffusion was explained by the authors as due to a probable addition compound with either the waxy coating materials or other components.

3.3.1.4 Meat products

The relative short life of fresh, unfrozen poultry has been an industry problem for some time. Fresh broilers in retail outlets normally have an initial contamination level of $10^4$ to $10^5$ cells/$cm^2$. Normally they can only be stored for 1 or 2 days at 3 - 5C and still maintain their freshness (Robach and Ivey, 1978; To and Robach, 1980). In a study by Robach (1979) the shelf life of fresh, whole broilers was extended by dipping freshly chilled carcasses into a 5% w/v solution of potassium sorbate for 30 seconds. The control birds were stored for 10 days when spoilage was evident, while sorbate-treated birds were stored for 19 days, at which time spoilage was evident.

The sensory properties of cooked chicken parts after dipping in 5 or 10% sorbate solutions were found acceptable by sensory panels (Cunningham, 1979).

In a study of turkey products, Robach et al. (1980) found that dipping and spraying were more effective than pumping it into the product, even when the total amount sprayed or dipped was lower than the total pumped. It was also noted that with increasing storage time microbial protection at the surface was lost, most probably due to preservative migration into the product.

### 3.3.2 Discussion

Uneven distribution of preservatives has been proposed, proved to be effective, shown to pose no organoleptic problems, and in some cases, its use has been authorized by regulatory agencies. Therefore FDA acceptance of the reduced preservative diffusion proposed to enhance microbial stability can be predicted.

Uneven distribution of preservatives has been proved very succesful for products with very short life (e.g. fresh poultry) or where the preservative could interfere with the production process (e.g. cheese ripening and yeast leavened bakery products).

The examples described showed also that the microbial stability improvement is generally limited by the diffusion of the preservative into the product. In order to expand this technique to other products it would be necessary to reduce the rate of diffusion as proposed in this study.

## 3.4 The reduced preservative diffusion approach

We have proposed to enhance the microbial stability of IMF's by the use of a high concentration of preservative on its surface. The overall concentration can still be within legal limits by lowering the concentration in the bulk, e.g. from 0.3% K-sorbate to 0.1%. The amount corresponding to this difference will be the amount used on the surface as described in Figure 4.

### 3.4.1 Theoretical considerations on mass transfer

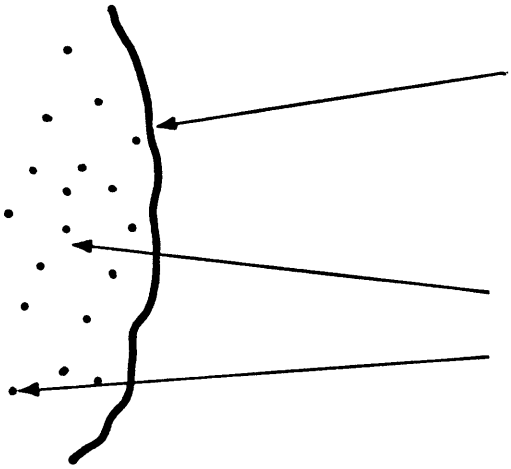
To facilitate the selection of coating procedure(s) and ingredient(s) we need: (1) a mathematical expression to estimate the mass transfer properties required to achieve the desired stability; and, (2) an experimental device where the potential coatings could be easily tested.

#### 3.4.1.1 Mass transfer estimations

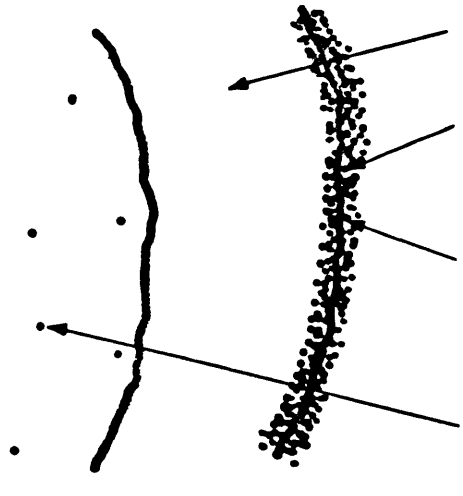
Before measuring mass transfer properties of coating materials, it was necessary to determine what rate would be required to achieve significant microbial stability improvements. Based on expressions derived in Appendix C required apparent diffusion coefficients have been summarized in Table 7. In order to evaluate the magnitude of these values, D values for different systems have been summarized in Table 8. From this comparison it seems that an appropriate coating will have to allow K-sorbate diffusion 1,000 times slower than food-like matrices.

Figure 4

SCHEMATIC REPRESENTATION OF MICROBIAL STABILITY ENHANCEMENT BY HIGH CONCENTRATION OF K-SORBATE DEPOSITED ON A HIGHLY IMPERMEABLE EDIBLE FOOD COATING




a. Uncoated food




b. Coated food

3.4.1.2 Experimental device for potential coating tests

The search for an appropriate coating procedure was facilitated by measurements of film permeability in a diffusion cell. As shown in Appendix D the use of a reference value, regenerated cellulose (dialysis membrane type 30F0, thickness = 30 microns, Union Carbide, Chicago, Illinois) provided an opportunity to obtain rapid, even though approximate measurement of a coating effectiveness. A direct measurement on the food model itself would have been much more laborious.

Table 7

APPARENT DIFFUSION COEFFICIENTS REQUIRED TO ACHIEVE SIGNIFICANT MICROBIAL STABILITY IMPROVEMENTS

| Number of days | D, $cm^2/sec$ | | |
|---|---|---|---|
| | h = 0.01 mm | h = 0.03 mm | h = 0.05 mm |
| | $x10^{-10}$ | $x10^{-9}$ | $x10^{-9}$ |
| 1 | 14.7 | 13.3 | 36.8 |
| 5 | 2.9 | 2.7 | 7.4 |
| 10 | 1.5 | 1.3 | 3.7 |
| 30 | 0.5 | 0.4 | 1.2 |

Table 8

D VALUES FOR DIFFUSION THRU A SOLID MATRIX

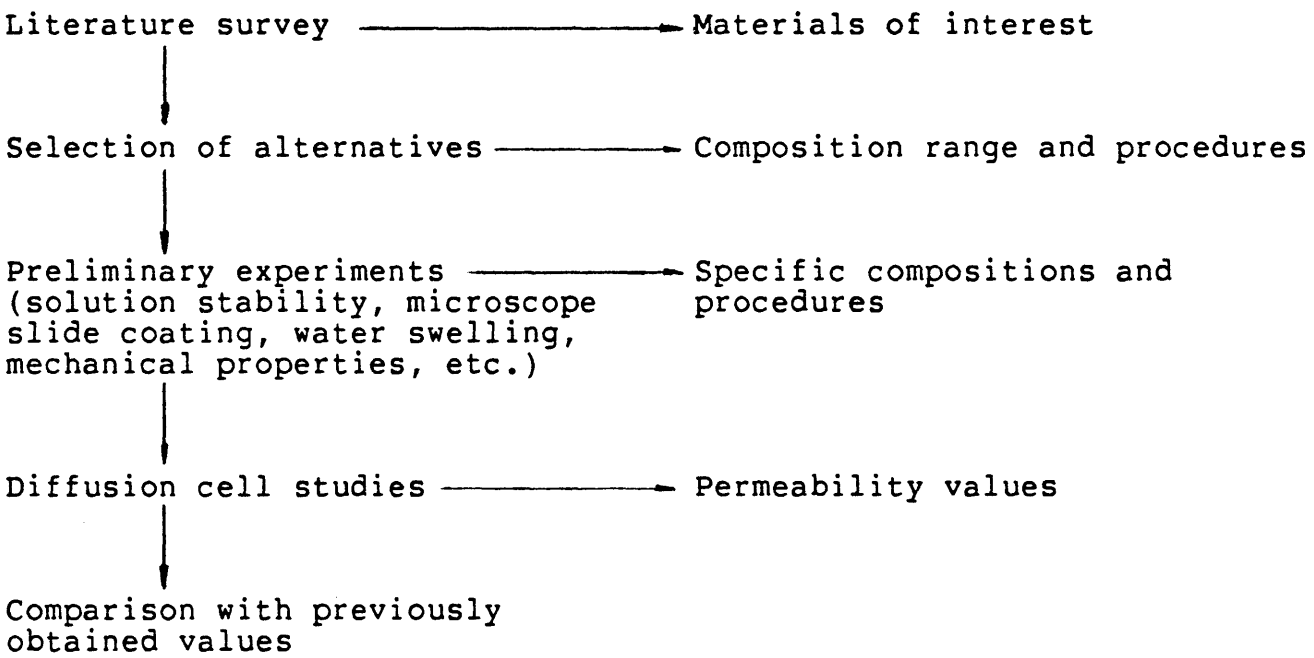
| Diffusing specy | Solid | D, $cm^2/sec$ | Reference |
|---|---|---|---|
| Water | fish muscle | $3\text{-}30x10^{-7}$ | Jason (1965) |
| Water | alfalfa wafer | $3\text{-}\ 8x10^{-6}$ | Bakker-Arkema et al. (1964) |
| Water | tree periderm tissues | $3\text{-}45x10^{-8}$ | White (1978) |
| Water | sugar beet | $3x10^{-7}$ | Vaccarezza et al. (1974) |

3.4.2 Formulation of effective coatings

The approach used in this study for the formulation of effective coatings have been summarized in Figure 5. Little specific information could be found in our literature survey. Particularly scarce was the information on transport properties. Formulation was facilitated by the following guidelines and the simple tests using the diffusion cell previously described. These guidelines concerned the food surface conditions, the diffusing molecule and the intended effect sought. They can be summarized as follows:

Figure 5

EXPERIMENTAL APPROACH TO THE SELECTION OF COATING COMPOSITIONS



a. All coatings had to be food grade and organoleptically acceptable.

b. The surface where the coating would be applied is at an $a_w$ in the IM range. Therefore we were interested in properties in this $a_w$ range. This eliminated materials that would experience severe water swelling.

c. Mass transfer would be slower in a coating with a very 'tight' microstructure, i.e. one leaving little free space for the diffusion of sorbic acid. This favored linear polymers over highly branched ones or with bulky side groups that would present steric hinderances to the desired tight structure. Numerous charged groups would have the same effect.

Once a coating composition is selected, preliminary trial and error experiments can be used to determine formulations that produce films with reasonable strength, flexibility and adhesivity. The final and critical step were the measurement of K values.

## 3.5 The reduced surface microenvironment pH approach

A second approach to IMF surface microbial stability enhancement proposed in this study was the establishment of a pH difference between food surface and food bulk. The increased resistance at reduced pH is associated with increased bacteriostatic effectiveness of lipophilic acids such as sorbic

acid at low pH. Since surface pH reduction increases surface availability of the most effective form of the preservative, we can predict that it should result in significant surface stability improvements.

Preliminary experimental work confirmed that food samples with a reduced surface pH are capable of resisting surface growth of *S.aureus* S-6 significantly longer than untreated controls, e.g. Figure 6. These tests showed also that as soon as the pH difference disappeared rapid growth occurred. Experimentally, a non permanent pH difference was achieved by inclusion of low molecular weight acids in a zein based coating. Diffusion was assumed to be the mechanism for pH equilibration.

### 3.5.1 Theoretical considerations

The Donnan model for semipermeable membranes suggests that it is possible to establish conditions for **permanent** pH differences. The objective of the following discussion will be to describe:

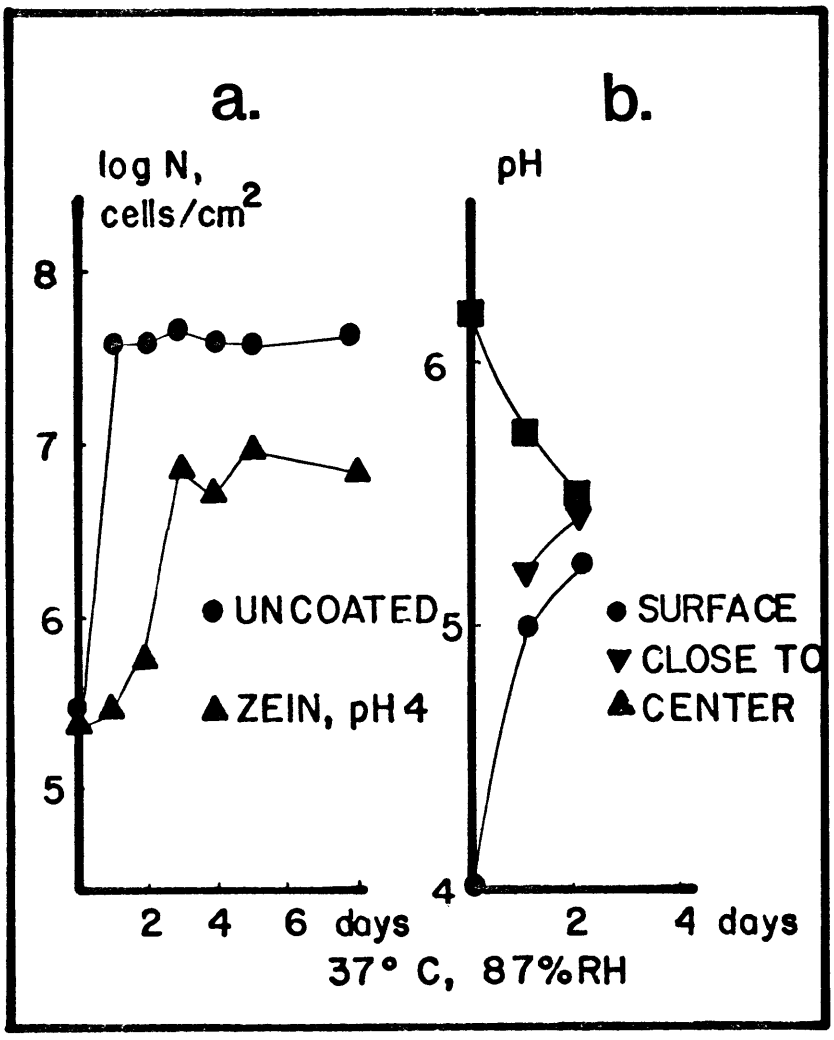
a. how this theoretical model can be interpreted for our particular application, and

b. how can it be used to identify the parameters which will allow us to establish the necessary conditions for maximum pH difference.

Figure 6

PRELIMINARY MICROBIAL TESTS AND pH DETERMINATIONS

IMF MODEL No.1 COATED WITH A pH 4 ZEIN SOLUTION




a. cell counts on coated and uncoated samples

b. pH values as function of location and time for coated samples

3.5.1.1 The Donnan equilibrium model

The Donnan equilibrium model describes the ionic concentration differentials created by the presence of a membrane separating two solutions. The membrane is assumed permeable to low molecular weight electrolytes but impermeable to a charged macromolecule. The rationale behind this modelling is that it could represent the situation of a charged macromolecule immobilized in the form of a food surface coating while other components, water and other solutes, particularly electrolytes, would be able to move freely from the food bulk to the surface and viceversa as schematized in Figure 7.

The first system to be dealt with (Hiemenz, 1977a) consists of water as the solvent, a charged macromolecule ($P^{-z}$) and a low molecular weight uni-uni-valent electrolyte ($M^+X^-$).

The specific situation to be considered in this analysis is that described in Figure 8. We shall arbitrarily designate the macromolecule, $P^{-z}$, i.e. consisting of a negatively charged macroion with a valence number -z. This macroion will be placed on side 1 of the chamber separated by the semipermeable membrane M-M', This membrane is impermeable to $P^{-z}$ but permeable to ions $M^+$ and $X^-$. At equilibrium $M^+$ and $X^-$ will be found on both sides of the membrane, but not in equal concentrations because of the presence of $P^{-z}$ on side 1 of the membrane. The condition for equilibrium is that the chemical potentials should be equal, therefore:

Figure 7

SCHEMATIC REPRESENTATION OF A DONNAN THEORY MODEL FOR THE ANALYSIS OF A pH DIFFERENCE BETWEEN SURFACE AND FOOD BULK

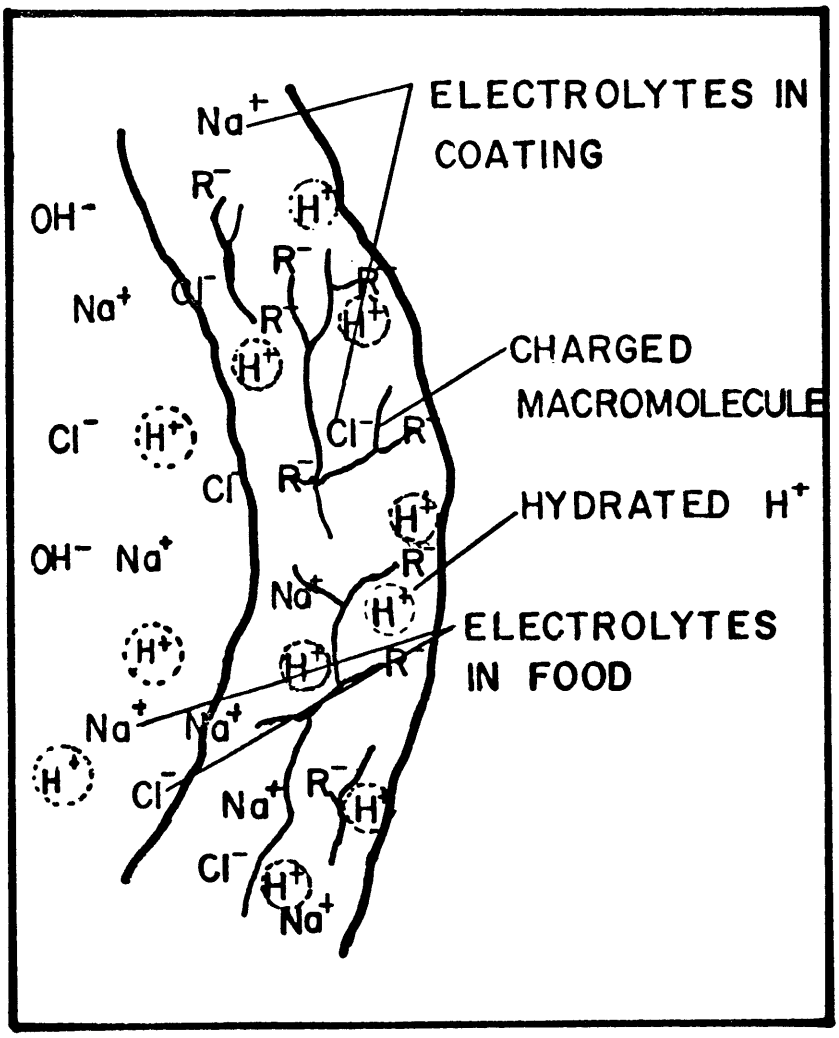
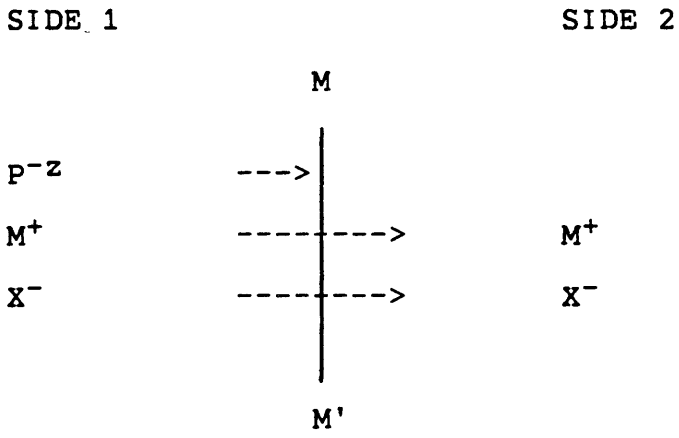


Figure 8

SCHEMATIC REPRESENTATION OF A SEMIPERMEABLE MEMBRANE ANALYSIS FOR AN IMPERMEABLE CHARGED POLYELECTROLYTE AND UNIVALENT PERMEABLE IONS



$$\mu_{MX/1} = \mu_{MX/2} \qquad (2)$$

But:

$$\mu_{MX/1} = \mu^{\circ}_{MX/2} + RT \ln a_{MX/1} \qquad (3)$$

$$\mu_{MX/2} = \mu^{\circ}_{MX/2} + RT \ln a_{MX/2} \qquad (4)$$

Therefore:

$$a_{MX/1} = a_{MX/2} \qquad (5)$$

Equation (5) can be further expanded using:

$$a_{MX} = a_{M^+} a_{X^-} \qquad (6)$$

Therefore:

$$a_{M^+/1} a_{X^-/1} = a_{M^+/2} a_{M^+/2} \qquad (7)$$

Expression (7) states that the ion activity product is constant on both sides of the membrane, although the activity of ions $M^+$ and $X^-$ is not. Remembering that:

$$a_{M^+} = \gamma_{M^+} m_{M^+} \qquad (8)$$

$$a_{X^-} = \gamma_{X^-} m_{X^-} \qquad (9)$$

$$\gamma_{\pm/MX} = \gamma_{M^+} \gamma_{X^-} \qquad (10)$$

where:

$\gamma_i$ = activity coefficient, component i

$m_i$ = molal concentration, component i

Assuming:

$$\gamma_{\pm/MX/1} \simeq \gamma_{\pm/MX/2} \tag{11}$$

we obtain:

$$m_{M^+/1}\, m_{X^-/1} = m_{M^+/2}\, m_{X^-/2} \tag{12}$$

Another restriction on the system is that both sides of the chamber have to be electrically neutral. Therefore:

$$z\, m_{P^{-z}} + m_{X^-/1} = m_{M^+/1} \tag{13}$$

$$m_{X^-/2} = m_{M^+/2} \tag{14}$$

From expressions 12 thru 14 two quadratic expressions can be obtained for the unknowns $m_{M^+/1}$ and $m_{X^-/1}$. These expressions were used to evaluate the concentrations of the low molecular weight ions in the compartment with the charged macromolecule, i.e. side 1, in terms of the valence z, the concentration of the macromolecule and the ionic concentration in side 2. The dependence of the ionic concentrations in chamber 1 is easier to visualize when numerical values are used as shown in Table 9. These calculations assumed a macromolecule with a charge z = -10 and a concentration ranging from $10^{-4}$ to $10^{-3}$. This is equivalent to a molecule with a m.w.= 10,000 used in the 1-10% concentration range.

Table 9

VALUES OF $m_{M^+}/1$ AND $m_{X^-}/1$ FOR TWO VALUES OF $m_{M^+}/2$ AND A RANGE OF VALUES FOR z $m_p$-z. ALSO INDICATED ARE THE LOW MOLECULAR WEIGHT ELECTROLYTE RATIOS BETWEEN SIDE 1 AND 2.

a) $m_{M^+}/2 = 10^{-3}$

| z $m_p$-z | $m_{M^+}/1$ | $m_{M^+}/1 \,/\, m_{M^+}/2$ | $m_{X^-}/1$ | $m_{X^-}/1 \,/\, m_{X^-}/2$ |
|---|---|---|---|---|
| x$10^3$ | x$10^3$ | | x$10^4$ | |
| 1 | 1.62 | 1.6 | 6.18 | 0.62 |
| 2 | 2.41 | 2.4 | 4.14 | 0.41 |
| 4 | 4.24 | 4.2 | 2.36 | 0.24 |
| 6 | 6.16 | 6.2 | 1.62 | 0.16 |
| 8 | 8.12 | 8.1 | 1.23 | 0.12 |
| 10 | 10.10 | 10.1 | 0.99 | 0.10 |

a) $m_{M^+}/2 = 10^{-2}$

| z $m_p$-z | $m_{M^+}/1$ | $m_{M^+}/1 \,/\, m_{M^+}/2$ | $m_{X^-}/1$ | $m_{X^-}/1 \,/\, m_{X^-}/2$ |
|---|---|---|---|---|
| x$10^3$ | x$10^2$ | | x$10^3$ | |
| 1 | 1.05 | 1.05 | 9.51 | 0.95 |
| 2 | 1.11 | 1.11 | 9.05 | 0.91 |
| 4 | 1.22 | 1.22 | 8.20 | 0.82 |
| 6 | 1.34 | 1.34 | 7.44 | 0.74 |
| 8 | 1.48 | 1.48 | 6.77 | 0.68 |
| 10 | 1.62 | 1.62 | 6.18 | 0.62 |

Table 9 shows that the concentration of the cation $M^+$ is higher in the phase containing the charged macromolecule while the reverse is true for the anion $X^-$. The difference becomes larger when the concentration of the charged macromolecule and/or the number of charged groups it carries is higher and when the concentration of the permeable electrolyte is lower. In other words, the more immobilized charged groups present, the more asymmetrically the simple electrolyte will be distributed.

The previous calculations have not considered the presence of water and the proton and hydroxyl ions that it forms, as shown in Figure 9. Since we are interested in pH values we should include them in our expressions (Grignon and Scallan, 1980; Scallan and Grignon, 1979; Donnan, 1934, 1924, 1911; Donnan and Guggenheim, 1932).

Let us now determine the equilibrium condition by considering the six particular cases of equilibrium deviations depicted in Figure 10. All mirror image equilibrium conditions were not considered since they would yield identical equations. We should also remember that:

$$a_{OH^-/1}\, a_{H^+/1} = K_W \qquad (15)$$

$$a_{OH^-/2}\, a_{H^+/2} = K_W \qquad (16)$$

Applying the equilibrium condition to Case 1:

$$0 = dG = \mu_{X^-/1}\,(dn_{X^-/1}) + \mu_{OH^-/1}\,(dn_{OH^-/1}) +$$

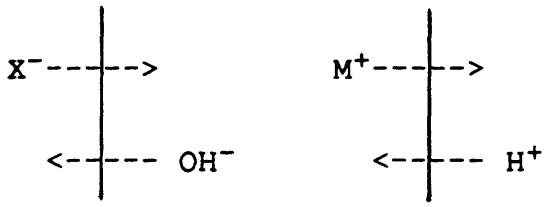
Figure 9

SCHEMATIC REPRESENTATION OF A SEMIPERMEABLE MEMBRANE
EFFECT ON pH

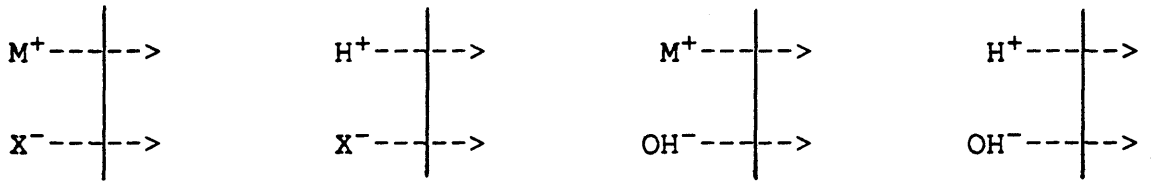
| COMPONENT | MOLAL CONCENTRATION | MOLAL CONCENTRATION |
|---|---|---|
| | | M |
| | SIDE 1 | SIDE 2 |
| $M^+$ | $K_w/y + n + zm_{p^{-z}} - y$ | $K_w/x + m - x$ |
| $X^-$ | n | m |
| $H^+$ | y | x |
| $OH^-$ | $K_w/y$ | $K_w/x$ |
| $p^{-z}$ | $m_{p^{-z}}$ | --- |
| | | M' |

Figure 10

EQUILIBRIUM DEVIATIONS USED FOR THE EQUILIBRIUM ANALYSIS OF A SEMIPERMEABLE MEMBRANE




Case 1 Case 2




Case 3 Case 4 Case 5 Case 6

$$\mu_{X^-/2}\,(dn_{X^-/2}) + \mu_{OH^-/2}\,(dn_{OH^-/2}) \qquad (17)$$

From mass balance and electroneutrality considerations:

$$dn_{X^-/1} = dn_{OH^-/1} = dn_{X^-/2} = dn_{OH^-/2} = dn \qquad (18)$$

Therefore:

$$\mu_{OH^-/1} + \mu_{X^-/2} = \mu_{OH^-/2} + \mu_{X^-/1} \qquad (19)$$

But:

$$\mu_i = \mu_i^o + RT \ln a_i \qquad (20)$$

Substituting in (19):

$$a_{X^-/2}\; a_{OH^-/1} = a_{X^-/1}\; a_{OH^-/2} \qquad (21)$$

Similarly for cases 2 thru 6 described in Figure 10:

$$a_{M^+/2}\; a_{H^+/1} = a_{H^+/2}\; a_{M^+/1} \qquad (22)$$

$$a_{X^-/2}\; a_{M^+/2} = a_{X^-/1}\; a_{M^+/1} \qquad (23)$$

$$a_{X^-/2}\; a_{H^+/2} = a_{X^-/1}\; a_{H^+/1} \qquad (24)$$

$$a_{M^+/2}\; a_{OH^-/2} = a_{M^+/1}\; a_{OH^-/1} \qquad (25)$$

$$a_{H^+/2}\; a_{OH^-/2} = a_{H^+/1}\; a_{OH^-/1} \qquad (26)$$

Not all of the above equations are independent of each other. It is possible to eliminate four of them: 21, 22, 25 and 26. Only for algebraic simplicity we shall substitute activities

by molal concentrations. From equations 15, 16, 23 and 24 the following series of equalities was obtained:

$$\frac{m_{M^+/1}}{m_{M^+/2}} = \frac{m_{X^-/2}}{m_{X^-/1}} = \frac{m_{H^+/1}}{m_{H^+/2}} = \frac{m_{OH^-/2}}{m_{OH^-/1}} = \lambda \qquad (27)$$

Let us now refer back to Figure 9 where electroneutrality considerations were used to determine expressions for $m_{M^+/1}$ and $m_{M^+/2}$. Substituting these expressions in (27):

$$\frac{K_W/y + n + zm_{P^{-z}} - y}{K_W/y + m - x} = \frac{m}{n} = \frac{y}{x} = \frac{K_W/x}{K_W/y} = \lambda \qquad (28)$$

This expression can be rearranged to obtain:

$$\frac{K_W/y + n + zm_{P^{-z}}}{K_W/x + m} = \lambda \qquad (29)$$

Substituting in (29) the following expressions derived from (28):

$$y = \lambda x \qquad (30)$$

$$m = \lambda n \qquad (31)$$

we obtain:

$$\lambda = \sqrt{1 + \frac{zm_{P^{-z}}}{K_W/y + n}} \qquad (32)$$

This expression gives the distribution constant for all permeable solutes in terms of the number of charges and the

concentration of the charged macromolecule, the proton and the anion concentration, all in side 1. It is important to emphasize that log $\lambda$ represents the pH difference between side 1 and 2, i.e. between coated surface and food bulk in the case of our intended application. From equation (30) we obtain:

$$\log \lambda = \log y - \log x = pH(\text{food bulk}) - pH(\text{coating}) \quad (33)$$

3.5.2 Applications to an IMF model

3.5.2.1 IMF model requirements

As shown in Figure 7, a negatively charged macromolecule can be immobilized in the form of a food surface coating, while other molecules, particularly electrolytes, can move freely. The key parameters in the expression for the pH difference between coated surface and food bulk are, electrolyte concentration in the food and the number of immobilized charged groups on the surface. This is further emphasized in Table 10 which reports the calculated pH differences as affected by these two parameters. It is quite apparent that significant $\Delta$pH values can only be achieved in an IMF system with low electrolyte content. To prove the validity and potential application of the pH difference concept, an IMF model had to satisfy this condition (IMF model No.2).

Table 10

pH VALUES AS AFFECTED BY THE CONCENTRATION OF CHARGED GROUPS AND THE CONCENTRATION OF ELECTROLYTES PRESENT IN A FOOD PRODUCT

| Charged groups, [M] | Electrolytes, [M] | log λ = ΔpH |
|---|---|---|
| 0.001 | 0.0100 | 0.02 |
| | 0.0010 | 0.21 |
| | 0.0001 | 1.00 |
| 0.010 | 0.0100 | 0.21 |
| | 0.0010 | 1.00 |
| | 0.0001 | 2.00 |

3.5.2.2 Selection of polyelectrolyte

Several food grade polyelectrolytes were considered for incorporation as negative carriers into a coating formulation. Among them we had pectic acid, xanthan gum, furcellaran and carrageenans.

A linear chain of anhydro-D-galacturonic acid units connected by $\alpha$ -(1-4) linkages is the basic structure of pectic substances. Some of the carboxylic acid groups are methylated and others are in the form of free acids or salts. The number of charged groups can be increased by altering the degree of methylation.

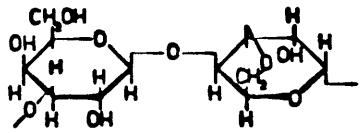
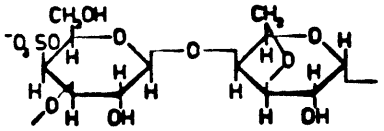
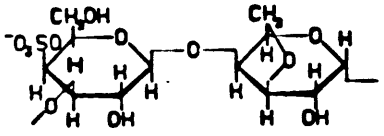
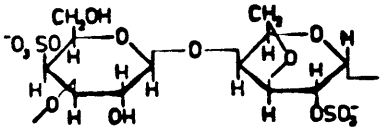
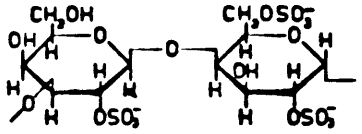
The molecular structure of xanthan gum consists of a main chain built up of $\beta$ -D glucose units linked through the 1 and 4 positions. The side chains consists of two mannose units and a glucuronic acid unit, shielding the backbone of xanthan gum and could be the major reason for the enzymatic resistance and the uniformity of chemical and physical properties.

Figure 11 shows the molecular structure of agarose, furcellaran and carrageenans. The idealized representations of these three seaweed galactans show them with their galactose residues linked alternatively by $\beta$-(1-3) and $\beta$-(1-4) linkages. The presence of electronegative sulfate groups and the 3,6 anhydro ring give them different properties. The presence of the ring confers hydrophobicity making the galactan less soluble, whereas sulfate groups confer hydrophilicity, making it more soluble.

The ideal polyelectrolyte should have a large number of strongly dissociated groups. Solubility and easiness of application should also be considered.

Figure 11

MOLECULAR STRUCTURE OF AGAROSE, FURCELLARAN AND CARRAGEENANS

| | | Sulfate content % |
|---|---|---|
| agarose |  | 0-3 |
| furcellaran |  | 12-16 |
| κ-carrageenan |  | 25 |
| ι-carrageenan |  | 30 |
| λ-carrageenan |  | 35 |

Sulfate content % is given as an indicator of substitution, e.g. λ-carrageenan has on the average about one sulfate group per repeating unit.

## 4. Experimental Procedures

### 4.1 Preliminary experiments

#### 4.1.1 The IMF model: composition, preparation and analysis

An IMF model was required to test the feasibility of the surface modification procedures proposed in this study. The product chosen was an IM cheese model developed in this laboratory (Motoki et al., 1982). Components are shown in Table 11. A brief preparation description is shown in Figure 12. Glycerol (Certified A.C.S.; Fisher Scientific Co., Fair Lawn, NJ), sorbitol (Pfizer Co., New York, NY) and sodium chloride (Baker Analytical Reagents, Phillipsburg, NJ) were dissolved in warm water (about 50C) with K-sorbate (Pfizer Co.) as the mycostatic agent. The proteins used were: isolated soybean protein (Ajinomoto USA., New York, NY) and sodium and calcium caseinates (Erie Casein Co, Erie, IL).

An emulsion paste was obtained by preblending 30% of the proteins in a Waring blender with the aqueous solution. The remainder of the protein was mixed with an oil phase composed of hydrogenated vegetable oil (Durkex 500, SCM Corp., New York, NY) and decaglycerol decaoleate (Glyco Chemical Inc., Greenwich, CT) and then emulsified using a food cutter (2 blades, 1450 rpm, Kitazawa Sangyo, Co., Tokyo, Japan). This emulsion was then blended with the emulsion paste using the same food cutter.

After emulsification, the mixture was adjusted to the desired pH with glucono-delta-lactone (GDL) (FMC Co.) or sodium

Table 11

COMPOSITION OF THE IMF MODEL SYSTEM

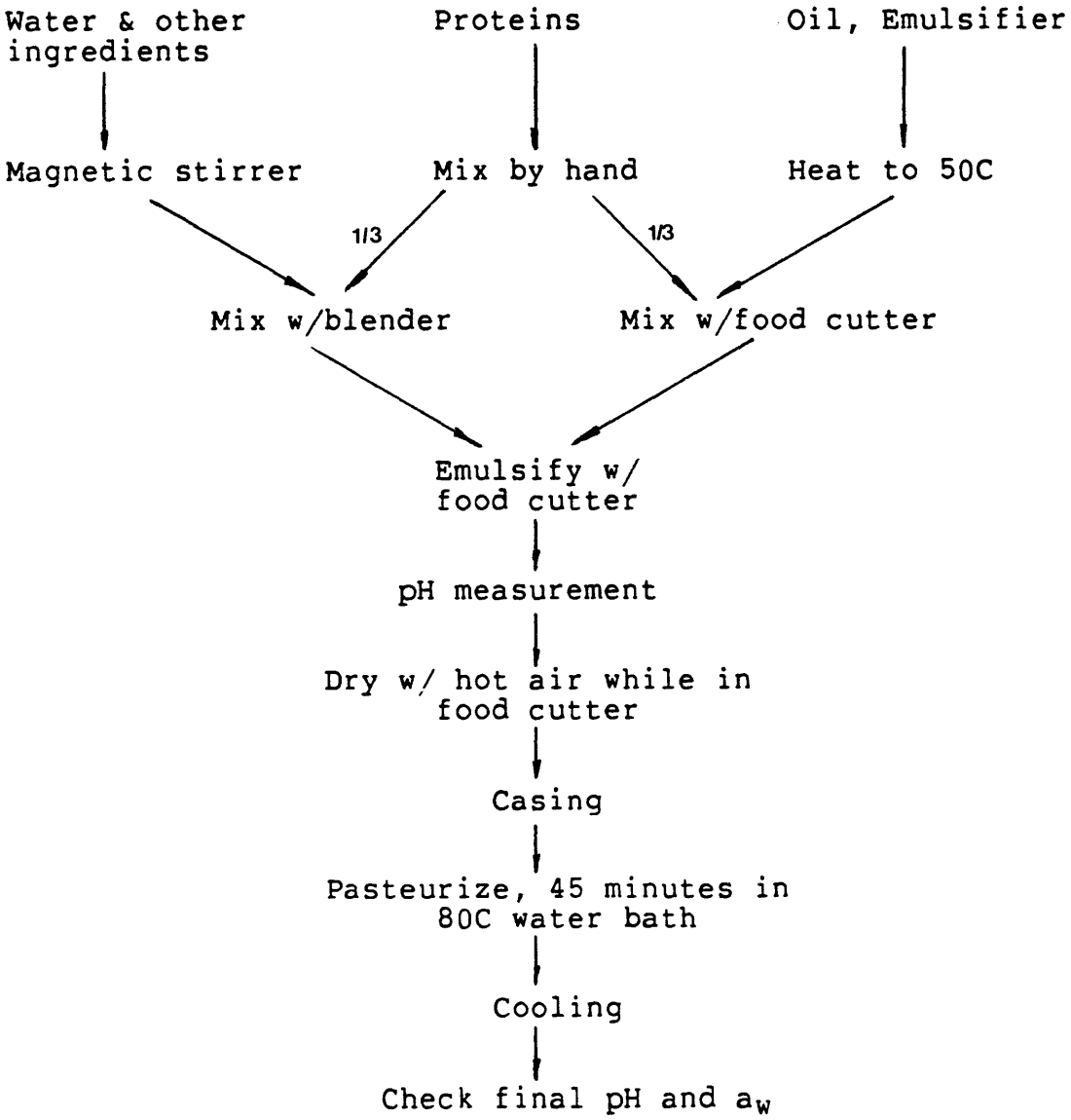
| Ingredient | %w/w |
|---|---|
| isolated soy protein | 26.1 |
| Na-caseinate | 5.9 |
| Ca-caseinate | 2.0 |
| hydrogenated vegetable oil | 34.0 |
| decaglycerol monooleate | .4 |
| salt | 4.8 |
| glycerol | 5.9 |
| sorbitol | 19.3 |
| K-sorbate | .4 |
| cheese flavor | 1.0 |
| monosodium glutamate | .2 |
| GDL/SAL | [1] |
| water | [2] |

[1] Glucono-delta-lactone (GDL) or Sodium Aluminum phosphate (SAL) is used to adjust the mixture to the desired pH.

[2] The initial water content is 100ml/100 g solids; this amount is reduced by drying the mixture w/ hot air so as to achieve the desired final $a_w$.

Figure 12

PREPARATION OF IMF MODEL SYSTEM



aluminum phosphate (Stauffer Chemical Co., Westport, CT). Some of the water was then evaporated by blowing warm air into the food cutter bowl. When the desired $a_w$ level was reached the emulsion was filled into a cellulose casing (Type 30F0, Union Carbide, Tarrytown, NY) with a hand press. Thereafter they were placed into seamless vinylidene chloride casing tubes (diameter 40 mm, Kreha Chemical Co., Tokyo, Japan).

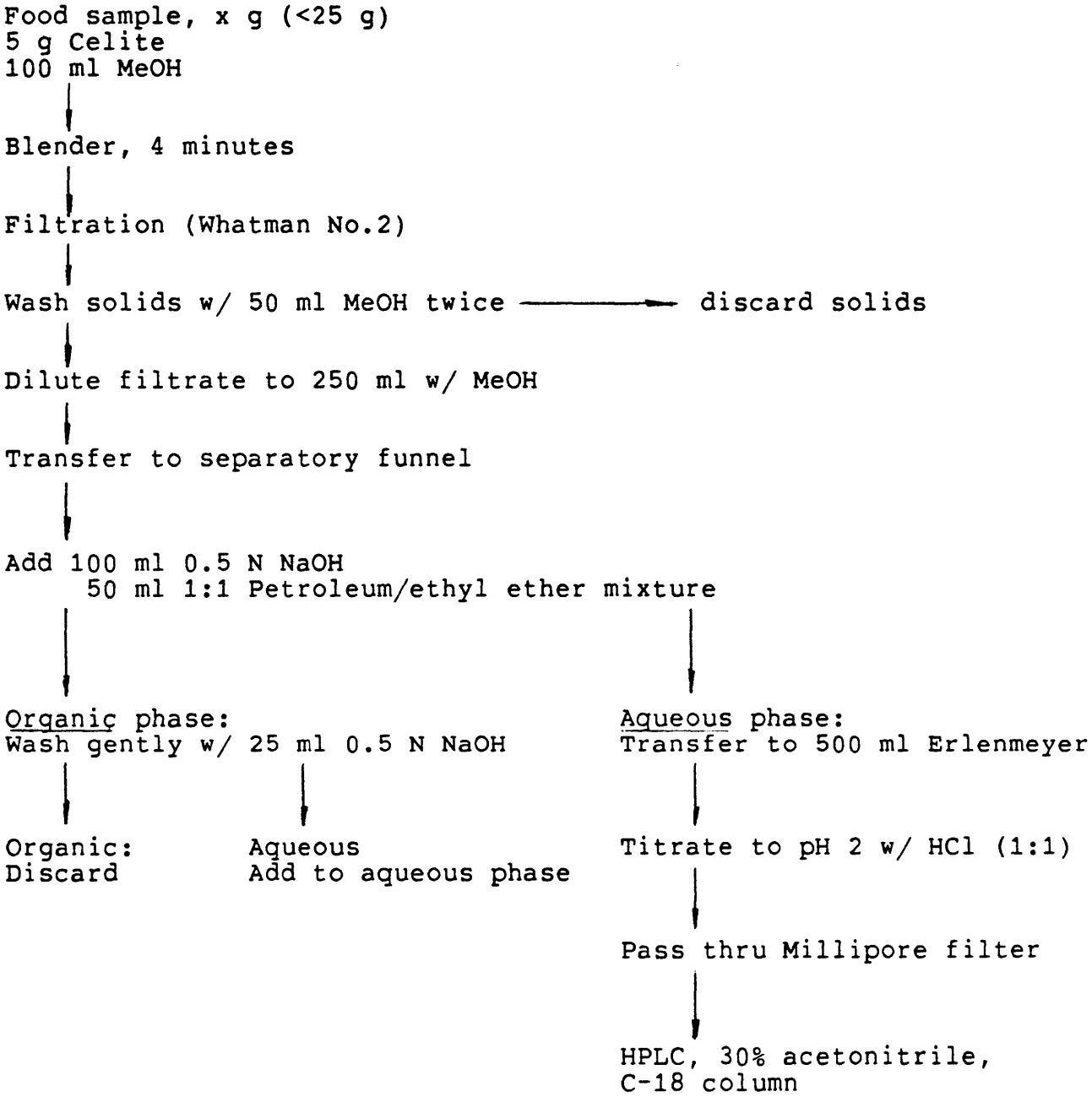
The emulsion was then pasteurized at about 85C for 45 minutes in a water bath. After cooling, pH was determined with a surface electrode probe (combination electrode 39507, Beckman Instruments, Inc., Cedar Grove, NJ).

Other important parameters were $a_w$, measured using an electric hygrometer (SINA Equihygroscope, Nova Sina, Zurich, Switzerland; marketed in the USA by Beckman Instruments); and, moisture content measured by the vacuum oven method. A relatively low drying temperature was selected to minimize humectant losses.

Finally, concentration of the preservative used in this model, K-sorbate was determined by HPLC. A simple procedure based on the paper by Park and Nelson (1981) was found to satisfy our needs (Figure 13). Analysis of an IMF model No.1 batch prepared with 0.2% K-sorbate showed excellent recovery values, (0.199 + 0.01)%, n = 8.

Figure 13

SORBIC ACID ANALYSIS BY HPLC



### 4.1.2 Preliminary microbial studies

The microorganisms used in this study were selected based on their resistance to low $a_w$ and importance in food microbiology. These are *S.aureus S-6*, *Aspergillus niger* and *A.tonophilus*.

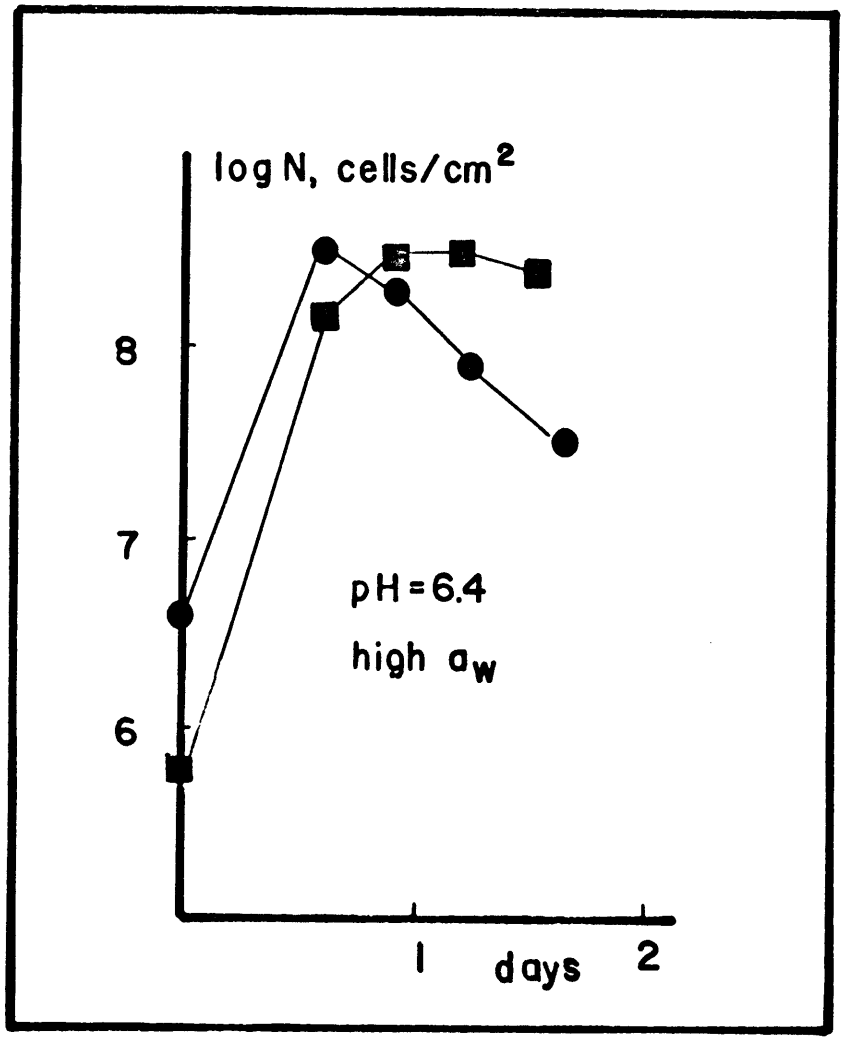
#### 4.1.2.1 Determination of inoculation conditions

Preliminary tests were required to determine *S.aureus* surface outgrowth. Food samples fabricated with optimal microbial growth conditions (i.e. neutral pH and high $a_w$) were inoculated at several levels by dipping them in glycerol solution cell suspensions. Growth was followed by plating on BHI (Difco, Detroit, MI) agar plates and expressed as viable counts/$cm^2$. As shown in Figure 14 the maximum growth observed was about $3x10^8$ cells/$cm^2$. This value was used as a guide for the design of future experiments. A complement to these tests was the determination of cell concentrations in the aqueous glycerol solutions used to inoculate food samples, so as to achieve the desired initial cell concentration. Similar preliminary test were also required for the mold challenge studies.

It is important to note that since we are interested only in the surface modification of IMF's, only the surface was inoculated. Later on, we will show that outgrowth affected only the surface. Therefore results could be expressed as cells/$cm^2$ or spores/$cm^2$.

Figure 14

SURFACE MICROBIAL CHALLENGE WITH *S.aureus* S-6 AT HIGH $a_w$ AND NEUTRAL pH



4.1.2.2 Growth response in the presence of high K-sorbate concentrations at various pH levels

This was another preliminary experiment required to provide necessary background information. Much information was found on the ability of K-sorbate to retard microbial growth (Gordon Greer, 1982; Blocher et al., 1982; Lynch and Potter, 1982; Lahellec et al., 1981; Reinhard and Radler, 1981; Ivey and Robach, 1978). However, no information was found on its effectiveness at levels above the legally authorized concentrations.

Various amounts of K-sorbate were dissolved in TSB (Difco, Detroit, MI) and pH was adjusted with HCl (0.1N). Results have been represented in Figure 15. At 0.3% K-sorbate, results are well in accordance to published work, which states that above pH = 5.5 and high $a_w$ level, the preservative is incapable of growth inhibition. From this experiment, it could be inferred, that an effective K-sorbate surface concentration at pH values closer to neutral should be approximately 0.5 to 1.0%.

4.1.3 Stability of the uncoated IMF model

Procedures for the microbial challenge tests have been summarized in Figure 16. Initial inoculation and growth (or death) of *S.aureus* was determined by plating on BHI agar plates. Initial inoculation levels of *A.niger* and *A.tonophilus* were obtained by plating on Saboureau dextrose agar (SDA, Difco)

Figure 15

HIGH K-SORBATE CONCENTRATION EFFECT ON *S.aureus* VIABILITY

Media utilized was TSB, pH adjusted w/0.1N HCl. Cell concentrations were determined at 19, 39, 49, 97 and 216 hours. Values reported correspond to increases over inoculation level (+), decreases (-) and no viable cells found (k).

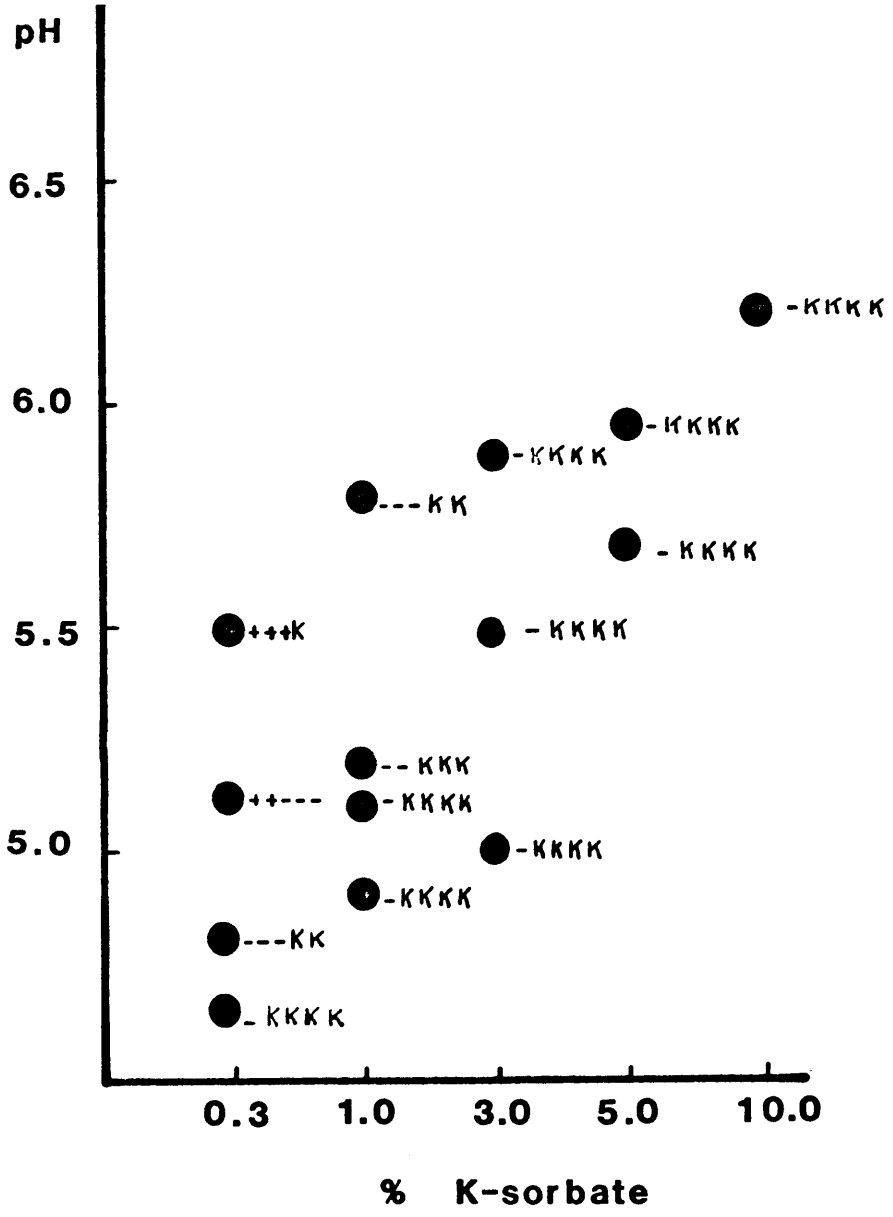
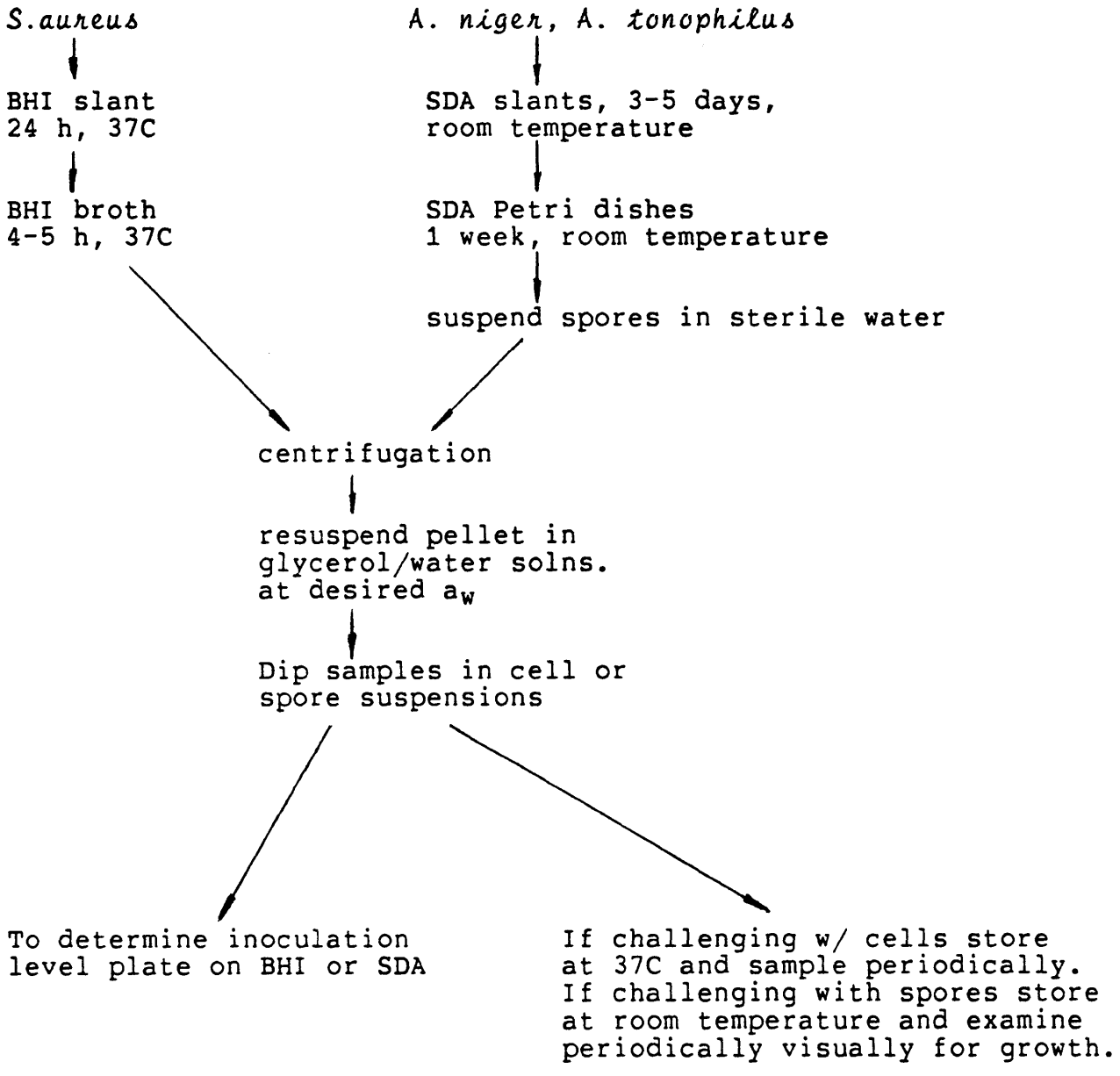


Figure 16

INOCULATION PROCEDURES FOR UNCOATED SAMPLES



plates. Mold outgrowth was determined by direct visual examination of inoculated samples.

Inoculated samples were placed in sterile Petri dishes and stored in a dessicator at a $a_w$ adjusted with sterile glycerol-water mixtures to that of the specific sample. Whenever possible, saturated salt solutions were used instead.

The objective of these tests was to determine the microbial growth potential of our particular IMF model surface as a function of the fabrication parameters: pH and $a_w$. The results served both as a guide for the design of experiments for coated samples and as the associated uncoated controls for these samples.

## 4.2 The reduced preservative diffusion approach

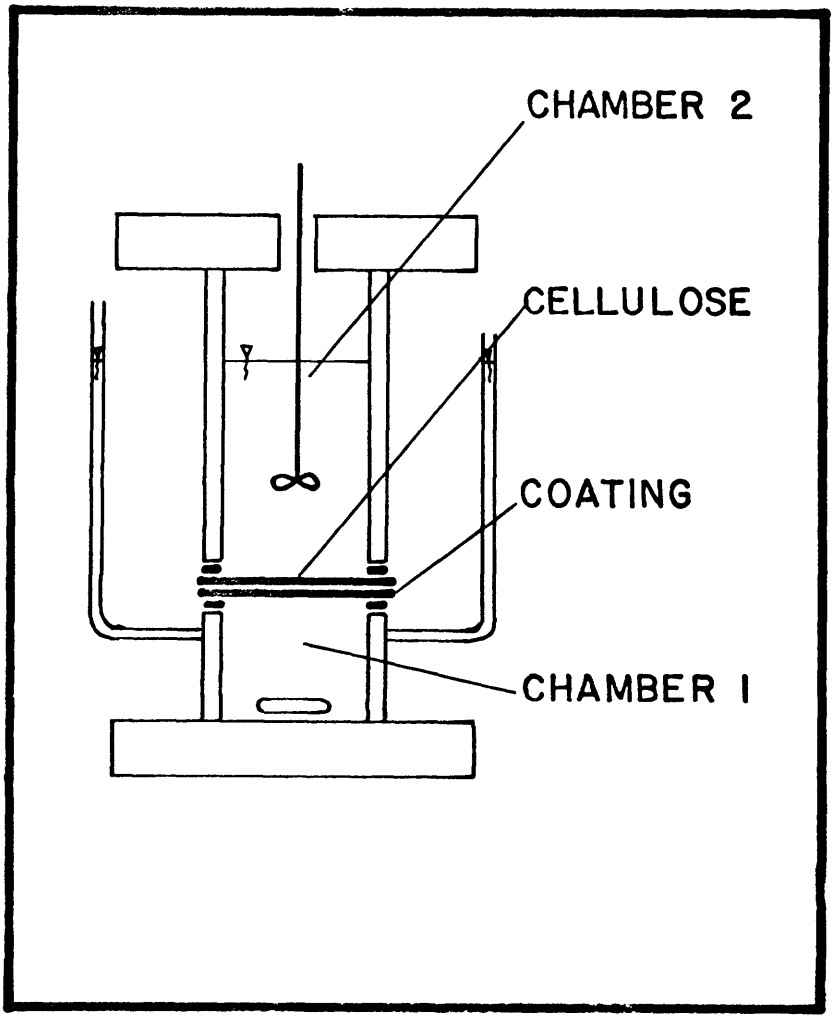
### 4.2.1 Permeability experiments

#### 4.2.1.1 Preliminary tests

Figure 17 shows the diffusion cell. A mechanical and a magnetic stirrer were provided to reduce resistance at the interfaces. The side tubes were used to load the cell with the high concentration solution, eliminate air bubbles and adjust levels to eliminate the influence of hydrostatic pressure on permeability. Samples were taken from the upper chamber and the preservative concentration was determined spectrophotometrically (at 268 nm, absorption maximum determined experimentally) with no need for an extraction procedure.

Figure 17

PERMEABILITY CELL USED IN THIS THESIS



The solvent used was 50% glycerol (w/w) which should give:

a. a $a_w$ level in the intermediate moisture range. This is important since we want a sorption status for the coating similar to actual use conditions.

b. a solvent viscosity in the range of solutions commonly found in IMF's (Perry, 1963, p. 3-199).

The advantages of regenerated cellulose were its availability, inertness, mechanical strength and low cost. Dialysis tubing made out of regenerated cellulose are very homogeneous, thus facilitating the obtention of reproducible results.

The diffusion cell was tested for its suitability to determine K values. As shown in Table 12, diffusion cell values agreed very well with those obtained with a dialysis sac arrangement.

4.2.1.2 Permeability tests on selected ingredients

Permeability tests on selected coating compositions were determined according to procedures schematized in Figure 18. Measurements on plain cellulose were often repeated to check for experimental artifacts (e.g. agitation speeds).

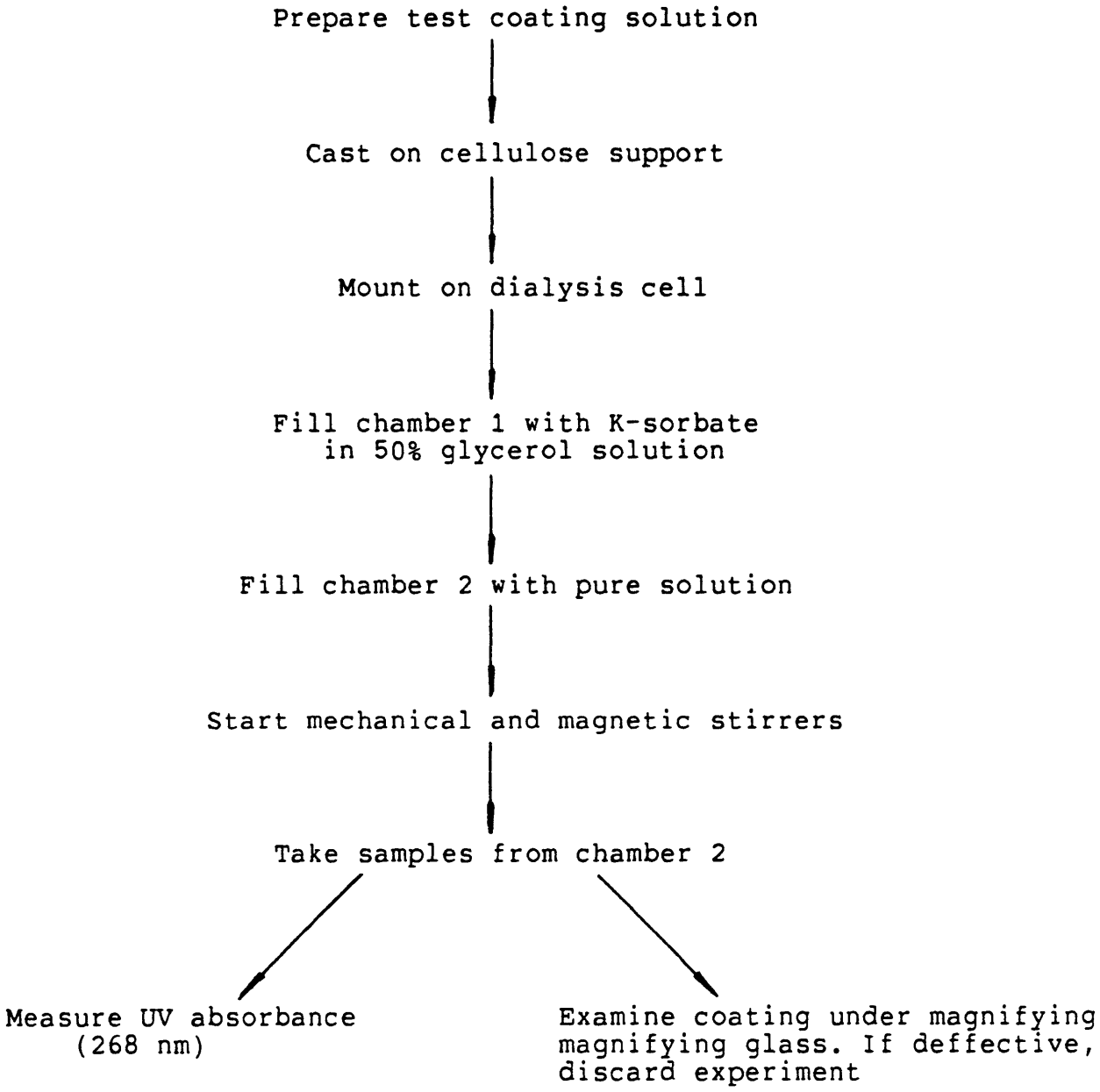
Table 12

COMPARISON OF K-VALUES

| Experimental device | K-values $x10^2$ $(mg/hr\ cm^2)/(mg/ml)$ |
|---|---|
| dialysis sac | 5.7 |
| | 4.1 |
| | 2.6 |
| | 4.2 |
| | $4.2 \pm 1.3$ |
| dialysis cell | 4.8 |
| | 4.8 |
| | 4.6 |
| | 4.7 |
| | 4.6 |
| | $4.7 \pm 0.1$ |

Figure 18

EXPERIMENTAL PROCEDURES TO DETERMINE K-VALUES FOR SELECTED COATINGS



4.2.2 Determination of sorbic acid stability

The objective of these tests was to determine the stability of sorbic acid at the high concentrations of intended use and to check for interactions with the IMF model.

Sorbic acid stability was assessed using the IMF model prepared as outlined in section 4.1.1. Initial concentrations were selected to cover the 0.3 to 5% (w/w, wet basis) range, values expressed as the potassium salt form. To simulate conditions existing in microbial stability tests, samples were also inoculated with *S.aureus* S-6.

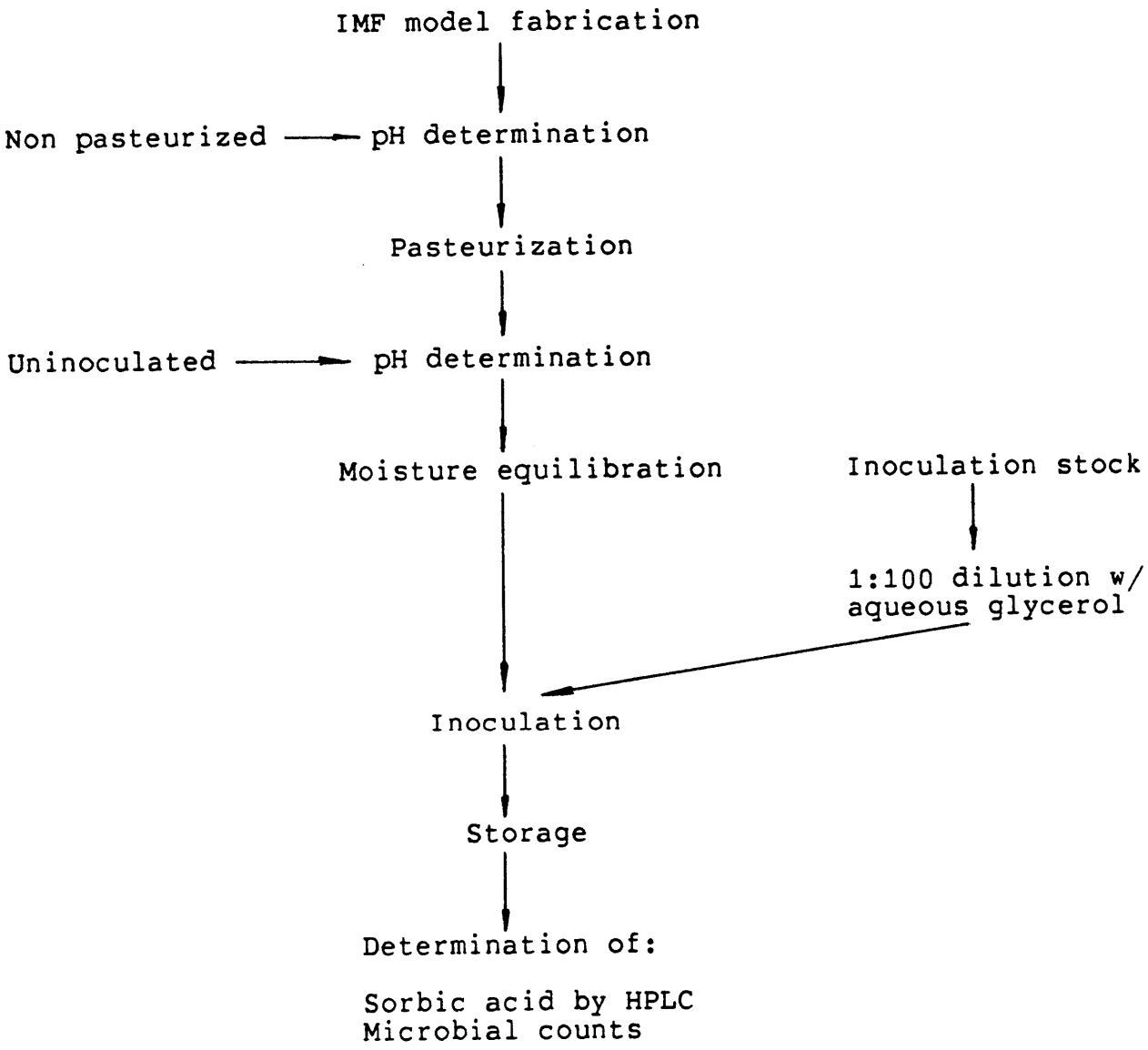
Experimental procedures outlined in Figure 19 include sorbic acid determinations by HPLC, pH by a Beckman surface pH electrode, and *S.aureus* growth response as described previously. It should be noted that these procedures included uninoculated pasteurized controls to determine if preservative losses could be related to microbial growth, as well as uninoculated not pasteurized controls to ascertain if heat treatment could affect sorbic acid stability.

4.2.3 Sorbic acid distribution studies

The objective of these tests was to show that zein coatings reduce preservative diffusion from coated food surface into food bulk. Therefore we had to determine how zein coatings affected the distribution of sorbic acid sprayed on the IMF model

Figure 19

DETERMINATION OF SORBIC ACID STABILITY PROCEDURE



surface. Moreover, we wanted to show that the distribution was a function of a coating mass transfer controlling parameter, e.g. thickness.

4.2.3.1 Microscopy studies

Nomarsky microscopy (Peil, 1982) was used to observe the general appearance of zein coatings as well as to estimate their thickness. $CO_2$ frozen samples were sliced into 10 microns sections and placed immediately on glass slides. To prevent artifacts caused by dehydration, samples were kept in a constant relative humidity chamber (over saturated $BaCl_2$) and examined within 24 hours.

4.2.3.2 Sorbic acid determination

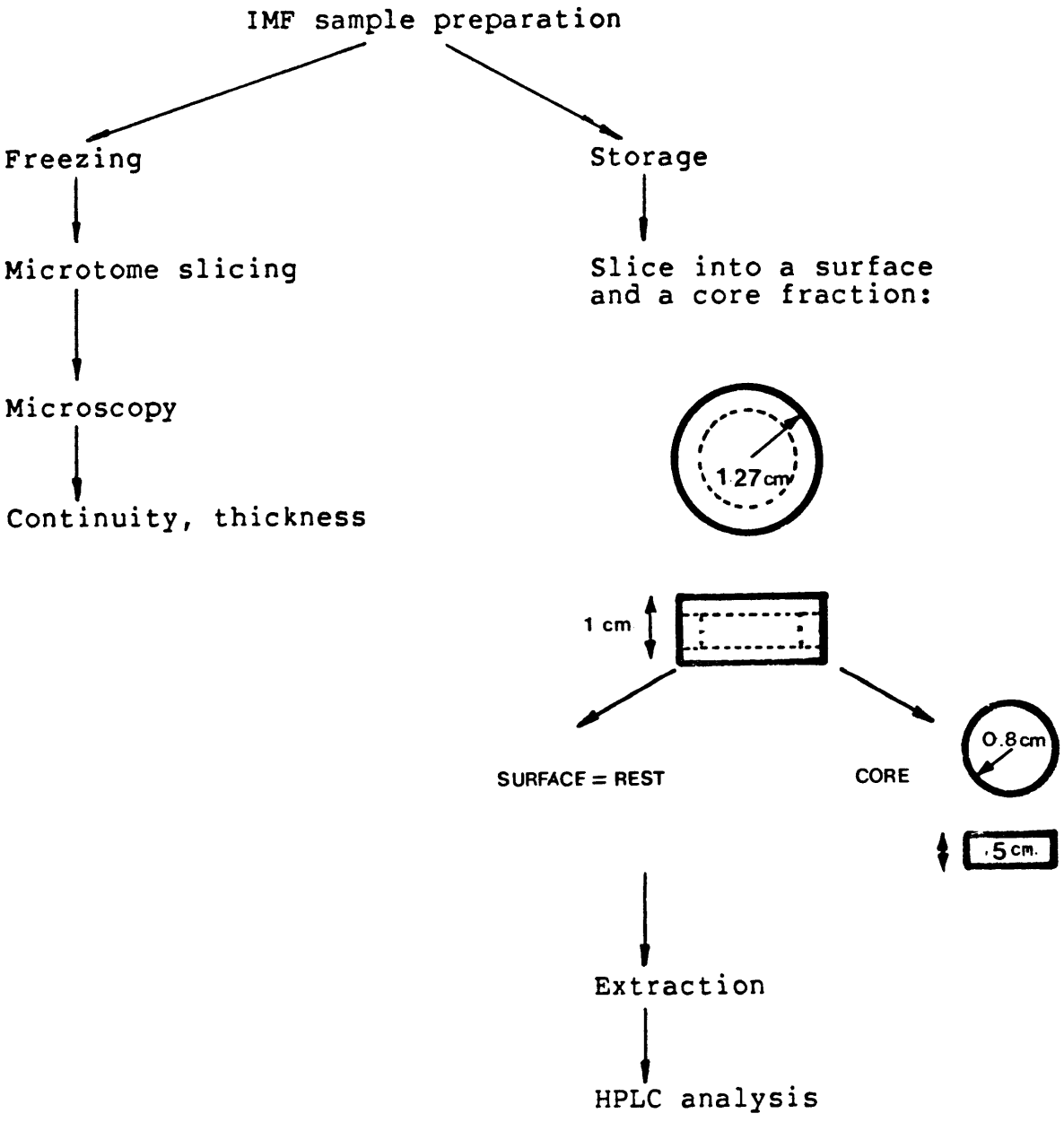
The effectiveness of zein coatings as a sorbic acid diffusion controlling barrier was studied by separating samples into a core and a surface piece, Figure 20. Sorbic acid concentration in each fraction was then determined by HPLC.

4.2.4 Microbial challenge tests

Microbial challenge studies were based on a worse situation condition. pH was close to neutral, 6.4, and food bulk contained only a minimum amount of preservative, 0.1% K-sorbate. Pasteurization, 2 hours at 80C in a water bath, was needed to insure low microbial background counts. Two different storage

Figure 20

EXPERIMENTAL PROCEDURES FOR THE DETERMINATION OF SORBIC ACID DISTRIBUTION



conditions were tested. In the first one, samples with a bulk $a_w$ = 0.88 were stored at 30C and 88% RH. In the second one, samples with bulk $a_w$ = 0.85, were stored at 30C with cycles of 12 hours at 85% RH and 12 hours at 88% RH.

Table 13 shows the IMF model ingredients and the composition of the solutions used for the zein and sorbic acid application on the model surface.

Preparation of surface treated samples required extreme care to: (i) maintain constant sample $a_w$; (ii) reduce microbial contamination; and, (iii) eliminate all the residual alcohol used as a solvent to apply zein and sorbic acid.

To maintain constant $a_w$, samples were kept in a constant RH chamber more that 95% of the preparation time. Air in the chamber was kept in circulation by the use of a fan. RH was controlled by the use of saturated salt solutions, $BaCl_2$ and $Sr(NO_3)_2$ for 88% and 85% RH, respectively. The chamber itself was placed in a constant temperature room.

To facilitate sample handling, individual pieces were placed on a sterile inoculation needles kept on needle holder (Figure 21).

To eliminate residual ethanol, samples were kept for at least 12 hours in the constant RH chamber after samples had been coated and after application of the preservative: sorbic acid.

Table 13

COMPOSITION OF IMF MODEL, COATING SOLUTIONS AND SORBIC ACID SOLUTIONS

| Component | Amount |
|---|---|
| 1. IMF model (1,000 g dry basis) | |
| A. water | 1050 |
| salt | 48 |
| glycerol | 59 |
| sorbitol | 193 |
| K-sorbate | 1.5 |
| B. isolated soybean protein | 260 |
| Na-caseinate | 60 |
| Ca-caseinate | 20 |
| C. hydrogenated vegetable oil | 340 |
| emulsifier | 4 |
| 2. Coating solution (100 g) | |
| zein | x |
| glycerol | 0.25x |
| Myvacet 7-00 | 1 |
| ethanol 190 proof | (balance) |
| 3. Sorbic acid solutions (100 g) | |
| sorbic acid | 10 |
| ethanol 190 proof | 90 |

Figure 21

FOOD SAMPLE HOLDER FOR ZEIN COATING, SORBIC ACID SPRAYING, SOLVENT REMOVAL AND INOCULATION

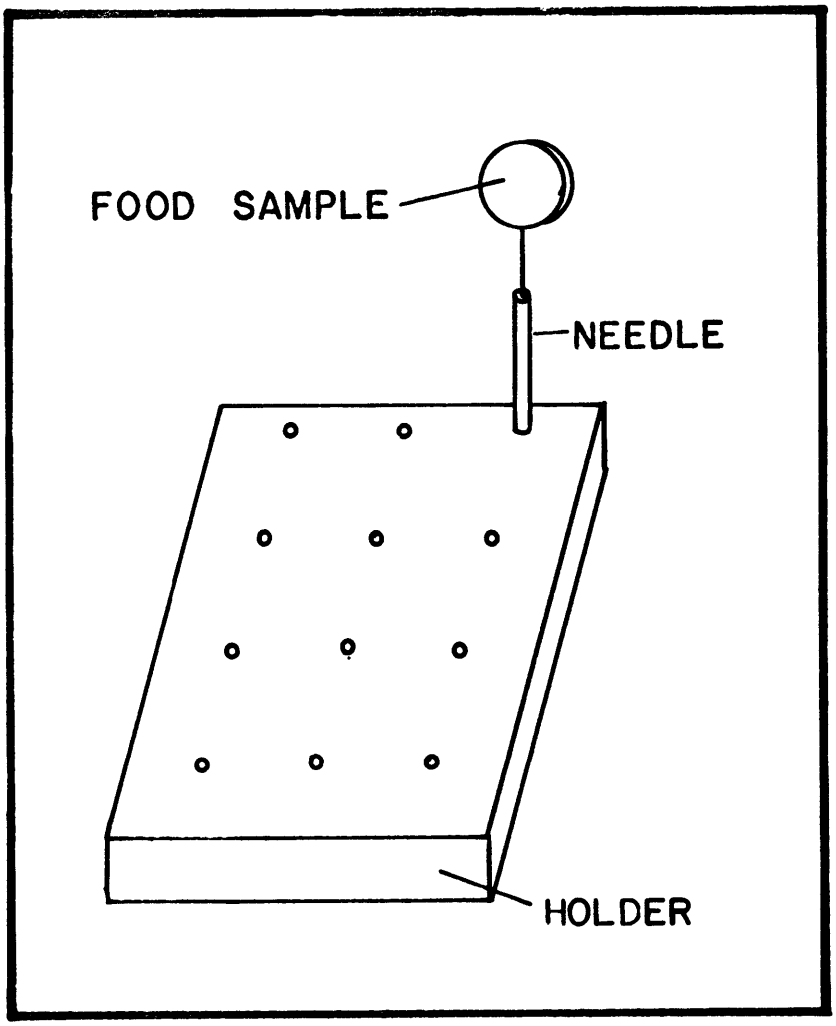
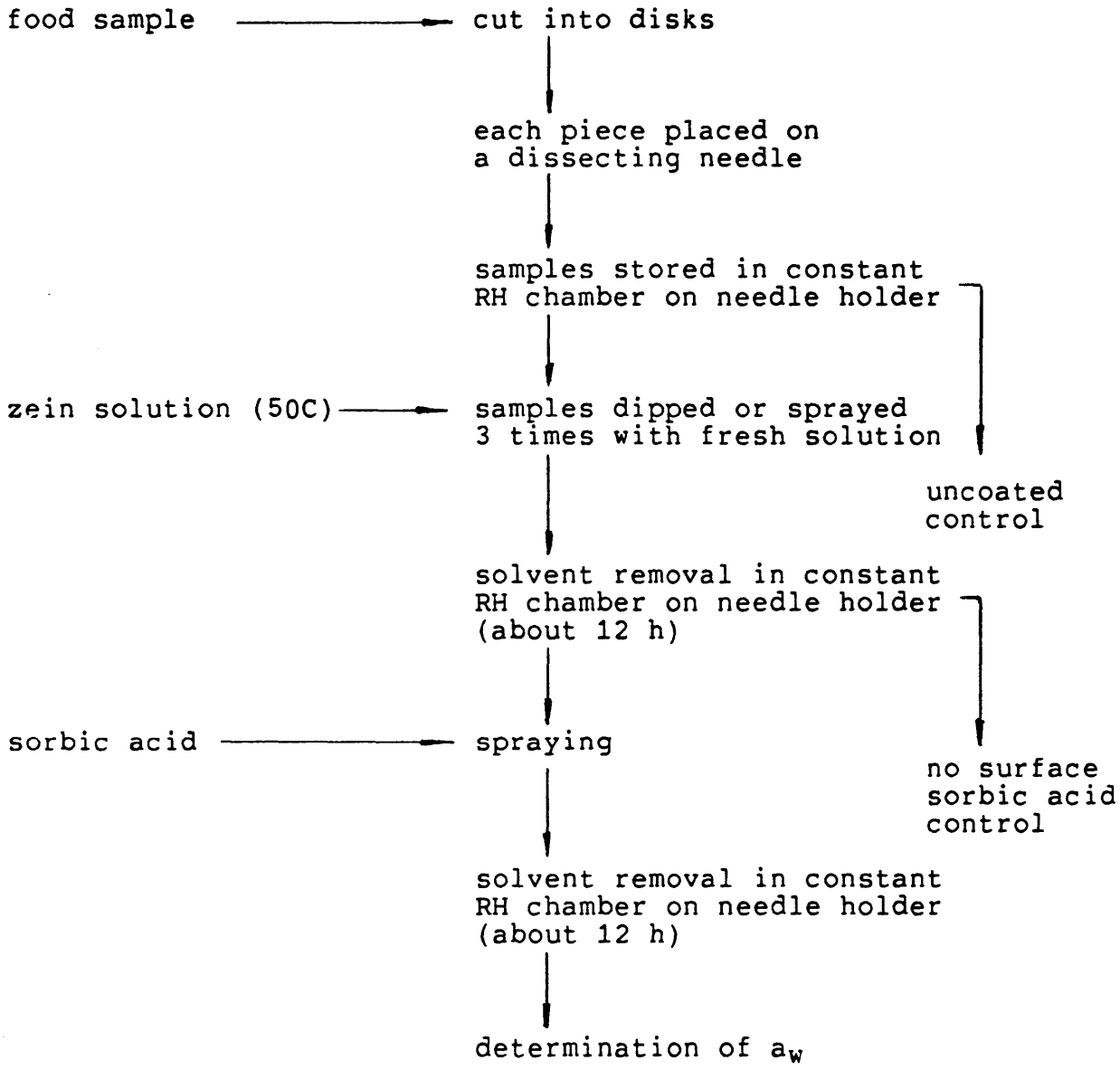


Figure 22

SAMPLE PREPARATION: CUTTING, ZEIN COATING AND SORBIC ACID SPRAYING



Experimental procedures based on the above considerations have been schematized in Figure 22. Casings were carefully removed and the cylinder thus obtained was cut into disks. Each disk was placed on a sterile dissecting needle and stored in the constant RH chamber for at least 12 hours.

Zein solutions at various concentrations in ethanol (190 proof) and constant zein:glycerol ratio (4:1) were then prepared to coat samples by dipping or spraying them three times. A fresh solution was used each time. Uncoated samples were separated for control purposes. Thereafter, coated and uncoated samples received a surface application of sorbic acid by spraying them with a 10% preservative solution in ethanol (190 proof). Again removal of residual ethanol was done by storage in the constant RH chamber for at least 12 hours. Untreated samples were kept for control purposes. Various samples were used to determine whether $a_w$ changes had occurred.

Figure 23 shows the inoculation procedure used for the microbial challenge of samples with various surface treatments. *S.aureus* S-6 stocks were kept in BHI tube slants at refrigeration temperature. Fresh cultures were prepared approximately every two weeks. An inoculation solution was prepared by transferring *S.aureus* from the slants to BHI broth. After incubation at 30C to mid-log phase (about 120 - 160 Klett units) the broth was diluted 100 times with 40% glycerol to reduce $a_w$ and cell concentration. Samples were sprayed with this cell suspension,

then placed in individual Petri dishes and stored in dessicators at 85 or 88% RH and 30C.

Sample treatments have been summarized in Table 14. These samples were used in the following studies:

a. Not inoculated samples after various surface treatments were used to determine background microbial load. Due to the nature of our tests we had to prove that it was possible to maintain the core of our samples as close as possible to sterility. Otherwise, our surface challenge studies would have no validity, i.e. outgrowth would not necessary mean that the coating had failed to enhance surface microbial stability.

b. Samples were also included to determine if it was possible to assume that the bulk of inoculated samples remained sterile and therefore cell numbers could be expressed as surface growth (i.e. cells/$cm^2$). Inoculated samples with various surface treatments and bulk $a_w$ 0.88 were stored at 30C and 88% RH. After significant growth had occurred the top layer was removed. Separate cell counts obtained for the core and surface fraction were used to determine core/surface cell numbers ratio.

c. Inoculated, uncoated samples with sorbic acid added to the surface were used as positive controls. Information on samples without added surface preservative is already available from section 4.1.3.

Figure 23

SAMPLE PREPARATION: INOCULATION PROCEDURE

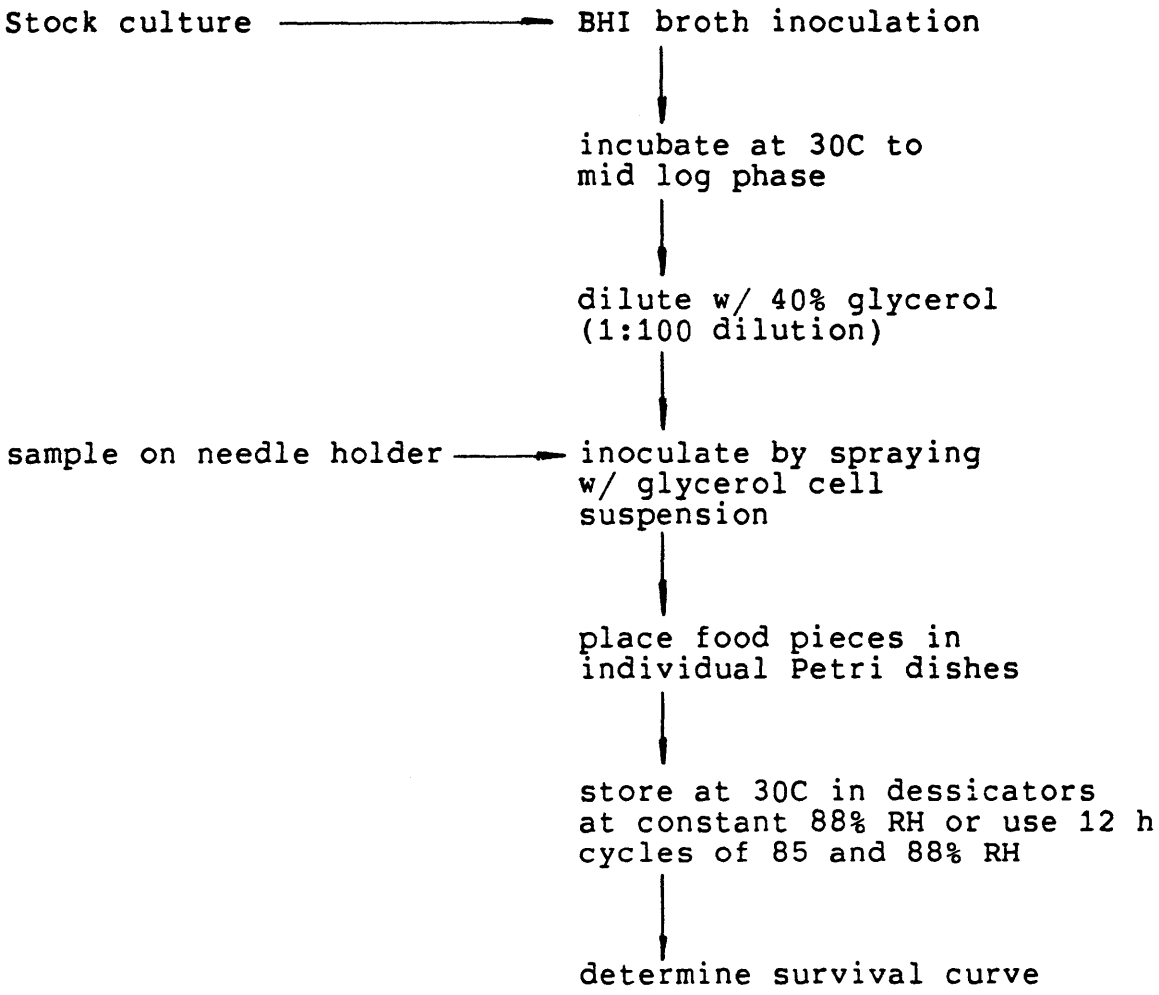


Table 14

IMF SURFACE TREATMENTS

| | |
|---|---|
| 1. Zein coating: | |
| | none |
| | coating by dipping |
| | coating by spraying |
| 2. Surface sorbic acid [1]: | |
| | none |
| | surface sprayed with 10% solution |
| 3. Inoculation: | |
| | uninoculated |
| | inoculated |
| 4. Bulk water activity: | |
| | 0.88 |
| | 0.85 |
| 5. Storage conditions: | |
| | constant RH |
| | RH cycles |

[1] All samples contained 0.1% K-sorbate in the bulk

e. Inoculated, coated samples with sorbic acid added to the surface were used to determine coating effectiveness. Two sets of experiments will be reported. In the first one, samples with a bulk $a_w$ = 0.88 were stored at 30C and 88% RH. The second one was a test on samples with bulk $a_w$ = 0.85 stored at 30C with cycles of 12 hours at 85% RH and 12 hours at 88% RH.

## 4.3 The controlled surface microenvironment pH approach

### 4.3.1 IMF model reformulations

After a theoretical analysis we concluded that it was possible to establish permanent reduced surface pH conditions if the IMF model had low total electrolyte concentration. Therefore

Table 15

LOW ELECTROLYTE IMF (IMF No. 2)

| Component | Amount, g |
|---|---|
| ISP | 26.1 |
| Caseinates | 7.9 |
| Hydrogenated vegetable oil | 34.0 |
| Emulsifier | 1.6 |
| Glycerol | 5.9 |
| Sorbitol | 47.0 |
| Sorbic acid | 0.4 |
| Water [1] | 100 to 56.2 |

[1] Moisture content reduced by air drying

### 4.3.2 Coating procedures

we had to reformulate our model as shown in Table 15 (IMF No.2). Sodium chloride was substituted by sorbitol with equivalent osmolality, while electrolytes present in the soybean isolate and caseinates were eliminated by dialysis. We had shown in Table 10

Table 16

PROTEIN FRACTION DIALYSIS REQUIREMENTS [1]

| protein concentration (g/g water)*100 | | [M] electrolytes | | equivalent permeate conductivity [4] micromho |
|---|---|---|---|---|
| IMF No.2 | Dialysis | IMF No.2 [2] | Permeate [3] | |
| 46.4 | 7.0 | <0.001 | <0.00015 | <1.5 |

[1] The objective of these calculations is to estimate permeate conductivity values based on protein concentration in the IMF model and the solution to be dialyzed (46.4 and 7.0%).
[2] Assuming that we are using a coating with 0.01 M charged groups and that the goal is a pH differential = 1
[3] Calculated using the ratio 46.4:7.0 to account for the concentration difference between IMF No.2 and dialysis solution (46.4 and 7.0%, respectively).
[4] Estimated using a NaCl standard curve.

Electrolyte removal was followed by electrical conductivity measurements. A NaCl standard curve was used to estimate electrolyte concentrations from electrolyte conductivity measurements. Measurements on the protein solutions themselves were not possible because proteins are conductive. Permeate measurements were used instead.

Table 17

λ-CARRAGEENAN COATING COMPOSITION

| Component | Amount, g |
|---|---|
| Deionized agarose | 1 |
| Deionized carrageenan | 1 |
| Sorbic acid | 1.2 |
| Propylene glycol, 40% solution | to 100 |

that significant pH differences between bulk food and treated surface were possible only when the total electrolyte concentration was kept below approximately 0.001 M. This restriction was used to estimate protein dialysis requirements as shown in Table 16.

The polyelectrolyte chosen for experimental tests was λ-carrageenan incorporated in an agarose gel as shown (Table 17). This polyelectrolyte was chosen for its solubility and high percentage of sulfate groups -- a strongly dissociated group. Agarose was chosen as the coating forming matrix for its lack of charged groups which explains why its gelling properties are independent of pH and salt concentration, important considerations in our study.

Best coatings were obtained when agarose was steamed for 30 minutes. Therefore, λ-carrageenan could not be added to the formulation until after heating. Sulfate groups can cause agarose hydrolysis as detected in preliminary experiments. Although we will show that pasteurization (2 hours at 80C) does not affect sorbic acid stability, it is safer to add it after heating. As with proteins, dialysis was used to eliminate electrolytes normally present in commercial reagent grade λ-carrageenan and agarose.

4.3.3 Microbial stability tests

Microbial challenge studies were again based on a worse situation condition. The $a_w$ of IMF model No.2 was 0.88 while bulk pH was 6.1. A standard homogeneous amount of preservative was incorporated (0.22% sorbic acid). Storage conditions were 30C and 88% RH (over saturated $BaCl_2$).

The effectiveness of a reduced surface pH was tested by inoculating the surface of coated samples with *S.aureus* S-6. As outlined in Figure 24, complementary tests included determination of pH difference between coated top and uncoated bottom, and sorbic acid stability studies using HPLC determinations.

# 5. Experimental results

## 5.1 Preliminary microbial challenge studies

### 5.1.1 Bacterial challenge studies

The possibility that λ-carrageenan could have an inherent effect on microbial growth was tested using media prepared as shown in Table 18. The variables of interest were λ-carrageenan concentration and initial pH. As indicated in Figures 25 and 26 neither one had a deleterious effect on *S.aureus*.

Challenge tests were conducted at various $a_w$ and pH levels. Results shown in Figure 27 indicate that even at the lowest $a_w$ tested increase in cell numbers were detected. However this increase may be due in part to recovery rather than growth. It is also interesting to note that stability was greatly increased by decreasing pH showing the increased effectiveness of sorbic acid.

### 5.1.2 Mold challenge studies

Results of the challenge study are summarized in Table 19. Nine Petri dishes, each containing three sample pieces, were observed for visible molding. A tenth dish was used to determine initial inoculation levels: 30-70 spores/$cm^2$ for *A.niger* and 20-30 for *A.tonophilus*. Table 19 shows that pH contributes significantly to the stability of the IMF tested. As the pH of the samples was decreased, the time required for detection of molding increased.

Figure 24

EXPERIMENTAL PROCEDURES FOR MICROBIAL STABILITY STUDIES ON THE EFFECT OF REDUCED SURFACE pH

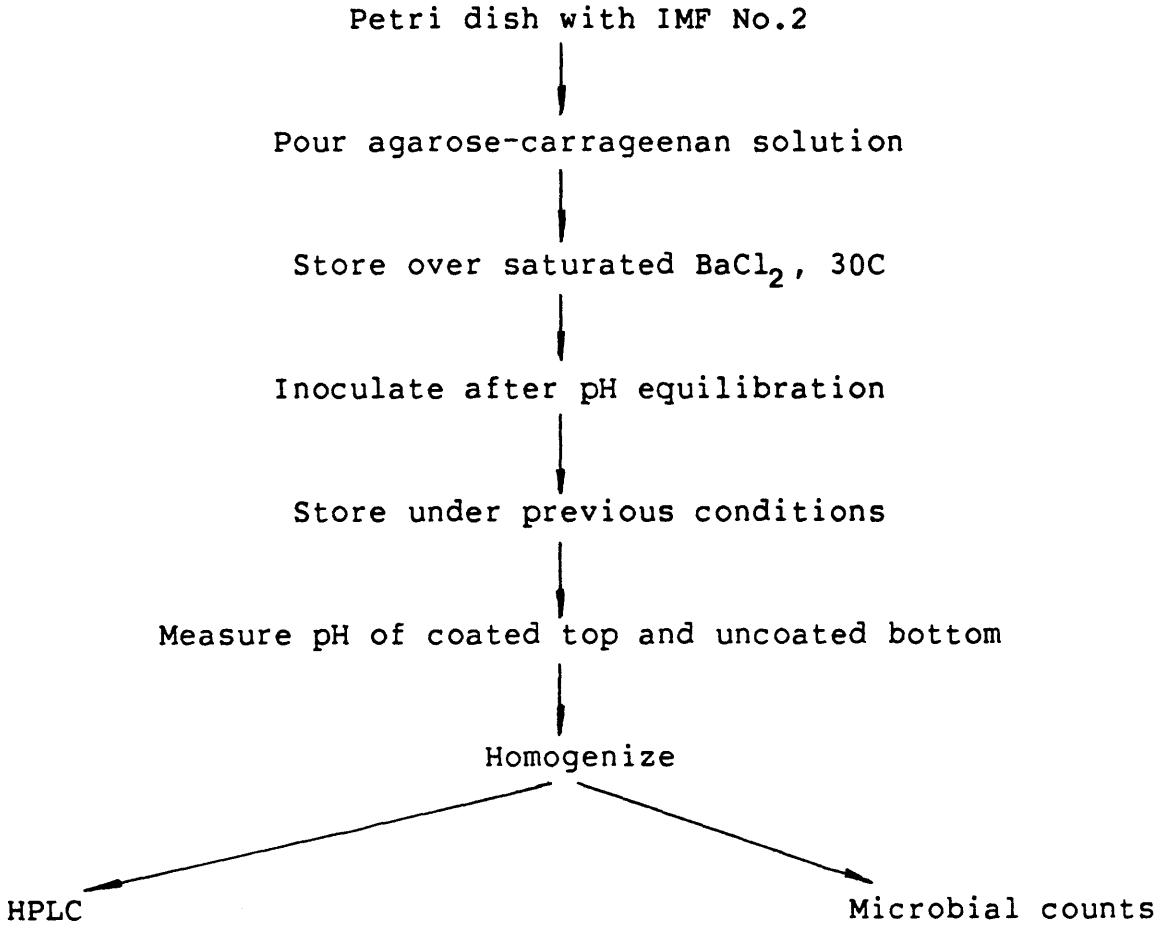


Table 18

MEDIA FOR PRELIMINARY TESTS ON THE POSSIBLE EFFECT OF λ-CARRAGEENAN ON MICROBIAL GROWTH

| Media (Results in Figure) | % carrageenan | initial pH |
|---|---|---|
| BHI (26) | 0 | 5.5 |
| | 0 | 7.3 |
| BHI + carrageenan (25) | 0.1 - 2.0 | 5.5 - 5.9 |
| | 0.1 - 2.0 | 7.2 - 7.3 |
| BHI + deionized carrageenan (26) | 1 | 5.5 |
| | 1 | 7.2 |

Figure 25

EFFECT OF λ-CARRAGEENAN ON *S.aureus* GROWTH

Experiments were ran with media prepared according to Table 18. At both pH, carrageenan concentration had no effect on *S.aureus* growth. Lines have been drawn through maximum and minimum values.

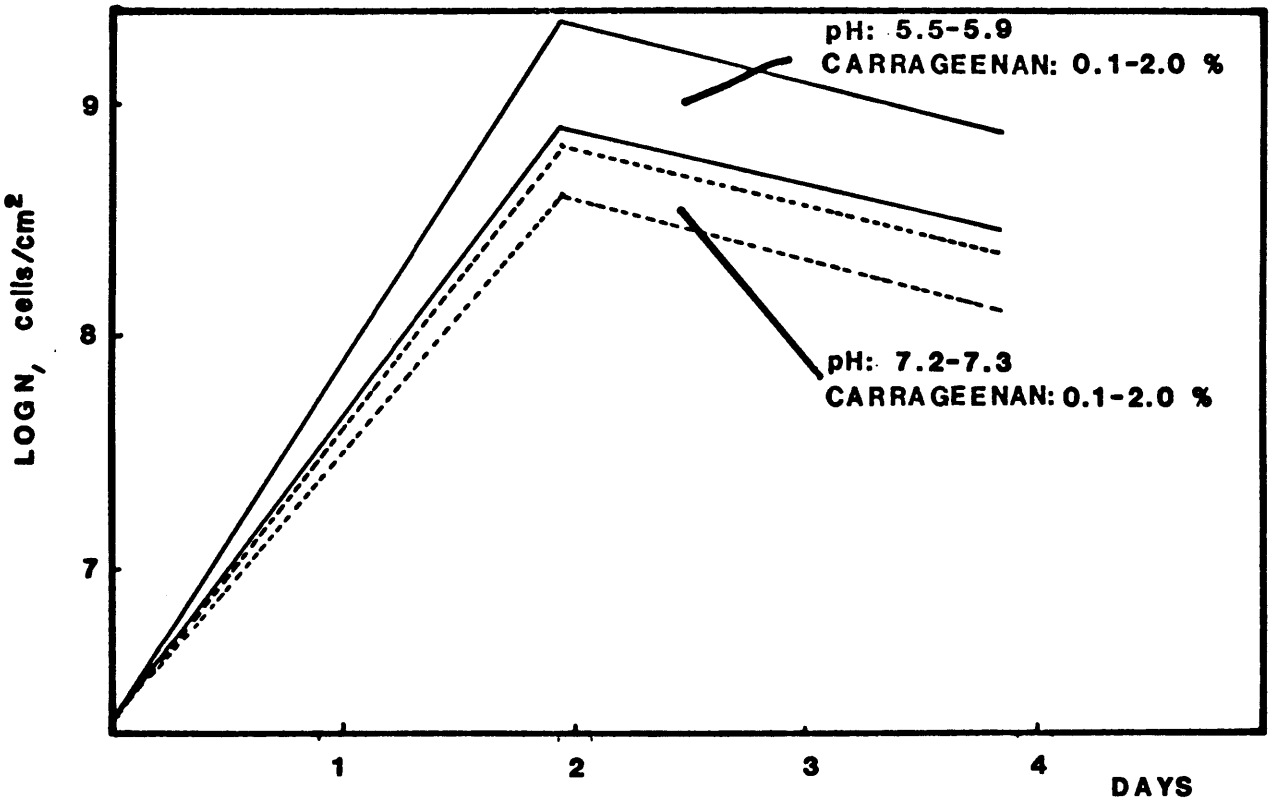


Figure 26

EFFECT OF DEIONIZED λ-CARRAGEENAN ON *S.aureus* GROWTH

Experiments were ran with media prepared according to Table 18. 1% carrageenan at pHi (initial pH) = 5.5 showed a minor growth delay, which was not further investigated.

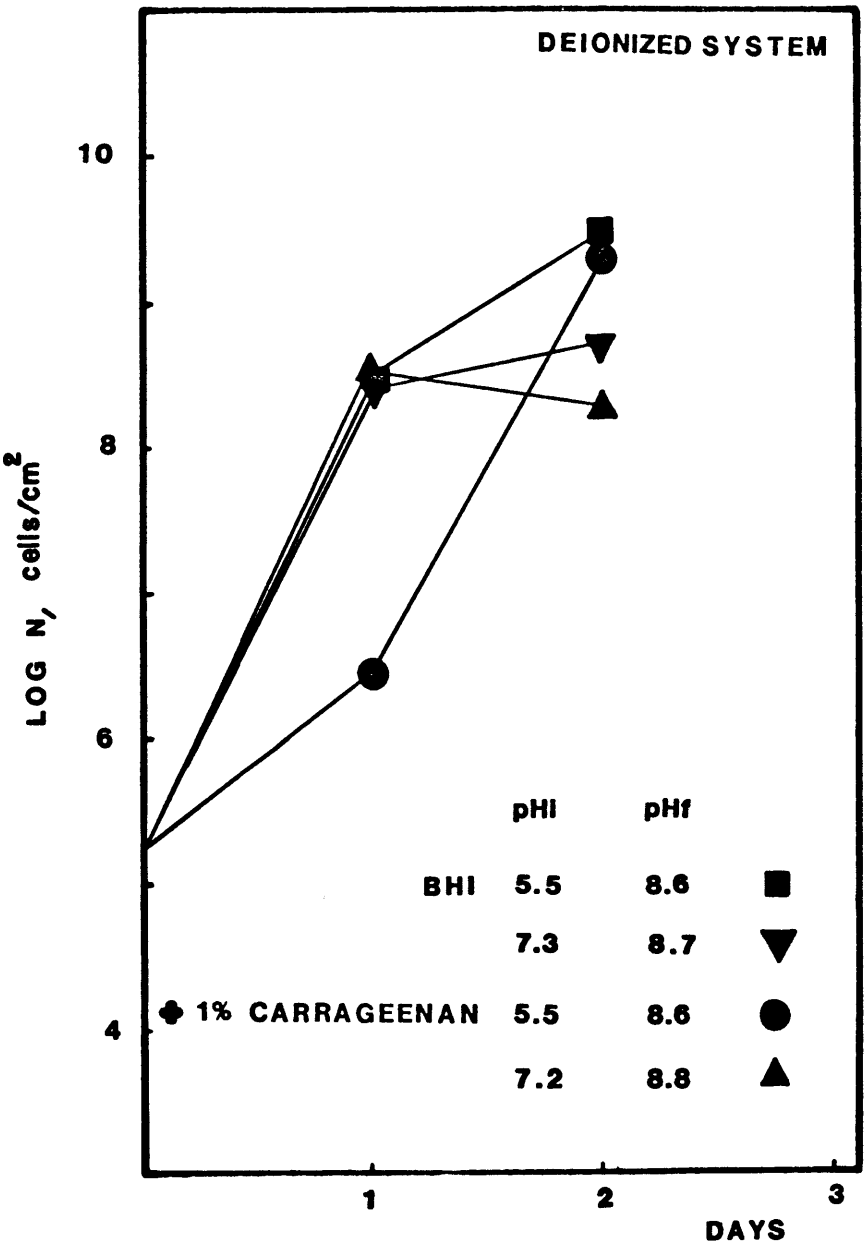
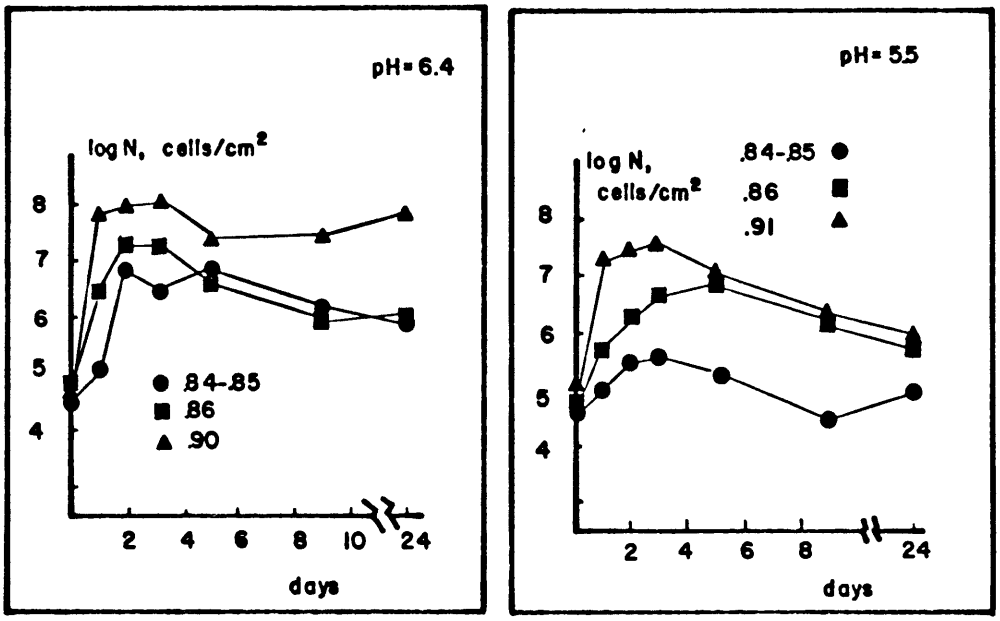


Figure 27

MICROBIAL STABILITY OF THE UNCOATED IMF (MODEL No.1)



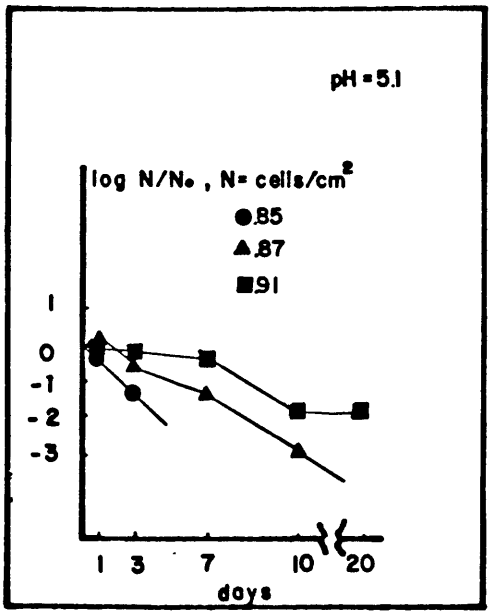



Table 19

NUMBER OF DAYS FOR MOLD GROWTH VISUAL DETECTION

| $a_w$ | pH | *A.tonophilus* | *A.niger* |
|---|---|---|---|
| 0.85 | 6.5 | 2 | 5 |
| | 6.0 | 40 | 9 |
| | 5.5 | >220 | 40 |
| 0.83 | 6.5 | 12 | 12 |
| | 6.0 | >220 | 15 |
| | 5.5 | >220 | >220 |

5.1.3 Conclusions

From both challenges it could be concluded that the effect of pH on stability is highly significant. In practice, however, substantial pH decrease is not possible, due to loss of acceptable taste and texture (Motoki et al., 1982).

5.2 The reduced preservative diffusion approach

5.2.1 Permeability experiments

It was very difficult to obtain good reproducibility within a particular treatment with coatings, even though results with uncoated cellulose gave good reproducibility. This did not

invalidate conclusions based on "order of magnitude" differences. All results obtained were summarized in Table 20. Specific sample preparation conditions have been outlined in Appendix E.

Best values were obtained for zein films. These determinations were repeated several times, with slight experimental modifications. Particularly, the experiment was repeated using different sorbic acid concentrations. As shown in Figure 28, a slight concentration dependence was observed. Based on these results and as indicated in Appendix D we showed that zein films had preservative diffusion 1,000 times smaller than the uncoated cellulose film.

### 5.2.2 Sorbic acid stability test

Table 21 shows that initial preservative affected pH of the model system giving values in the 6.3 to 6.8 range. No attempt was made to correct for this pH variation.

Sorbic acid determination expressed as percentage of initial concentrations are shown in Appendix F and summarized in Figure 29. Surface growth at 0.57 and 1.06% K-sorbate have been plotted in Figure 30. At the higher concentrations, 2.10 and 4.24%, total counts per sample remained below detectable levels during the length of the test (about 40 days).

Table 20

SUMMARY OF K-VALUES FOR VARIOUS COATING MATERIALS AS DETERMINED BY A DIFFUSION CELL

| Coating material | Initial concentration differential, (mg/ml) | K-values $x10^4$ $(mg/hr\ cm^2)/(mg/ml)$ |
|---|---|---|
| uncoated cellulose | 10 | 470 ± 10 |
| cellulose coated with [1]: | | |
| zein [2] | 7.9 - 110.2 | 3.4 ± 2.2 |
| gelatin hot melts | 100 | 170<br>210<br>210 |
| caustic amylose coagulated with ammonium sulfate | 100 | 80 (HVII-20-40)<br>90 (HVII-20-SA)<br>120 (HVII-20G-40) |
| caustic amylose coagulated by acid salt mixture | 100 | 120 (HVII-20-S)<br>110 (HVII-20G-PS)<br>140 (HVII-20G-S) |
| low DS amylose ester | 100 | 260 (dipped)<br>320 (sprayed) |

[1] specific coating compositions are given in Appendix E.
[2] individual values have been plotted in Figure 28

Figure 28

VARIATION OF K-VALUES AS A FUNCTION OF INITIAL K-SORBATE CONCENTRATION DIFFERENCE

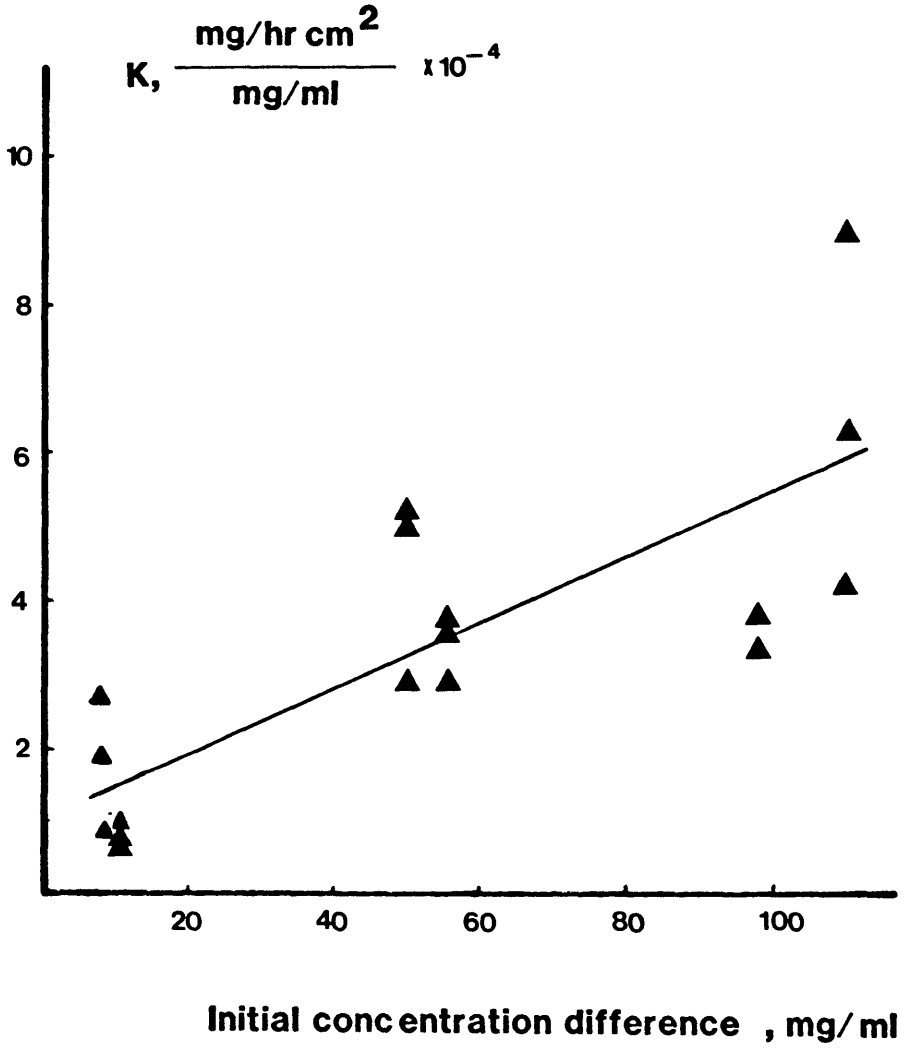


Table 21

SORBIC ACID STABILITY STUDY, INITIAL CONCENTRATIONS

| pH [1,4] | % K-sorbate at time 00 [2] | %K-sorbate at time 0 [3,4] |
|---|---|---|
| 6.3 ± 0.06 (n=4) | 0.55 | 0.57 |
| 6.5 ± 0.03 (n=4) | 0.95 | 1.06 ± 0.11 (n=2) |
| 6.6 ± 0.02 (n=4) | 1.98 | 2.10 ± 0.07 (n=2) |
| 6.8 ± 0.04 (n=4) | 4.02 | 4.24 ± 0.03 (n=2) |

[1] corresponds to pasteurized and not pasteurized samples which showed no significant differences
[2] time 00 denotes time 0 before pasteurization
[3] time 0 denotes time after pasteurization and moisture equilibration. During sample preparation, moisture removal was stopped before reaching final desired value. Final moisture conditions were then achieved by storage over saturated $BaCl_2$ solution. This explains the slightly lower values for unpasteurized samples.
[4] n = number of replicates

Results shown in Figure 29 indicate that sorbic acid is stable under testing conditions. After approximately 40 days storage at 37C and 88% RH, in the dark, losses of less than 25% were detected at all four concentrations tested. Moreover, there is no initial concentration effect on preservative stability.

Figure 29

SORBIC ACID DETERMINATION, PERCENTAGE OF INITIAL CONCENTRATION

Except for controls, all samples were inoculated with $10^6$ to $10^7$ cells/$cm^2$. Controls were either pasteurized [□] or not [△]. Initial concentrations, 0.57 to 4.24%, were determined after pasteurization and moisture equilibration. This is a summary of the values reported in Appendix F, by drawing lines thru average points.

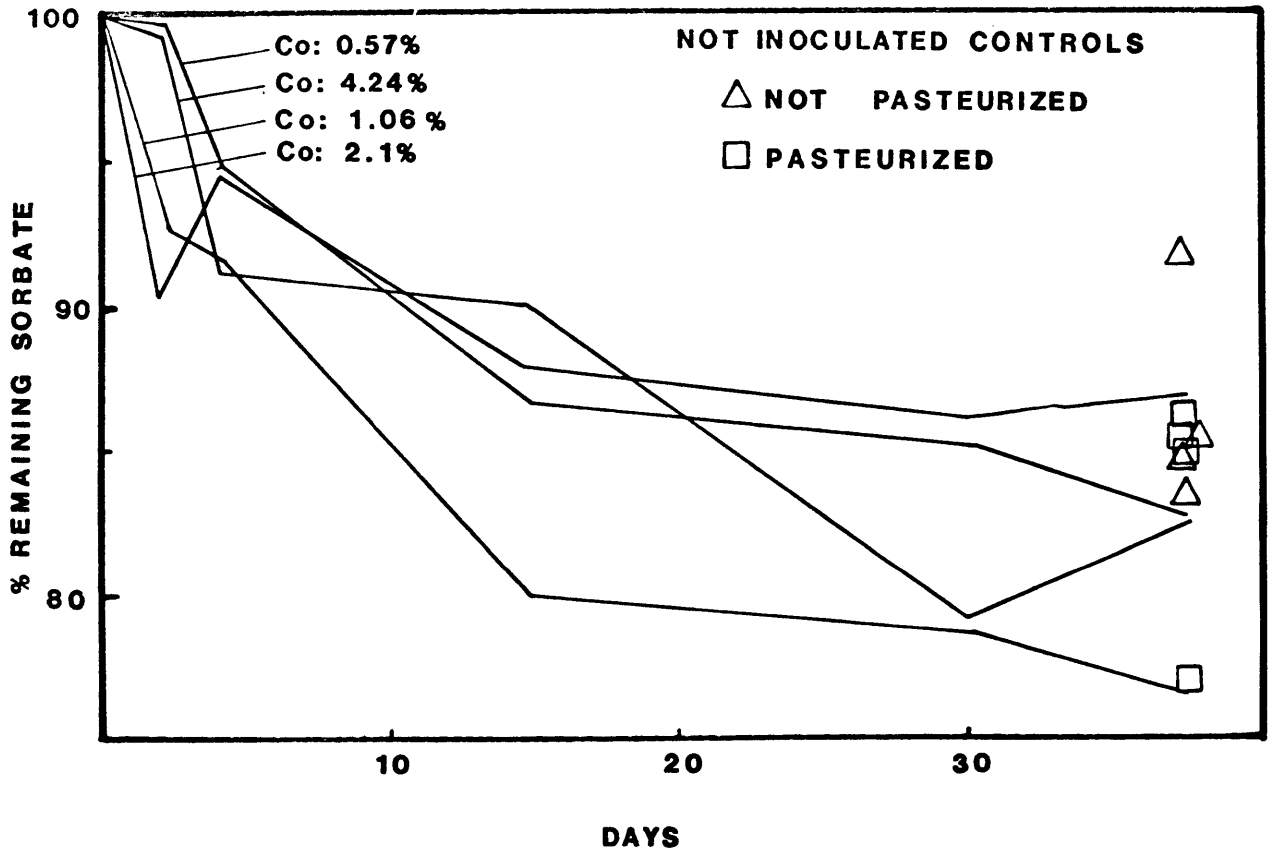
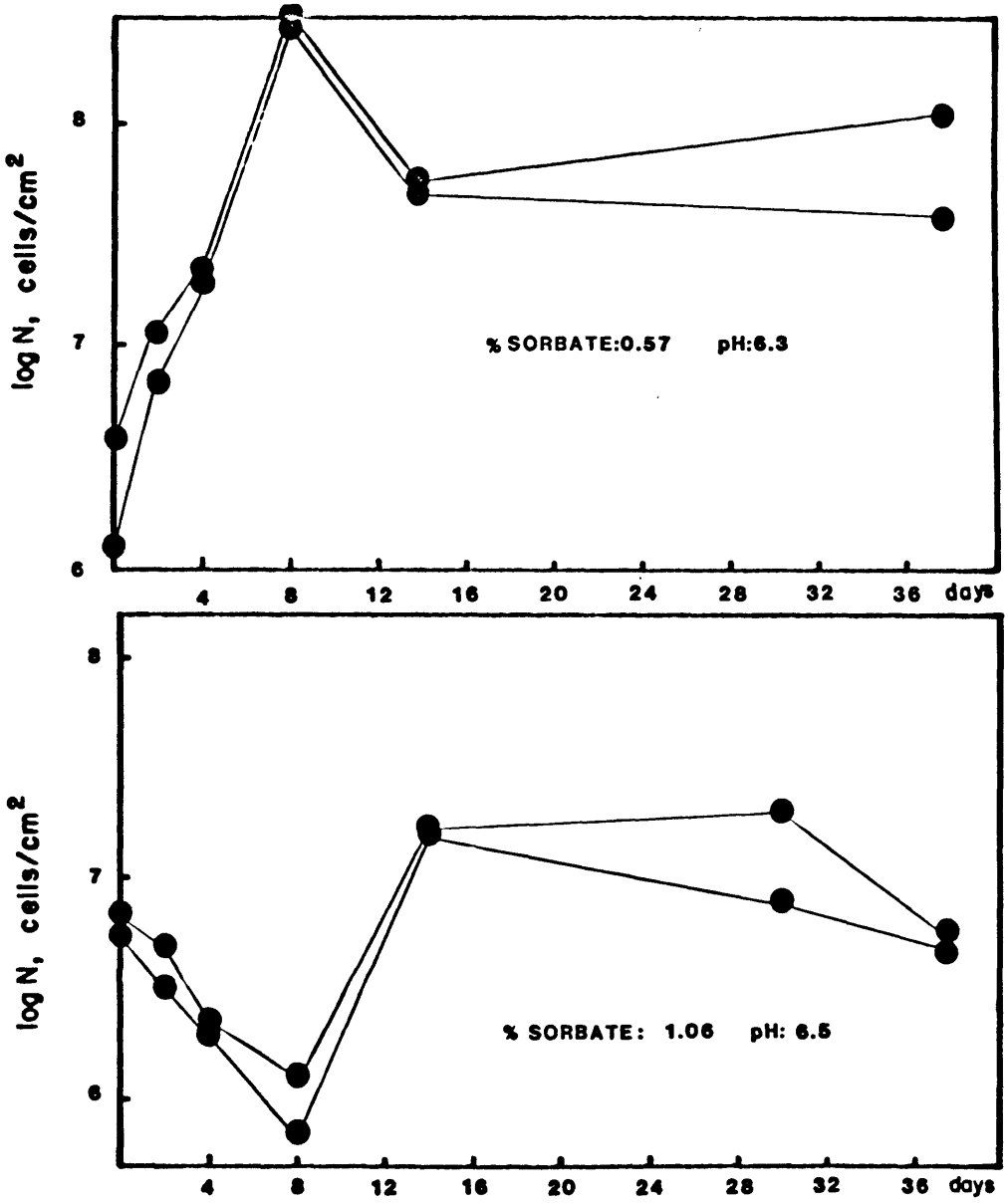


Figure 30

MICROBIAL GROWTH RESPONSE AT HIGH K-SORBATE CONCENTRATIONS




Experiment was ran in duplicates. Lines have been drawn through maximum and minimum values. Samples with higher sorbic acid concentrations, inoculated at the same level were bactericidal. No viable counts were detected after inoculation.

Table 22

EFFECT OF PASTEURIZATION ON SORBIC ACID STORAGE STABILITY

REMAINING %SORBIC ACID AFTER 38 DAYS STORAGE AT 37C, RH = 87.5%

| %K-sorbate | Non-pasteurized | Pasteurized [1] |
|---|---|---|
| 0.57 | 84.7 | 84.7 |
| 1.06 | 83.4 | 84.7 |
| 2.10 | 91.8 | 86.0 |
| 4.24 | 84.7 | 76.8 |
| | 86.1 $\pm$ 3.8 | 83.1 $\pm$ 4.2 |

[1] 2 hours in a 80C water bath

As shown in Table 21, heat treatment (2 hours at 80C) resulted in no detectable sorbic acid losses. Moreover, it had no effect upon storage (Table 22).

Finally, we compared uninoculated with inoculated samples to determine if presence of *S.aureus* plays a role in sorbic acid degradation. Growth response was a function of initial sorbate concentration. At 0.57%, significant outgrowth was detected, at 1.06%, initial decrease with later growth were observed (Figure 30), while 2.10 and 4.24% were lethal. None of

these behaviors had an effect on sorbic acid stability (Figure 29).

### 5.2.3 Sorbic acid distribution studies

#### 5.2.3.1 Microscopy studies

Photomicrographs in Figure 31 show a continuous coating with minimal thickness variation. Thickness measurements show that each application resulted in similar thickness increments.

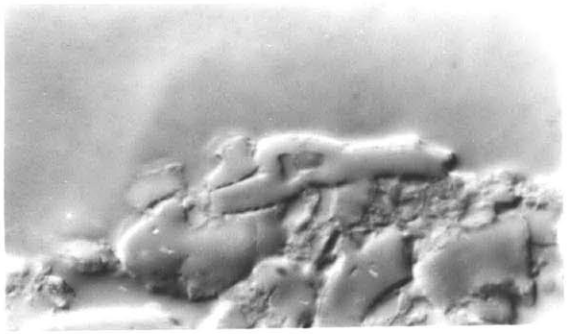
#### 5.2.3.2 Sorbic acid determinations

Sorbic acid determinations showed variation in the amount of sorbic acid deposited on each individual sample as shown in Table 23. Therefore data were normalized for analysis. Sorbic acid core concentrations were divided by the total amount deposited on each individual piece. These normalized values were then plotted as a function of sampling time and surface treatment.

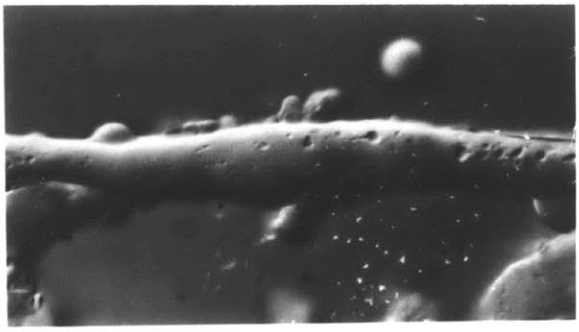
As shown in Figure 32 zein coatings reduced sorbic acid core concentrations significantly. This represents the rate reduction in the process of diffusion of sorbic acid deposited on the surface of these samples into the food core, due to the barrier properties of zein films. Moreover, the thickness, i.e. the number of zein spray applications had also a strong effect. A more quantitative analysis will be presented in the following paragraph.

Figure 31

NOMARSKY MICROSCOPY STUDIES

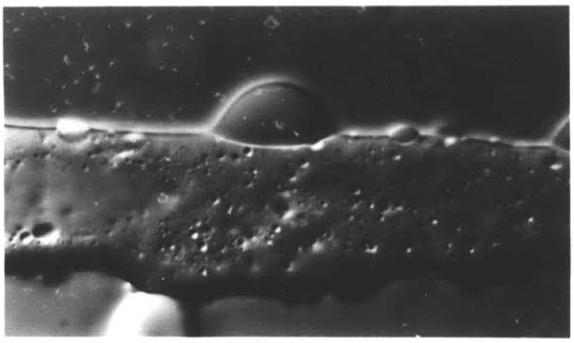


a. Uncoated control (0X)



12.0 ± 2.3 microns

b. One zein spray application (1X)



26.5 ± 0.9 microns

c. Two zein spray applications (2X)

38.0 ± 1.8 microns

d. Three zein spray applications (3X)

Table 23

SORBIC ACID DEPOSITED ON EACH INDIVIDUAL SAMPLE PIECE

| Time, h | Pooled samples [1,3] mg | Recovery controls [2] mg |
|---|---|---|
| 2 | 18.9 ± 6.8 (n=5) | - |
| 18 | 22.6 ± 5.3 (n=6) | - |
| 44 | 17.7 ± 2.1 (n=6) | 13.7 |
| 68 | 15.4 ± 6.1 (n=6) | 14.9; 16.4 |
| 118 | 20.0 ± 3.9 (n=5) | 12.9; 15.0 |
| 168 | 21.5 ±10.2 (n=5) | 13.6 |
| 228 | 14.8 ± 4.0 (n=6) | 12.3; 9.1 |
| | 18.6 ± 6.1 (n=39) | |

[1] Corresponds to uncoated and coated samples
[2] These controls were included to determine if losses occurred when separating samples into a core and a surface fraction.
[3] n = number of samples

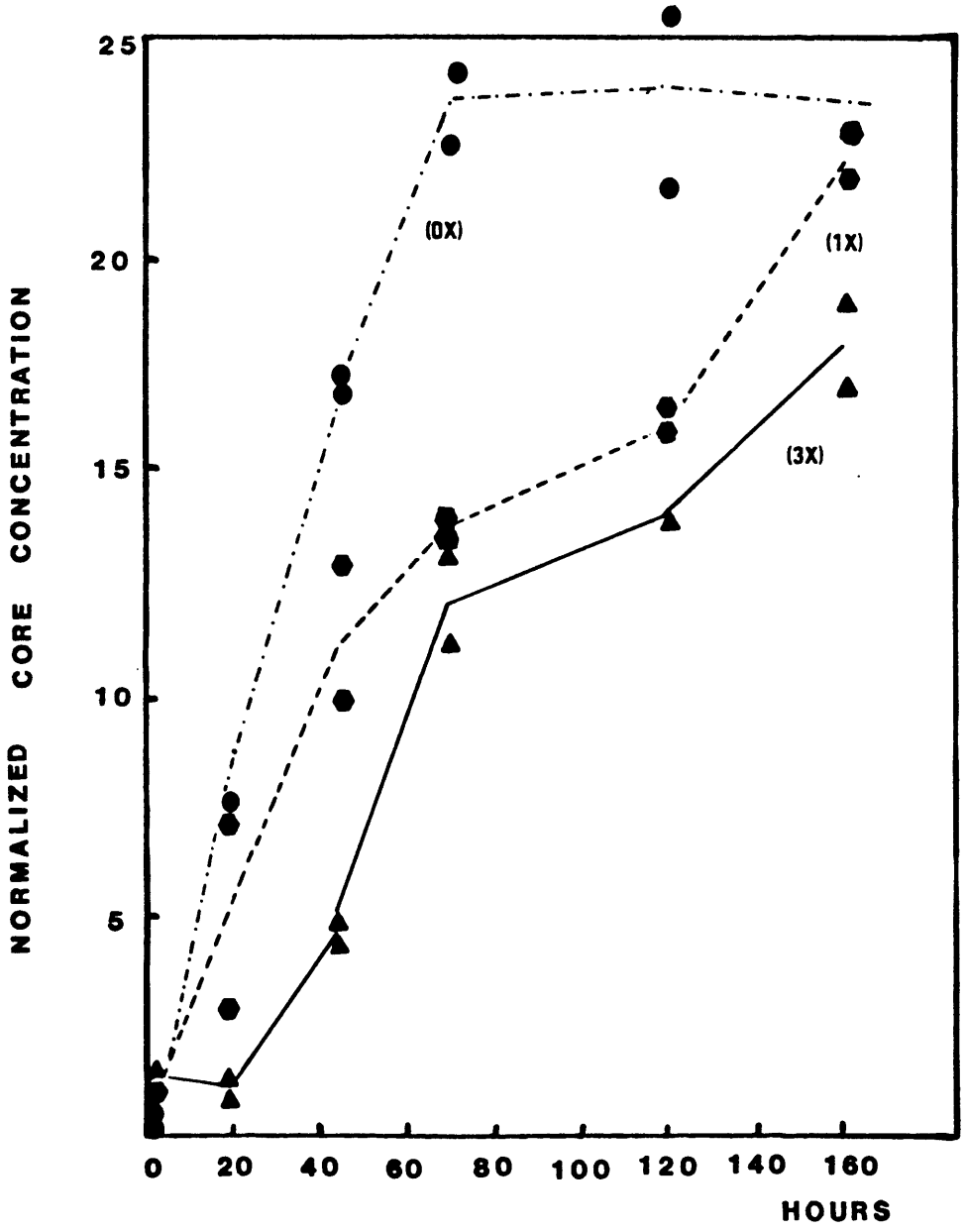
Apparent diffusion coefficients for sorbic acid in the food model and zein coating were evaluated using data from Figure 32. As shown in Appendix H, the average values were ($1.0 \pm 0.1 \times 10^{-6}$, n = 6), ($3.3 \pm 0.7 \times 10^{-9}$, n = 5) and ($6.8 \pm 0.9 \times 10^{-9}$, n = 6) $cm^2/sec$ for samples 0X, 1X and 3X.

The value obtained for the uncoated IMF model, $1 \times 10^{-6}$ $cm^2/sec$, agrees well with the one obtained by Guilbert *et al*

Figure 32

SORBIC ACID DISTRIBUTION STUDIES

Effect of coatings on normalized core concentrations defined as core concentrations divided by total amount deposited on each individual piece. Experiment was ran in duplicates, lines have been drawn through average values.



(1983). Their value, determined in an IM agar model at the same $a_w$, 0.88, was $2.0 \times 10^{-6}$ $cm^2/sec$.

The calculated $D_f/D_c$ ratios were 300 (1X) and 150 (3X), compared with values of 1,000 with respect to cellulose film measured with the permeability cell. The difference may be due to several factors. The surface of the IMF model is rich in topographical features such as pores, valleys, etc. which makes it more difficult to cover than cellulose. Second, the assumptions made to analyze Figure 32, unidimensional diffusion and core concentration measured representing the situation in the piece center, introduce error leading to lower D values. The values are however quite comparable given the orders of magnitude of diffusion reduction with respect to cellulose.

### 5.2.4 Microbial challenge tests

#### 5.2.4.1 Background microbial load

Background microbial load tests for various surface treatments have been summarized in Table 24. Cell counts did reach detectable counts after approximately a week storage for uncoated samples and only much later (2 to 3 weeks or more) for coated samples. The difference seems to indicate that sample cores were free of contaminants and that surface contamination occurred during sample preparation and/or sample removal. Since the surface of coated samples had enhanced growth inhibiting

Table 24

MICROBIAL BACKGROUND LOAD TESTS
NUMBER OF DAYS BEFORE VARIOUS NOT-INOCULATED
SAMPLES REACHED DETECTABLE COUNTS [1]

| Treatment [2] | days |
|---|---|
| Uncoated | 6.5 (or less?) |
| Uncoated w/.2% sorbic acid added as dip | 6.5 (or more) |
| Coated w/ 3 20% zein solution dips w/.2% sorbic acid added as spray | 16 |
| coated w/ 3 12% zein solution dips w/.2% sorbic acid added as spray | 16 |

[1] About 1,000 cells/$cm^2$
[2] All samples were stabilized with 0.1% K-sorbate. Some samples received a surface application equivalent to an additional 0.2%. Bulk $a_w$ was 0.88 and storage conditions were 88% RH and 30C.

properties, it is to be expected that they will have lower counts for longer periods of time.

5.2.4.2 Location of microbial growth

Coated and uncoated inoculated samples that had shown 3 to 4 log cycles increases above inoculation level were used to

Table 25

LOCATION OF GROWTH TESTS
COMPARISON OF SURFACE AND CORE FRACTION COUNTS [1]

| Treatment | Surface counts x $10^7$ | Core counts x $10^5$ | % total counts |
|---|---|---|---|
| uncoated | 6.7 | 2.6 | .40 |
| uncoated w/ .2% sorbic acid as dip | 5.6 | 0.14 | .03 |
| coated w/8-12-20% zein as dips w/ .2% sorbic acid as dip | 24.0 | 140 | 5.80 |
| coated w/3 12% zein as dips w/ .2% sorbic acid as dip | 16.0 | 25 | 1.60 |

[1] samples taken after 11 days storage at 30C and 88% RH. Samples were peeled to separate a surface and a core fraction, a slightly more difficult procedure for coated samples. All samples contained 0.1% K-sorbate. Some samples received a surface application equivalent to an additional 0.2%.

determine if growth was occurring only on the surface of food samples. Table 25 shows that it is safe to assume that growth is limited to sample surfaces. Significant cell numbers were detected in the core, but they represent an experimental artifact. It is very difficult to separate a sterile core from a

heavily contaminated surface without cross-contamination. This operation was slightly more difficult for coated samples.

5.2.4.3 Determination of coating effectiveness

a. Tests at high relative humidity

These tests correspond to samples with bulk $a_w$ = 0.88 and stored at 30C and constant 88% RH. To allow for comparisons between different treatments a stability limit was defined as follows: samples are no longer considered stable if cell counts are one cycle above inoculation level.

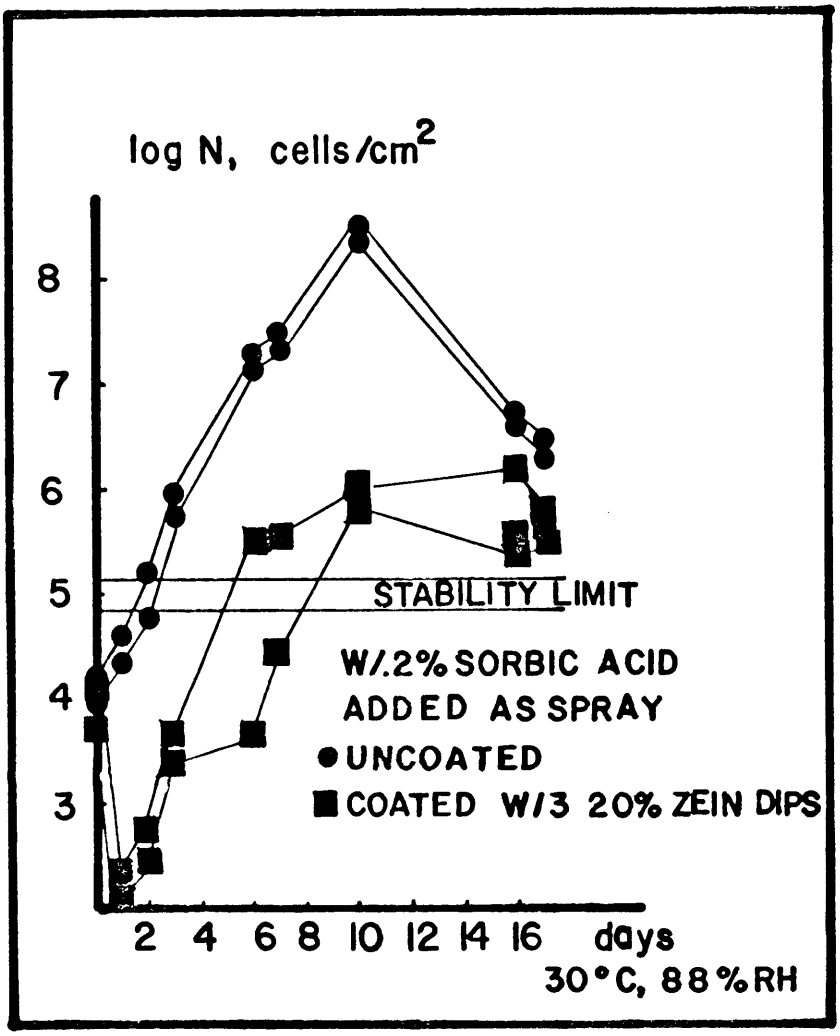
At this point it is important to mention that cell numbers, reported as time 0 inoculation levels, were obtained about 4 hours after inoculation. That is why, inoculation levels for effective surface treatments, those that even show initial bactericidal effect, are lower than those obtained for positive controls. That is also why we have chosen as initial inoculation values those measured for positive controls.

Figure 33 shows the effectiveness of samples coated by dipping in 20% zein solutions. Stability period can be estimated as being between 5 and 8 days. There is also a significant bactericidal effect during the initial storage period.

Figure 34 shows the stability tests of samples coated by spraying them 3 times with 12% zein solutions. Stability period has increased to about 10 to 16 days.

Figure 33

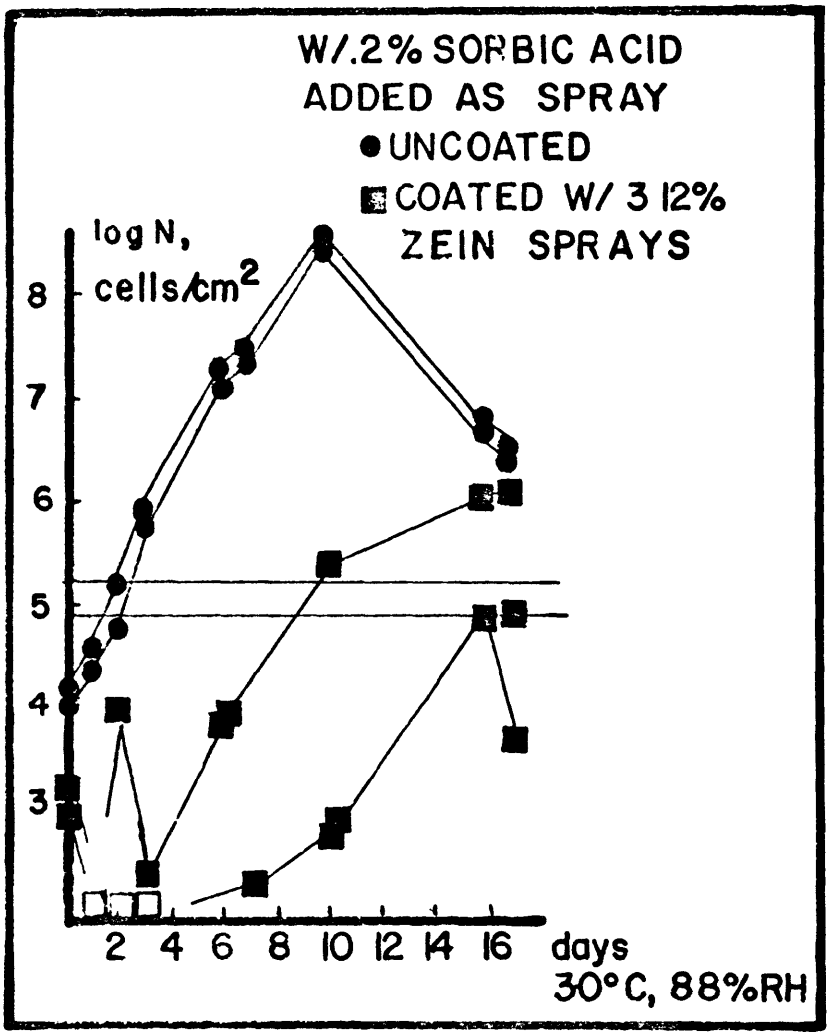
STABILITY TEST: ZEIN DIPPED COATED SAMPLES, BULK $a_w$ = .88, CHALLENGED WITH *S.aureus* (CONSTANT RH)




Samples were taken in duplicates or triplicates. Empty symbols are used to indicate whenever no counts were detected, <1,000 counts/cm$^2$. Lines have been drawn through maximum and minimum values. The two parallel lines represent the stability limit defined in the text.

Figure 34

STABILITY TEST: ZEIN SPRAYED COATED SAMPLES, BULK $a_w$ = 0.88, CHALLENGED WITH *S.aureus* (CONSTANT RH)




Samples were taken in duplicates or triplicates. Empty symbols are used to indicate whenever no counts were detected, <1,000 counts/$cm^2$. Lines have been drawn through maximum and minimum values. As indicated in Figure 33, the two parallel lines represent the stability limit defined in the text.

b. Tests with cycles of low and high relative humidity

These tests correspond to samples with bulk $a_w$ = 0.85 stored at 30C and exposed to cycles of 12 hours at 85% RH and 12 hours at 88% RH. Figure 35 shows the effectiveness of samples coated with 20% zein dips, a repetition of preparation conditions reported in Figure 33. The only difference was that storage conditions tested were less demanding. The change in bulk $a_w$ and storage RH conditions resulted in an increased stability period: 10 to 15 days.

Figure 36 shows the stability test of samples sprayed with 12% zein stored under the less demanding conditions. In this case the stability period seems to be longer than, or about the length of the testing period, 28 days.

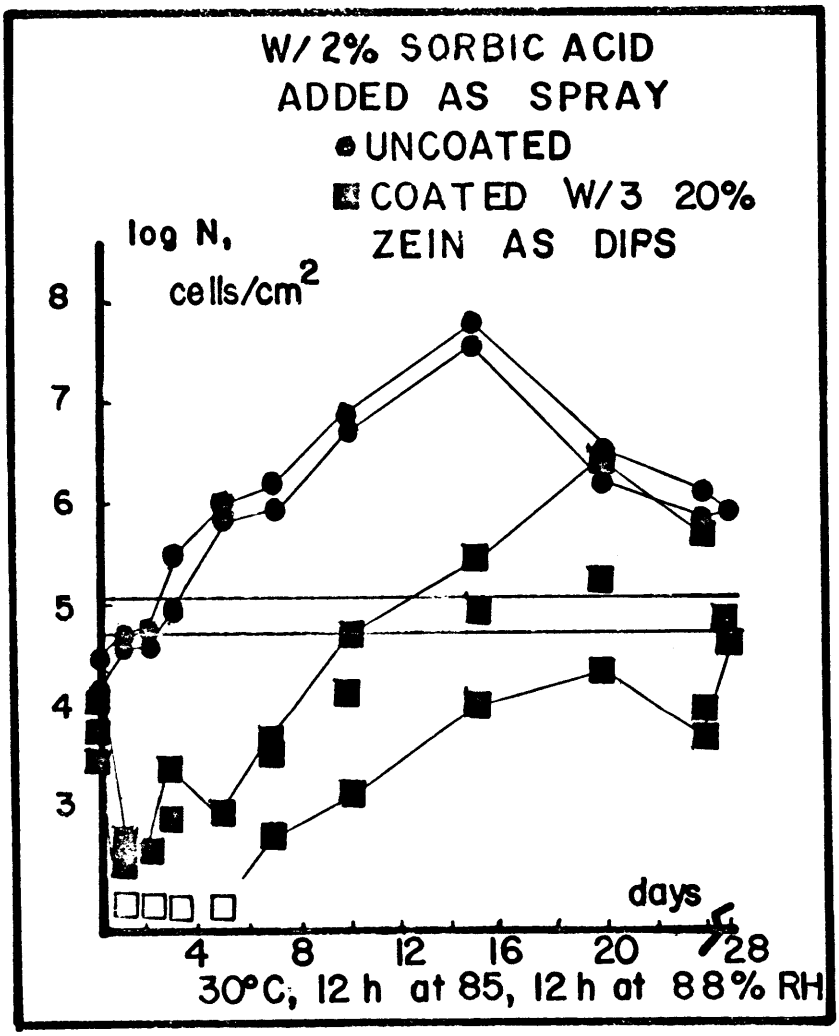
5.2.5 Conclusions

Improved microbial stability was achieved when zein coatings were applied to the IMF model. This result is consistent with predictions based on diffusion cell experiments and sorbic acid distribution studies.

At this point it is important to emphasize that the microbial response to the surface environment can not be predicted with accuracy. An attempt to describe the surface environment has been schematized in Figure 37, where we show the highly dynamic conditions faced by microorganisms. We have a

Figure 35

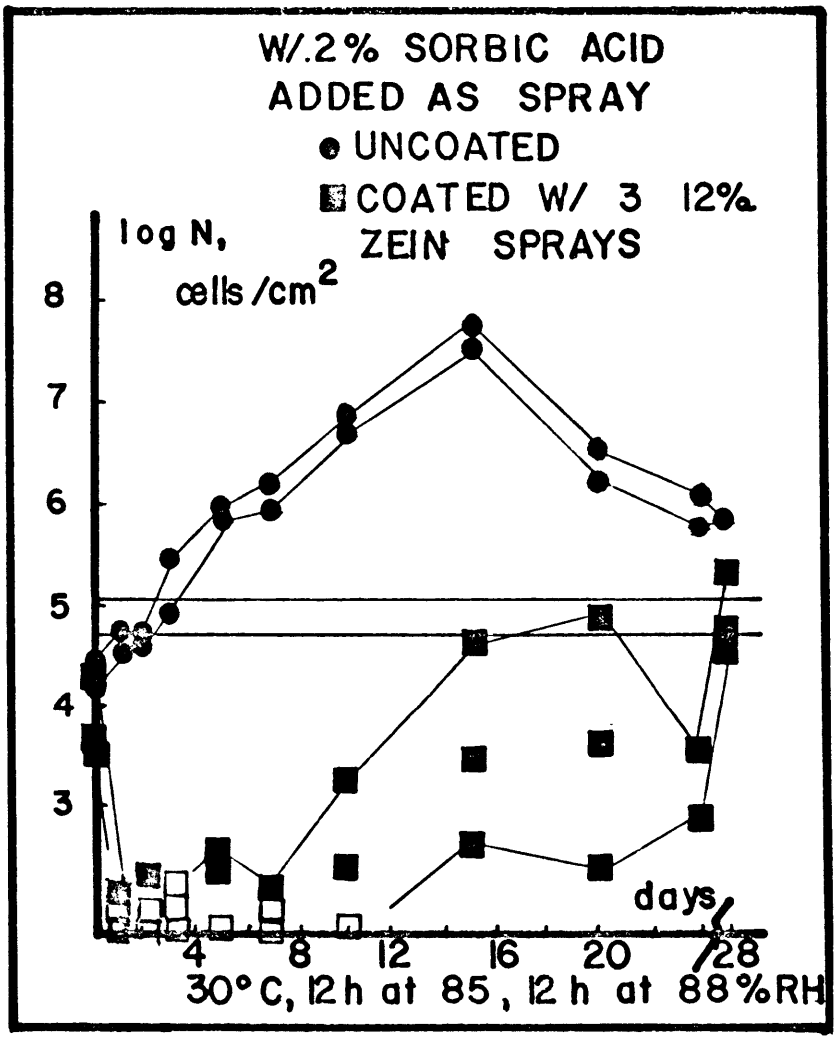
STABILITY TEST: ZEIN DIPPED COATED SAMPLES, BULK $a_w$ = 0.88, CHALLENGED WITH *S.aureus* (RH CYCLES)




Samples were taken in duplicates or triplicates. Empty symbols are used to indicate whenever no counts were detected, <1,000 counts/cm$^2$. Lines have been drawn through maximum and minimum values. As indicated in Figure 33 the two parallel lines represent the stability limit defined in the text.

Figure 36

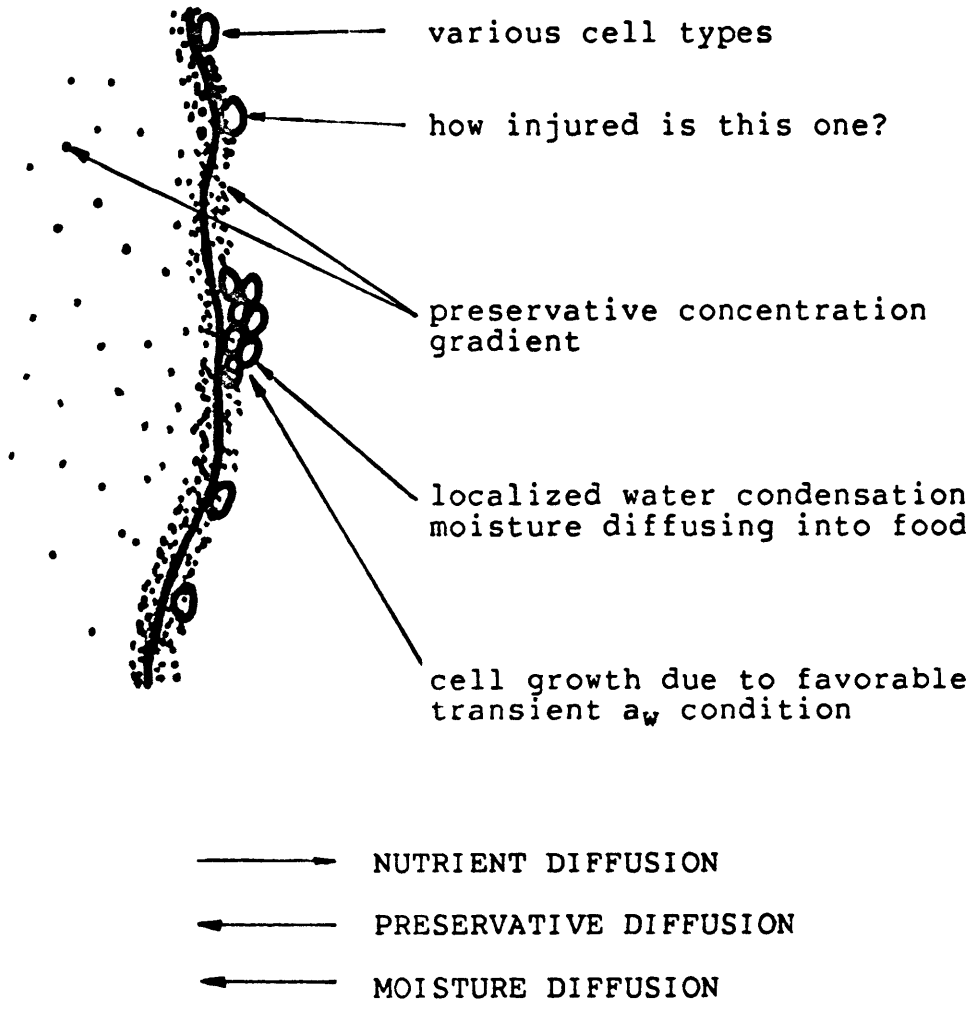
STABILITY TEST: ZEIN SPRAYED COATED SAMPLES, BULK $a_w$ = 0.88, CHALLENGED WITH *S.aureus* (RH CYCLES)




Samples were taken in duplicates or triplicates. Empty symbols are used to indicate whenever no counts were detected, <1,000 counts/cm$^2$. Lines have been drawn through maximum and minimum values. As indicated in Figure 33 the two parallel lines represent the stability limit defined in the text.

Figure 37

SCHEMATIC REPRESENTATION OF THE HIGHLY DYNAMIC AND HETEROGENEOUS CONDITIONS ON THE SURFACE OF A COATED FOOD



preservative concentration gradient, nutrients diffusing from food bulk to surface and water diffusing into the food after an event of localized condensation. Moreover, the physiological status of the microorganism will depend on the history of their locations. Therefore, a precise mass transfer model could not predict microbial stability. The final test of a potentially effective coating had to be the microbial challenge with a microorganism of interest.

Further analysis of these microbial tests allowed the following statements:

a. Spraying of zein solutions on food samples is a more effective treatment than dipping. The exact nature of this difference was not determined.

b. Microbial test showed large differences between individual values. The cause behind this variation has been traced to variability in the amount of sorbic acid deposited on coated surfaces as shown in Appendix G.

c. Samples with bulk $a_w$ = 0.88 and pH = 6.4, stored at constant 88% RH and 30C represent extreme testing conditions. Such conditions were chosen to accelerate the obtention of results. Samples with lower bulk $a_w$, 0.85, challenged with cycles of low and high RH, 85 and 88% RH, cycles of 12 hours, gave significantly longer stability periods. This cycling test is closer to situations found in commercial distribution of

IMF's. Unfortunately, no model is available to extrapolate from these results, the consequences of other abuse conditions. Only qualitative comments are possible.

d. The severeness of the above test should not be underestimated. Surface challenge conditions, in terms of $a_w$, pH and temperature were capable to support outgrowth of *S.aureus*. The only hurdle was the high concentration of sorbic acid existing on the surface of treated samples.

## 5.3 The reduced surface microenvironment pH approach

### 5.3.1 Dialysis experiments

Dialysis experiments yielded protein fractions with the characteristics summarized in Table 26. Using this data and equation (32) we predicted that we could prepare coated IMF samples with a food surface/food bulk pH difference of approximately 0.5 pH units.

Dialyzed protein solutions were steamed for 15 minutes and then freeze dried. Three IMF sample runs were prepared and labelled according to the ISP batch source, i.e. A, B and C.

### 5.3.2 Microbial challenge tests

Using ISP from batch A, IMF No. 2 was prepared and subjected to microbial challenge. As shown in Figure 38, pH equilibration was not achieve instantaneously. About 3 to 4 days

Table 26

ELECTROLYTE ELIMINATION BY DIALYSIS
FINAL PERMEATE CONDUCTIVITY MEASUREMENTS

| Protein | Conductivity, micromho | Equivalent NaCl $\times 10^{-3}$ |
|---|---|---|
| ISP, batch A [1,2] | 30 - 40 | 3 - 4 |
| batch B [3] | 20 | 2.0 |
| batch C [4] | 18 | 1.8 |
| Na-caseinate [5] | 2 | 0.2 |
| Ca-caseinate [5] | 2 | 0.2 |

[1] Each batch consisted of a 7 g/l (100 g total protein) solution dialyzed against 20 l deionized distilled water, which was changed as frequently as required.
[2] Protein solution as is dialyzed in a cold room (about 7 days).
[3] Protein solution adjusted to pH 8.0, dialysis in a cold room, pH neutralization by dialysis against 0.0001 M HCl (about 4 days).
[4] Dialysis at 60C (about 12 hours).
[5] Each batch consisted of a 7 g/l (50 g total protein) solution dialyzed against 6 l deionized distilled water, which was changed as frequently as required.

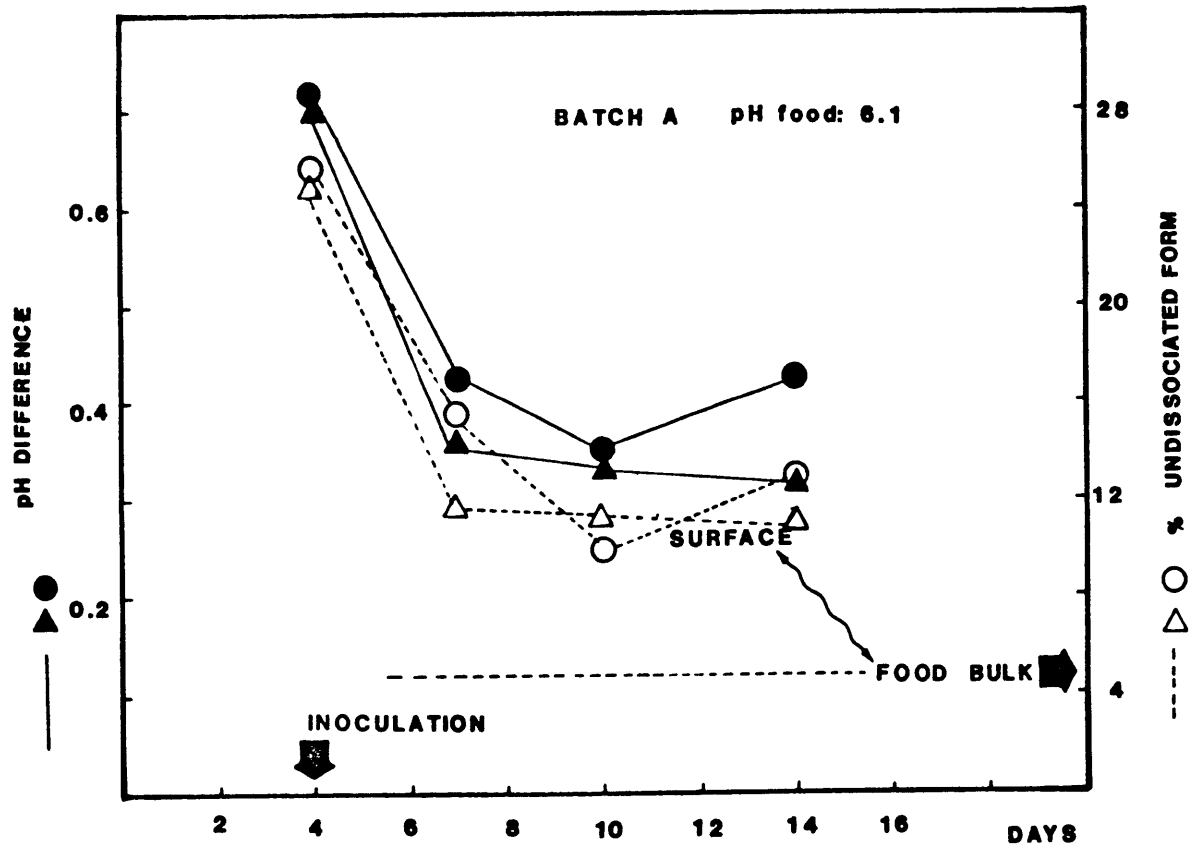
were needed to reach a stable difference. The final pH difference was 0.3 to 0.5 pH units, i.e. close to the calculated value: 0.5.

Figure 38

MEASUREMENT OF THE pH DIFFERENTIAL ESTABLISHED ON IMF MODEL No.2 AND ESTIMATION OF ITS EFFECT ON THE SURFACE AVAILABILITY OF UNDISSOCIATED SORBIC ACID

[ ●, ▲ ] are ΔpH values measured as described in the text. [ ○, △ ] are calculated % undissociated sorbic acid at the surface pH conditions. This value should be compared to the one calculated for the bulk pH conditions [ ---→ ]. As indicated, samples were inoculated four days after coating application.

Experiment was ran twice in duplicates or triplicates. pH measurements were done with a surface pH electrode, which gave ΔpH with a maximum error ± 0.1 pH units.



In Figure 38 we have also represented the % of undissociated sorbic acid corresponding to bulk and localized reduced pH surface conditions. The difference between these two curves visualizes the strong stabilizing effect achieved by reducing surface pH. The pH conditions established on the surface have more than doubled the concentration of the undissociated, i.e. the active form of the preservative.

After ΔpH equilibrium, samples were inoculated with *S.aureus* with the results shown in Figure 39. Inoculated uncoated samples were used as positive controls.

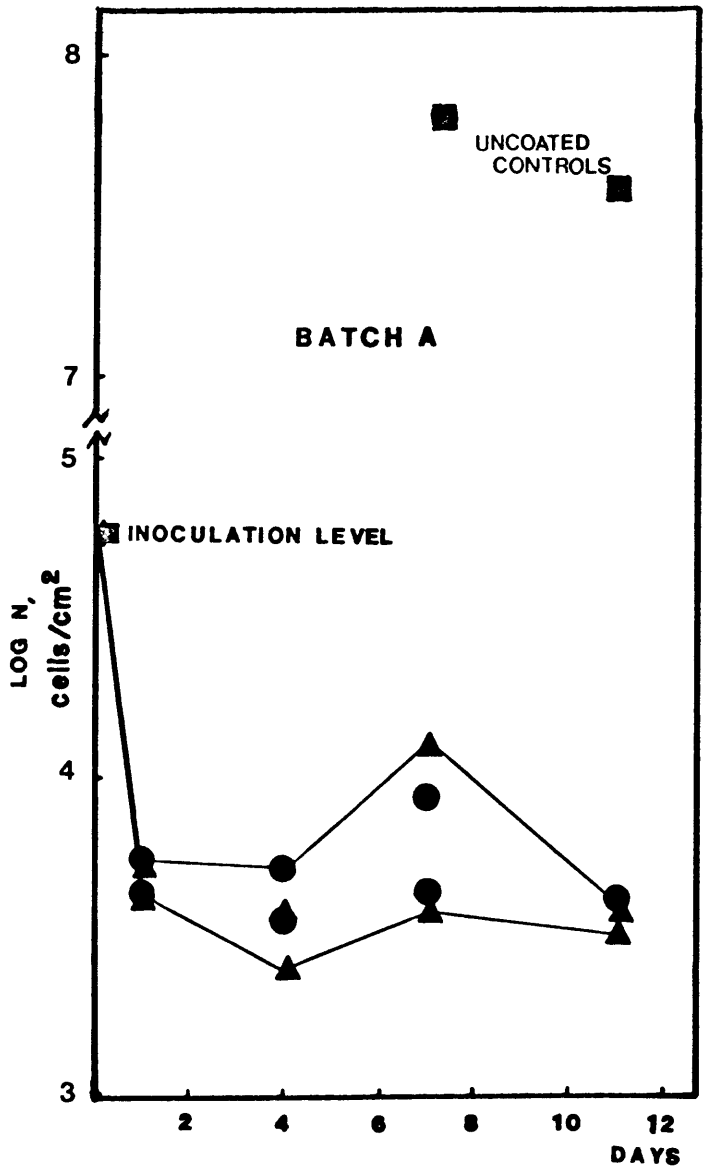
ISP from batches B and C were used for more extended microbial tests with the ΔpH values shown in Figure 40. Again samples were inoculated after an equilibration period of about 4 days. Based on conductivity measurements the similarity to Figure 39 was expected, and confirms the prediction power of the Donnan equilibrium model.

Growth occurred 21 days after inoculation, i.e. 25 days after surface treatment. Growth of unidentified bacteria and fungi was observed. At the same time, the pH difference disappeared. Multiple samples taken at day 27 confirmed this finding. The observation could not be explained as a ΔpH disappearance caused by an "equilibration" between surface and food bulk. As shown in Figures 38 and 40 it is clear that stable ΔpH conditions had already been established. Moreover, as shown in section 5.3.3 sorbic acid was stable at testing conditions.

Figure 39

MICROBIAL CHALLENGE STUDIES: EFFECT OF REDUCED SURFACE pH

*S.aureus* counts on uncoated controls and samples coated with λ-carrageenan

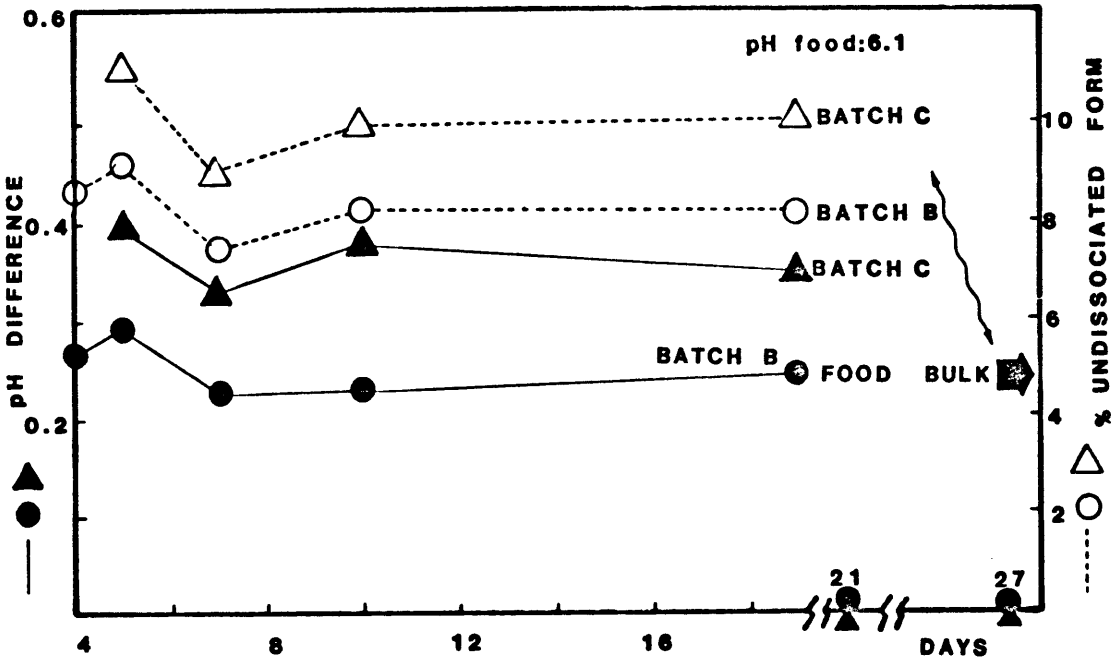



Experiment was ran twice in duplicates. Lines have been drawn through maximum and minimum values and should be compared with two uncoated controls.

Figure 40

MEASUREMENT OF THE pH DIFFERENCE ESTABLISHED ON IMF MODEL No. 2 AND ESTIMATION OF ITS EFFECT ON THE SURFACE AVAILABILITY OF UNDISSOCIATED SORBIC ACID, EXTENDED TEST

Experiment was ran in duplicates or triplicates. pH measurements were done with a surface pH electrode, which gave ΔpH with a maximum error ± 0.1 pH units.



Most likely the explanation lies in unavoidable microbial contamination collected during the long sample preparation process. It should be noted that at testing conditions **food bulk** is not stable.

### 5.3.3 Sorbic acid stability

The possibility that sorbic acid stability could be affected by the presence of λ-carrageenan was also considered. As shown in Figure 41 no major sorbic acid losses were detected. We should note that the outgrowth of a mixed microbial population interfered with sorbic acid recovery determinations, as shown by samples taken at days 21 and 33.

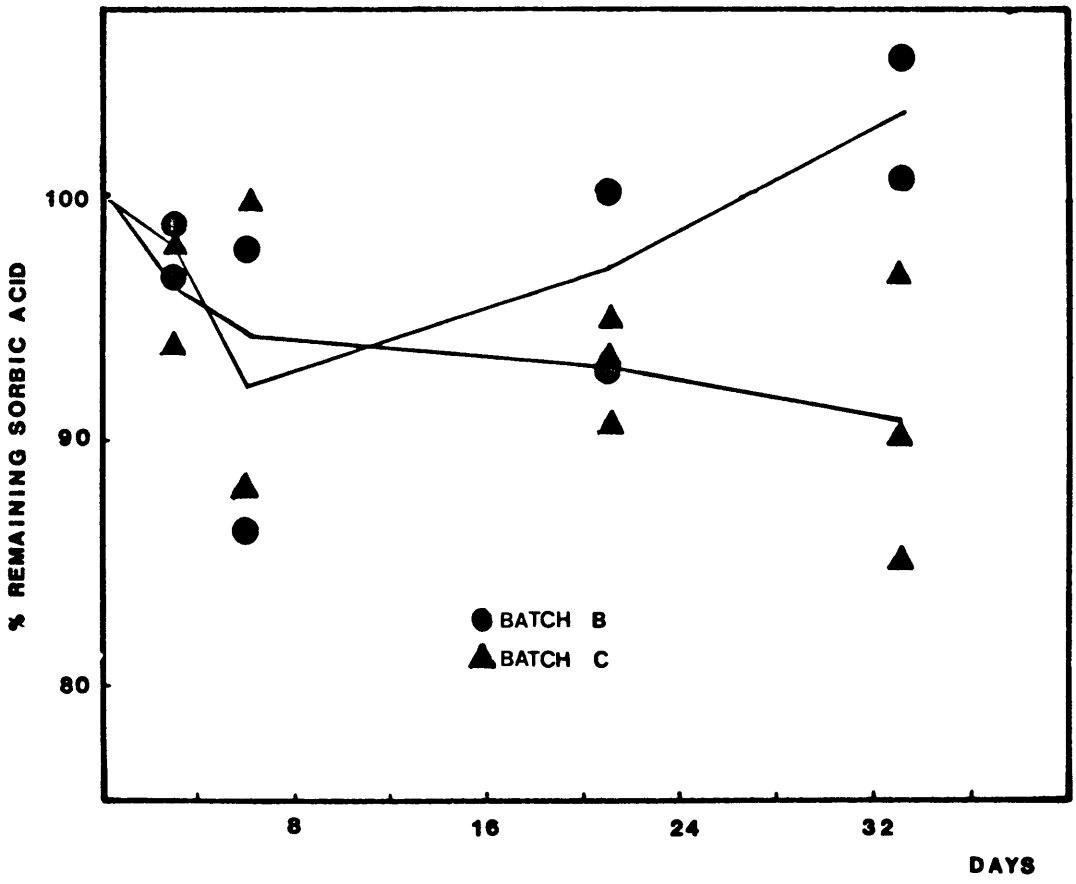
### 5.3.4 Conclusions

We have shown in these experiments that: (1) it is possible to establish a pH differential between surface and food bulk. Moreover, measured values were in the range predicted by the Donnan theory model; (2) surface microenvironment pH resulted in improved microbial stability as shown by the resistance of treated food surfaces to outgrowth of *S.aureus* S-6; and, (3) sorbic acid was stable under our experimental conditions.

Figure 41

SORBIC ACID STABILITY IN THE PRESENCE OF λ-CARRAGEENAN

All samples were inoculated with $10^5$ cells/$cm^2$. Initial concentration was 0.22% and values reported correspond to individual values obtained in two experimental runs.



# 6. Discussion

## 6.1 General

The general aim of this thesis was to find solutions to microbial stability problems associated with unstable temperature environments normally found in commercialization and consumer use of IMF products. We showed that they affect localized $a_w$ food surface conditions. This would explain why shelf life would end unexpectedly due to surface microbial growth.

The stability of an untreated IMF model (No.1) was studied as a function of $a_w$, pH (section 5.1) and preservative concentration (section 5.2.2).

As expected $a_w$ levels higher than 0.85 resulted in instability. The specific $a_w$ value limit was a strong function of product pH, showing the increased effectiveness of sorbic acid. Unfortunately low pH products are not always organoleptically acceptable (Motoki et al., 1982).

Higher K-sorbate levels also increased microbial stability. Tests at $a_w$ = 0.88, showed that somewhere between 0.5 and 1% K-sorbate provided stability. However, for safety reasons, levels above 0.3% are not allowed.

Therefore we decided to investigate approaches that would allow us to create surface conditions of high K-sorbate concentration or reduced pH.

## 6.2 Increased stability due to increased undissociated sorbic acid concentration in the surface

Improved stability was achieved by adding more K-sorbate on the surface and reducing preservative diffusion into the food, or by making more of the active form available by reducing surface pH. Therefore sorbic acid stability studies under our conditions of use were required.

We showed that, in samples with 0.57 to 4.24% K-sorbate losses less than about 25% were detected after 40 days storage (section 5.2.2). Samples with surface applications of 1 $mg/cm^2$ showed no detectable losses after 10 days storage (section 5.2.3.2). Finally sorbic acid stability in the presence of λ-carrageenan was also investigated. Less than 10%% losses were detected after about 30 days storage (section 5.3.3). These results were consistent with published findings (Saxby et al., 1982; Bolin et al., 1980; Arya, 1980; Heintze, 1974, 1971).

## 6.3 The reduced preservative diffusion approach

The use of high surface K-sorbate concentrations required the development of a coating acting as a diffusion

barrier. A coating composition was identified thru permeability cell experiments. Zein films were found to show apparent diffusion constants 1,000 times smaller than cellulose dialysis film used as a reference value (section 5.2.1). Sorbic acid distribution studies confirmed the barrier properties of zein films. We found that sorbic acid apparent diffusion coefficients in zein were between 150 and 300 times smaller than in the IMF itself. The values were 3.3 to 6.8 x $10^{-9}$ $cm^2$/sec and 1 x $10^{-6}$ $cm^2$/sec, in zein and IMF model respectively (section 5.2.3).

The effectiveness of zein films acting as barrier was confirmed by extensive microbiological tests. Zein coated and uncoated IMF model samples were challenged with *S.aureus* S-6 and stored under constant high and under low-high RH cycles conditions. As shown in Table 27 zein coated samples were stable for 28 or more days, while uncoated models were stable only for 2-3 days.

As shown in Figure 37 it is difficult to extrapolate from our testing conditions to other product abuse situations found in commercial practice. There are no models, nor enough information, to account for the highly heterogeneous and dynamic conditions existing on the surface.

Table 27

MICROBIAL STABILITY IMPROVEMENTS AS A FUNCTION OF VARIOUS SURFACE TREATMENTS

Number of days to reach stability limit defined as the number of days for a tenfold increase over initial *S.aureus* inoculation level.

| Treatment | Days |
|---|---|
| a. Tests w/samples w/bulk $a_w$ = 0.88 stored at 30C and 88% RH | |
| uncoated w.2% sorbic acid added as spray | 2 |
| coated w/3 20% zein as dips w/.2% sorbic acid as spray | 5 - 8 |
| coated w/3 12% zein sprays w/.2% sorbic acid as spray | 10 - over 16 |
| b. Tests with samples w/bulk $a_w$ = 0.85 exposed to cycles of 12 hours at 85% RH and next 12 hours at 88% RH | |
| Uncoated w/.2% sorbic acid as spray | 3 |
| coated w/3 20% zein as dips w/.2% sorbic acid as spray | 10 - 15 |
| coated w/3 12% zein sprays w/.2% sorbic acid as spray | 20 - over 28 |

## 6.4 The reduced pH surface microenvironment approach

The use of reduced surface pH conditions required the development of a mathematical model capable of predicting the feasibility and experimental conditions for a desired ΔpH objective. This information was provided by the use of the Donnan equilibrium model for semipermeable membranes. As shown in sections 4.3.1 and 4.3.2 the requirements were an IMF with low total electrolyte and a coating immobilizing a large polyelectrolyte. To satisfy these conditions an IMF with about 0.005 M total electrolyte concentration and a coating composed of deionized λ-carrageenan and deionized agarose was used to establish a pH differential between surface and bulk in the order of 0.5 pH units (section 5.3.1). Such a product was found to be stable for up to 20 days when stability was lost apparently due to microbial contamination of the food bulk which was not stable under testing conditions: pH = 6.1, $a_w$ = 0.88, sorbic acid = 0.22% (section 5.3.2). As indicated in Figures 38 and 40 surface stability was achieved by the increased availability of undissociated sorbic acid due to reduced surface pH. In the bulk only 4.8% corresponded to the active form while on the surface the value was around 12%. Therefore surface preservative availability increased 2.5 times without increasing total preservative use in the IMF formulation.

## 7. Summary and conclusions

1. Changing environment conditions to which IMF products are exposed during production, storage, distribution and use, are important microbial stability factors. An analysis showed that they result in local surface condensations. We concluded that we could solve this problem by surface treatments leading to improved surface stability.

2. An IMF model was tested for microbial stability. Information on microbial stability as a function of pH, $a_w$ and K-sorbate concentration was collected when challenged with *S.aureus* S-6, *A. niger* and *A.tonophilus*.

3. Sorbic acid was used as the major improver of surface stability. It was shown that the acid is stable under our experimental conditions including microbial growth, preservative concentration variations, mode of application (surface versus homogeneous incorporation), processing conditions and IMF formulation variations (IMF No.1 and 2, use of zein and λ-carrageenan coatings). None of these treatments had an effect on its stability, confirming findings reported in the literature.

4. An approach to improved surface stability using a high preservative surface concentration maintained by a highly impermeable food coating was developed. General guidelines,

that allowed us to select materials to be tested for mass transfer properties, were established.

5. Permeability tests showed that zein based coatings were the most promising coating alternative and were selected for coated IMF surface microbial challenges with *S.aureus*.

6. Sorbic acid distribution studies confirmed the barrier properties of zein films. Estimated apparent diffusion coefficients ranged between 3-7 x $10^{-9}$ $cm^2$/sec. These values were 150-300 times smaller than the value measured for IMF model, 1 x $10^{-6}$ $cm^2$/sec. The latter one agreed well with published work.

7. Several combinations of coating and preservative surface application were challenged with $10^5$ cells/$cm^2$ under various conditions. These tests showed that:

   a. Periods of stability were strong function of testing conditions. No model is available to extrapolate results from one condition to another.

   b. When the IMF model was prepared with $a_w$ = 0.88 and pH = 6.4, challenged with *S.aureus* and then stored at 30C, 88% RH, it showed 5 - 8 days stability for samples coated by dipping in 20% zein.

   c. When the same test was repeated on samples sprayed with 12% zein we doubled stability to 10 - 16+ days.

d. When the IMF model was prepared with $a_w$ = 0.85 and pH = 6.4, challenged with *S.aureus* and then exposed to cycles of 12 hours at 85% RH and 12 hours at 88% RH, all at 30C, stability periods were 10 to 15 days and 20 to 28 days for zein dipped and sprayed samples, respectively.

8. Preliminary tests showed that reduced surface pH enhanced microbial stability. In these experiments pH reduction was not permanent and correlated well with loss of microbial stability.

9. A theoretical model was analyzed for the establishment of permanent pH differences: Donnan equilibrium model for semipermeable membranes. This model showed that it was possible to create significantly reduced surface pH conditions (>0.5 pH units) if the total electrolyte concentration was reduced below 0.005 [M] and if samples were coated with 1% of a polyelectrolyte: λ-carrageenan. An alternative to electrolyte reduction, use of a coating highly impermeable to electrolytes, was not explored.

10. Experimental tests confirmed the prediction power of the Donnan equilibrium model. Surface pH reduction in the order of 0.3 to 0.5 pH units were measured experimentally. Such a pH reduction increased surface availability of undissociated sorbic acid 2.5 times.

11. The effectiveness of surface pH reduction was established thru microbial challenge tests with *S.aureus* S-6. Under extreme testing conditions, $a_w$ = 0.88, RH = 88%, T = 30C, %sorbic acid = 0.22, stability was greater or equal to 20 days.

## 8. Suggestions for future work

We believe that the important suggestions relate to exploration of: (a) what work is needed to improve solutions found; and, (b) what work is needed to utilize our findings.

### 8.1 The reduced preservatives diffusion approach

Food coatings acting as diffusion barriers could provide interesting applications. Therefore, we should find answers to the following questions:

1. What are the mechanical properties of zein coatings under use conditions?
   Although some literature information is available we should find out whether zein coatings can withstand the mechanical abuse normal to food distribution operations.

2. What is the maximum coating thickness that is organoleptically acceptable?
   Succesful microbial challenge studies here reported utilized zein coatings only 0.03 to 0.04 mm thick. Since thicker coatings would extend microbial stability significantly we should find out what is the maximum acceptable to a consumer.

3. What would be consumers attitude towards coated product?
   If we tell consumers that, product x has been "corn protein

coated for better stability", thicker coatings would probably be accepted.

4. Would it be possible to make zein coatings more acceptable? Preliminary microbial tests using sorbic acid incorporated in zein films were found to be ineffective. Although we did not investigate the reason for this behavior, we can assume that a major sorbic acid fraction remained encapsulated, and was unavailable to affect microbial growth. This suggests that we could be able to encapsulate flavoring agents making the coating not only acceptable, but even desirable, from an organoleptic point of view.

5. Are there other products that could benefit from our surface treatment?
   The possibility of applying zein coatings to other products should not be ignored. For example, improving microbial stability of fresh poultry would be of great commercial interest.

## 8.2 The reduced pH surface microenvironment approach

The main difficulty in the application of this technology lies in the low total electrolyte concentration limitation. The total potential of this approach will be realized only if we find answers to the following questions:

1. What food products can be produced with low electrolyte concentrations?

   Most IMF products contain large amounts of NaCl and other salts and therefore surface pH reductions can not be achieved. It will be interesting to find food components that can be used to create an IMF with total electrolyte concentrations below 0.005 M.

2. What coating could be used to isolate eletrolytes present in IMF's?

   If it is not possible to eliminate electrolytes present in foods, an alternative would be to precoat samples with a barrier impermeable to electrolyte transport.

3. What is the organoleptic effect of food surfaces with reduced pH?

   The organoleptic response of consumers to food with pH differences between surface and food bulk will have to be determined before continuing further studies.

4. Are there other products that could benefit from surface pH reductions?

   As stated previously, we see no need to restrict applications to IMF's. Other food applications should also be explored.

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# APPENDIX A

## An example of surface condensation problems

As an example of surface condensation problems let us analyze what happens to an individual package when it is loaded from a warehouse, T(1) = 65F, into a railroad car in the winter, T(2) = 35F.

The objective of these calculation is to show that cooling of the food pieces is mostly due to evaporation. The package inside walls will accumulate moisture condensations that will lead to drops on a few food surface spots. This will lead to localized conditions of high $a_w$ and therefore to microbial outgrowth.

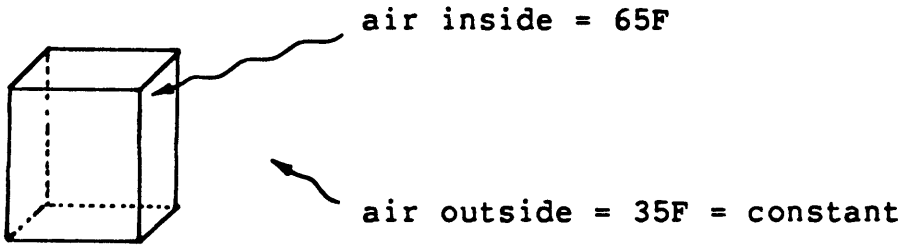
Calculations will be simplified by treating all rate processes separately, i.e. steps a thru e discussed in the following paragraphs. This will allow us to identify which one is the rate limiting process and how long growth promoting conditions will last.

Let us assume the following values:

| | |
|---|---|
| package dimensions: | 5"x3"x2" |
| net weight/package: | 1 lb |
| food pieces, spheres, r : | 0.5" |
| food pieces/package: | 66 |
| product density : | 50 $lb/ft^3$ |

| | |
|---|---|
| product conductivity: | 0.4 Btu/hr ft F |
| specific heat: | 0.7 Btu/lb F |
| package heat conductivity: | 0.05 Btu/hr ft F |
| package thickness: | 0.08" |
| air, natural convection, h: | 0.8 Btu/hr $ft^2$ F |
| air, specific heat: | 0.25 Btu/lb F |
| air, conductivity: | 0.016 Btu/hr ft F |
| air, density: | 0.081 $lb/ft^3$ |

a. Assume that we have a package filled only with air




Further, assume that there is no convection inside the package which would be the worst case situation. Using Gurney-Lurie graphs (McAdams, 1954) it can be estimated that air temperature inside the package will reach 36F in less than a minute.

b. We can now assume that air inside the package, surrounding food pieces, is at 35F. Let us now calculate how long it takes a food particle to cool under down these conditions if there is no moisture evaporation at all. In this case the heat transfer resistance ratio (surface/bulk) is 12 which allows us to use the

simplified equation described in McAdams (p. 41, 1954). The following values were thus obtained:

| time h | product - air temperature F |
|---|---|
| .1 | 25.5 |
| 1.0 | 5.7 |
| 2.0 | 1.2 |

c. Using a heat balance we calculated the amount of water needed to cool down an individual food piece = 0.14 g/food piece (i.e 9.24 g/package). This amount is large to cause significant localized $a_w$ increases but small enough to be provided by only a thin surface layer. It should be noted that while evaporation will come from the surface of all food pieces, condensation will occur only on a few localized spots.

d. Following calculations similar to example 21.2-1 (Bird et al., 1960) the instantaneous initial rate of evaporation was estimated as 0.72 mg water/sec. Assuming an average equal to 0.36 mg water/sec we estimated that it would take the surface only about 6 minutes to evaporate all the water needed to provide this amount and therefore the necessary cooling effect. This value should be compared to the one obtained in part b, 2 hours.

e. Based on the results c and d we can simplify the cooling analysis by assuming that due to evaporation the surface will be at 35F. We can now calculate how long it will take the whole food piece to cool down. Using again Gurney-Lurie graphs (McAdams, 1954) we estimated that under this assumption the food piece core will reach 36F in about 10 minutes. This is obviously an underestimation caused by assuming that every preceding stage occurred instantaneously. It shows however, that cooling and therefore surface evaporation will be relatively fast.

f. Finally we tried to estimate the time length that a water drop could keep a food surface at $a_w$ leading to microbial outgrowth. Disappearance of the water drop will be due to many processes: diffusion into the food, spreading over the surface, evaporation to the headspace, etc. The simplest one to calculate was the diffusion process. Assuming that this was the only mechanism and that we were dealing with 0.2 ml drop, an IMF with $a_w = 0.80$ and microorganisms capable to grow down to 0.85, we estimated that disappearance of growth allowing conditions could take up to 50 hours. This is obviously an overestimation. Inclusion of the other rate processes should reduce it by one order of magnitude. However, even a few hours will have serious microbial stability consequences.

Summarizing we can conclude that heat transfer processes involved in food cooling are much faster than moisture reequilibration. Therefore every time that the product goes thru

temperature cycles large amounts of surface moisture will be released in an analogous process to evaporative cooling. This will lead to significant moisture condensations and therefore to microbial stability difficulties.

## APPENDIX B

Measured and predicted equilibrium moisture content comparison

[1]

| Product [1] | Composition | g | $M_i$ [2] | $M_c$ [3] | error [4] |
|---|---|---|---|---|---|
| 1 | meat solids | 59.9 | 23.6 | | |
| IM meat | meat fat | 10.6 | - | | |
| $a_w$ = 0.83 | Frodex, 42DE | 14.5 | 32.0 | | |
| | water | 15.0 | | 22.1 | 26% |
| 2 | meat solids | 51.3 | 23.6 | | |
| IM meat | meat fat | 9.1 | - | | |
| $a_w$ = 0.83 | glycerol | 25.0 | 142.0 | | |
| | NaCl | 2.6 | 381.0 | | |
| | water | 25.0 | | 52.1 | 56% |
| 3 | meat solids | 26.6 | 23.6 | | |
| IM meat | meat fat | 6.7 | - | | |
| $a_w$ = 0.83 | glycerol | 25.0 | 142.0 | | |
| | NaCl | 3.7 | 381.0 | 93.1 | 40% |
| 4 | fish solids | 22.0 | 15.0 | | |
| IM deep | fish fat | .7 | - | | |
| fried | glycerol | 20.1 | 142.0 | | |
| cat fish | NaCl | 4.1 | 381.0 | | |
| $a_w$ =0.83 | fat | 13.2 | - | | |
| | water | 39.9 | | 79.0 | 19% |
| 5 | fish solids | 20.9 | 15.0 | | |
| IM deep | fish fat | .1 | | | |
| fried | glycerol | 21.5 | 142.0 | | |
| $a_w$ = 0.83 | NaCl | 3.6 | 381.0 | | |
| | water | 31.5 | | 69.2 | 50% |
| 6 | glycerol | 12.5 | 133.0 | | |
| IM irish | corn syrup | 5.5 | 36.0 | | |
| stew gravy | sucrose | 5.0 | 48.0 | | |
| $a_w$ = 0.85 | NaCl | 3.0 | 424.0 | | |
| | egg yolk | 10.0 | 16.0 | | |
| | seasoning | 2.1 | 50.0 | | |
| | fat | 38.4 | - | | |
| | water | 23.5 | | 47.5 | 55% |

| | | | | | |
|---|---|---|---|---|---|
| 7 | carrot solids | 6.3 | 29.0 | | |
| IM carrots | glycerol | 51.1 | 78.0 | | |
| $a_w$ = 0.77 | NaCl | 2.1 | 295.0 | | |
| | prop. glycol | .9 | 96.0 | | |
| | water | 39.3 | | 80.3 | 24% |
| 8 | carrot solids | 34.2 | 28.0 | | |
| IM carrots | glycerol | 51.1 | 78.0 | | |
| $a_w$ = 0.77 | NaCl | 2.1 | 295.0 | | |
| | prop. glycol | .7 | 92.0 | | |
| | water | 27.5 | | 56.7 | 59% |
| 9 | n/fat dry milk | 12.8 | 30.0 | | |
| IM sauce | glycerol | 9.8 | 116.0 | | |
| (ham) | egg yolk | 10.0 | 16.0 | | |
| $a_w$ = 0.86 | corn syrup | 2.1 | 37.0 | | |
| | prop. glycol | 1.0 | 163.0 | | |
| | NaCl | 1.0 | 449.0 | | |
| | others | 1.3 | 50.0 | | |
| | fat | 47.7 | - | | |
| | water | 14.2 | | 28.5 | 12% |
| 10 | n/fat dry milk | 12.8 | 30.0 | | |
| IM sauce | glycerol | 11.8 | 116.0 | | |
| (chicken) | egg yolk | 10.0 | 16.0 | | |
| $a_w$ = 0.86 | corn syrup | 2.0 | 37.0 | | |
| | NaCl | 1.0 | 449.0 | | |
| | others | 1.2 | 50.0 | | |
| | fat | 47.2 | - | | |
| | water | 14.1 | | 29.6 | 80% |
| 11 | isol.soy prot. | 26.1 | 34.0 | | |
| IM cheese | Na caseinate | 5.8 | 29.0 | | |
| analog | Ca caseinate | 2.0 | 29.0 | | |
| $a_w$ = 0.86 | oil phase | 34.4 | - | | |
| | NaCl | 4.8 | 449.0 | | |
| | glycerol | 5.9 | 116.0 | | |
| | sorbitol | 19.3 | 58.0 | | |
| | others | 1.7 | 50.0 | | |
| | water | | | 52.4 | 5% |

---

[1] Product and compositions reported by Chirife (1978) with the exception of No. 11, the model system used in this study. Fat was estimated using food composition tables (Bowes and Church, 1975).

[2] Equilibrium content for each ingredient was obtained from published isotherms (Iglesias and Chirife, 1976b; Chirife et al., 1980; Troller and Christian, 1978; Benmergui et al., 1979; Rasekh et al., 1971; Copper et al.,1968; Iglesias and Chirife, 1976 d; Heldman et al., 1965; Hansen, 1976; Berlin,

1979).

[3] Calculated moisture content (Lang and Steinberg, 1980):

$$M_C = \Sigma W_i \, M_i / \Sigma \, M_i \qquad \text{(b-1)}$$

[4] % error was defined as:

$$(M_C - M_m)/M_m \; 100 = \%\text{error} \qquad \text{(b-2)}$$

where $M_m$ = measured moisture content calculated from composition data.

## APPENDIX C

### Mass transfer estimations

Approximations for required D-values can be based con the following equation (Crank, 1975, equation 2-7):

$$C(x) = \frac{M}{(\pi Dt)^{1/2}} \exp(-x^2 / 4Dt) \qquad (c\text{-}1)$$

Since we are interested in surface concentrations, we can assume small values for x, therefore:

$$\exp(-x^2/4Dt) \simeq 1 - x^2/4Dt \qquad (c\text{-}2)$$

which gives us:

$$C(x) = \frac{M}{(\pi Dt)^{1/2}} (1 - x^2/4Dt) \qquad (c\text{-}3)$$

An average 'surface' concentration can be calculated as as follows:

$$\bar{C}(0,\Delta x) = \text{average between } x=0 \text{ and } x=\Delta x$$
$$= 1/2 \; ( \; C(x=0) + C(x=\Delta x) \; ) \qquad (c\text{-}4)$$
$$C(x=0) = M/(\pi Dt)^{1/2} \qquad (c\text{-}5)$$

Therefore:

$$\bar{C}(0, \Delta x) = \frac{1}{2} \frac{M}{(\pi Dt)^{1/2}} \qquad (c\text{-}6)$$

The average value at time 0 is given by the following expression:

$$C_o(0,\Delta x) = M/\Delta x \qquad (c\text{-}7)$$

Let us define reduction in average surface concentration as:

$$f = C(0,\Delta x)/C_o(0,\Delta x) \qquad (c\text{-}8)$$

$$= \frac{\Delta x}{(\pi Dt)^{1/2}} \left(2 - \frac{\Delta x^2}{4Dt}\right) \qquad (c\text{-}9)$$

This equation can be simplified to yield:

$$D = \frac{\Delta x^2}{f^2 \pi t} \qquad (c\text{-}10)$$

Assuming that $\Delta x$ is the coating thickness h we can estimate required D-values for a given desired stability period with the results shown in Table 7 (f = 0.05).

## APPENDIX D

### 1. Use of permeability values to estimate coating effectiveness

Under well stirred conditions, the resistance to mass transfer in a permeability cell will be due only to film properties. A constant concentration difference between diffusion cell reservoirs facilitates the analysis of experimental data. This was done by using

$c_1(t=0)$ = concentration in reservoir 1 = 0

$c_2(t=0)$ = concentration in reservoir 2 = large

The use of large reservoirs compared to the area of mass transfer allowed us to measure a constant permeability rate equal to:

$$N = DA(c'_2 - c'_1)/x \qquad (d\text{-}1)$$

where:

N = flux of K-sorbate

D = apparent diffusion constant

A = area of mass transfer

$c'_i$ = concentration in the film which is in equilibrium with bulk solution concentration $c_i$, equilibrium expressed as $c'_i = kc_i$

x = a diffusion distance

Assuming constant k we obtain:

$$N = K A (c_2 - c_1) \qquad \text{(d-2)}$$

where:

$$K = kD/x \qquad \text{(d-3)}$$

If we evaluate this expression for the uncoated and coated reference material, i.e. r = cellulose and rc = coated cellulose, we obtain:

$$K_r = k_r D_r / x_r \qquad \text{(d-4)}$$

$$K_{rc} = k_{rc} D_{rc} / x_{rc} \qquad \text{(d-5)}$$

Assuming no interface resistance between coating and cellulose and k values independent of testing material, i.e.

$$k_{rc} = k_r = k_c \qquad \text{(c = coating alone)} \qquad \text{(d-6)}$$

We know that:

$$\frac{x_{rc}}{D_{rc}} = \frac{x_r}{D_r} = \frac{x_c}{D_c} \qquad \text{(d-7)}$$

where:

$$x_{rc} = x_r + x_c \qquad \text{(d-8)}$$

We can express x as:

$$x_c = f\, x_r \qquad \text{(d-9)}$$

Thus:

$$\frac{1 + f}{D_{rc}} = \frac{fD_r + D_c}{D_c D_r} \qquad \text{(d-10)}$$

We are interested in $D_c << fD_r$, thus:

$$1 + f = fD_{rc}/D_c \qquad \text{(d-11)}$$

From (d-4), (d-5) and (d-9) we obtain:

$$\frac{K_{rc}}{K_r} = \frac{D_{rc}\, x_r}{D_r\, x_{rc}} = \frac{D_{rc}}{(1 + f)D_r} \qquad \text{(d-12)}$$

From (d-11) and (d-12) we obtain:

$$\frac{D_c}{D_r} = \frac{f\, K_{rc}}{K_r} \qquad \text{(d-13)}$$

2. Estimation of zein coating effectiveness

The effectiveness of zein films was evaluated by comparing experimental K-values obtained at the same initial $\Delta c$ conditions (10 mg sorbic acid/ml solution) for zein coated and uncoated cellulose films. The values were:

$K_r = 4.7 \times 10^{-2}$ (mg/hr cm$^2$)/(mg/ml) (Table 12)

$K_{rc} = 1.5 \times 10^{-4}$ (mg/hr cm$^2$)/(mg/ml) (Figure 28)

$x_r = 0.003$ cm (product specification)

$x_c = 0.00088 \pm 0.00012$ cm (SEM determination)

Substituting in equation (d-13) we found that D(zein) was 1070 smaller than D (cellulose).

## APPENDIX E

Preparation and composition of coatings tested

a. Zein films with the following compositions were tested:

| | |
|---|---|
| zein, x = 8 to 20 | x g |
| glycerol | 0.25x g |
| Myvacet 7-00 | 1 g |
| ethanol 190 proof | balance to 100 g |

Ethanol, glycerol and Myvacet were heated up to 50-60C. Zein was then slowly added under constant agitation. Cellulose films were then sprayed or dipped with this solution while still hot (50C).

b. Gelatin hot melts with the following compositions were tested:

| Polyol | g | Gelatin | g |
|---|---|---|---|
| propylene glycol | 80 | Type B, 175 Bloom | 20 |
| ethylene glycol | 80 | Type B, 175 Bloom | 20 |
| propylene glycol + Myvacet 7-00 | 80<br>1 | Type B, 175 Bloom | 20 |

Polyol and plasticizer were heated to 90C under medium agitation, and then gelatin was slowly added to the vortex. The solution was mixed for 30 minutes at 90C. Cellulose films wer then coated and let dry at room temperature for approximately 12 hours.

c. Caustic amylose coagulated with ammonium sulfate films with the following compositions were also tested:

| Code [2] | Starch soln | | NaOH soln | | Coagulation bath |
|---|---|---|---|---|---|
| | Amylose g | water ml | NaOH g | water ml | $(NH_4)_2SO_4$ |
| HVII-20-40 | 30 | 106 | 4.8 | 14.4 | 40% soln |
| HVII-20-Sat | 30 | 106 | 4.8 | 14.4 | saturated soln |
| HVII-20G-Sat | 30 | 96[2] | 4.8 | 14.4 | saturated soln |

[1] Amylose types used in preliminary trials: Amylose AVII (American Maize products, Co.); Hylon V and Hylon VII (National Starch and Chemicals Corp.). Hylon VII (HVII) showed the best film forming properties.

[2] contained 5 g glycerol were added as a plasticizer.

Amylose, water and glycerol were stirred at room temperature for 1 hour. While stirring, the NaOH solution was added. Stirring was continued for about 3 hours. The solution was then cloth filtered and let rest for 12 hours for elimination of air bubbles. Cellulose was coated with this solution and then dipped in the coagulation bath. The final step was a wash with

50% glycerol. They were then let dry for about 4 to 12 hours at room temperature.

d. Caustic amylose coagulation by acid salt mixture was used to prepare the films shown in the following table:

| Code | Amylose g | Water ml | NaOH g | Water ml | Coagulation bath |
|---|---|---|---|---|---|
| HVII-20-S | 30 | 106 | 4.8 | 14.4 | S=$H_3PO_4$(15 g)+ $Na_2HPO_4$(30 g)+ $H_2O$ (55 ml) |
| HVII-20G-PS | 30 | 96[1] | 4.8 | 14.4 | P=$H_2SO_4$(12 g)+ $Na_2SO_4$(30 g) + $H_2O$ (64 ml) and then S too |
| HVII-20G-S | 30 | 96[1] | 4.8 | 14.4 | S |

[1] 5 g glycerol were added as a plasticizer

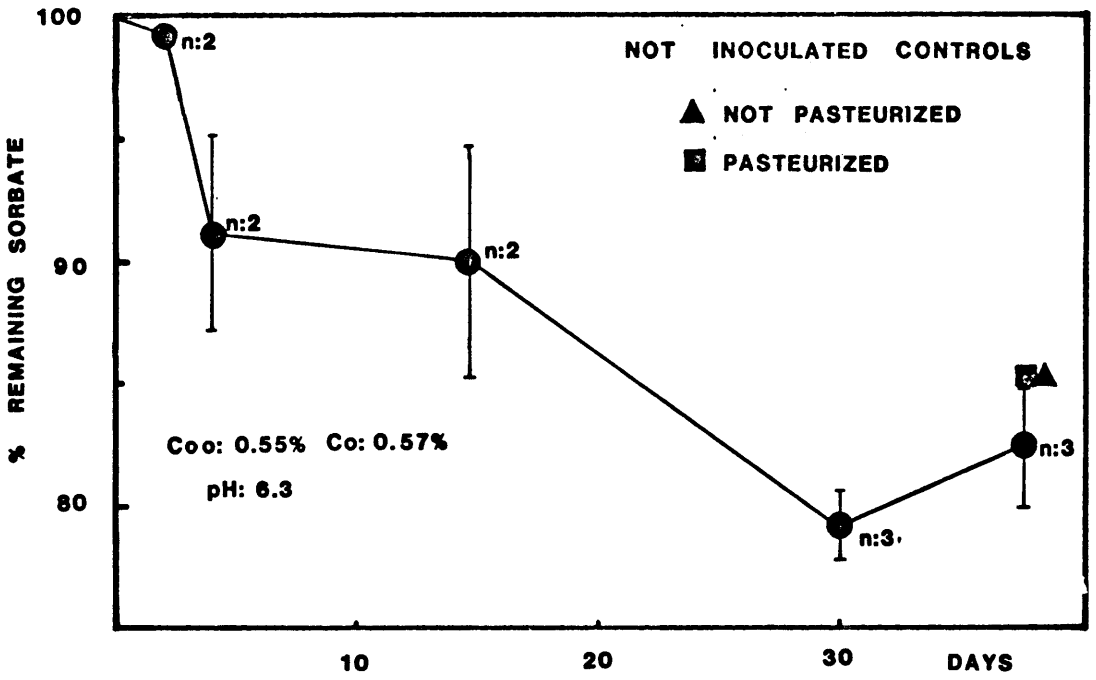
The preparation of amylose coated cellulose supports was identical to the one described in part c.

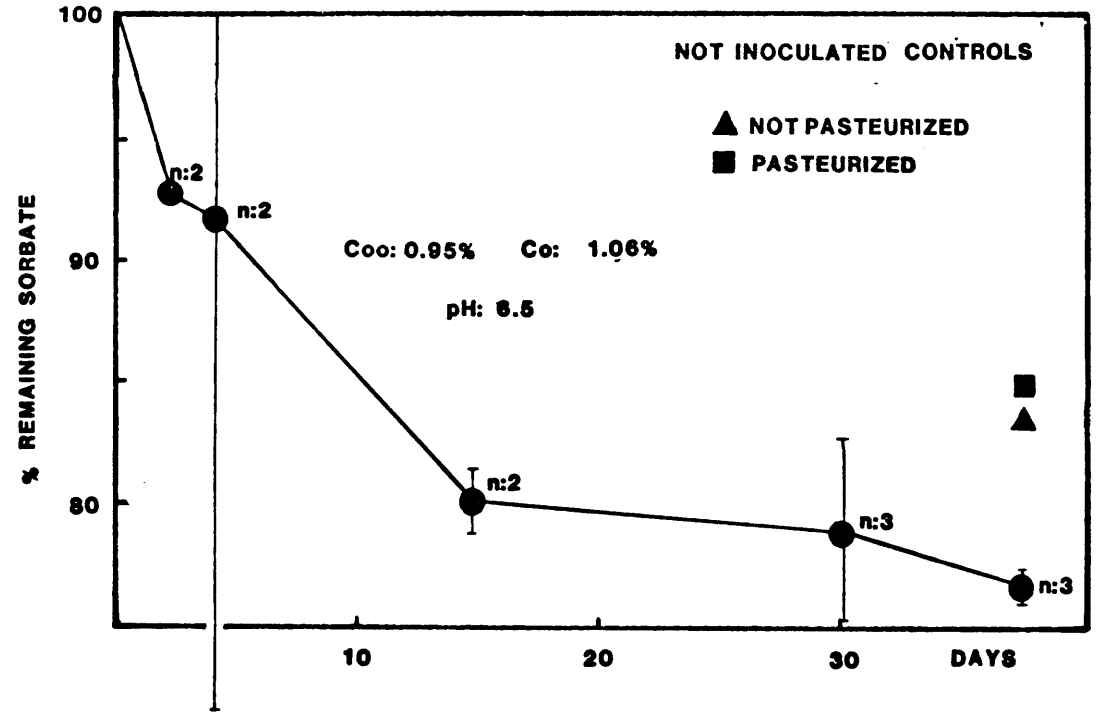
e. Water soluble amylose esters with low degree of substitution were also tested. High amylose starch may be made more water soluble by esterification of some of the free hydroxyl groups. A limited amount of branching disrupts the linearity of the amylose molecule producing a derivative which can be easier dispersed in water. The conditions used in this study to prepare this

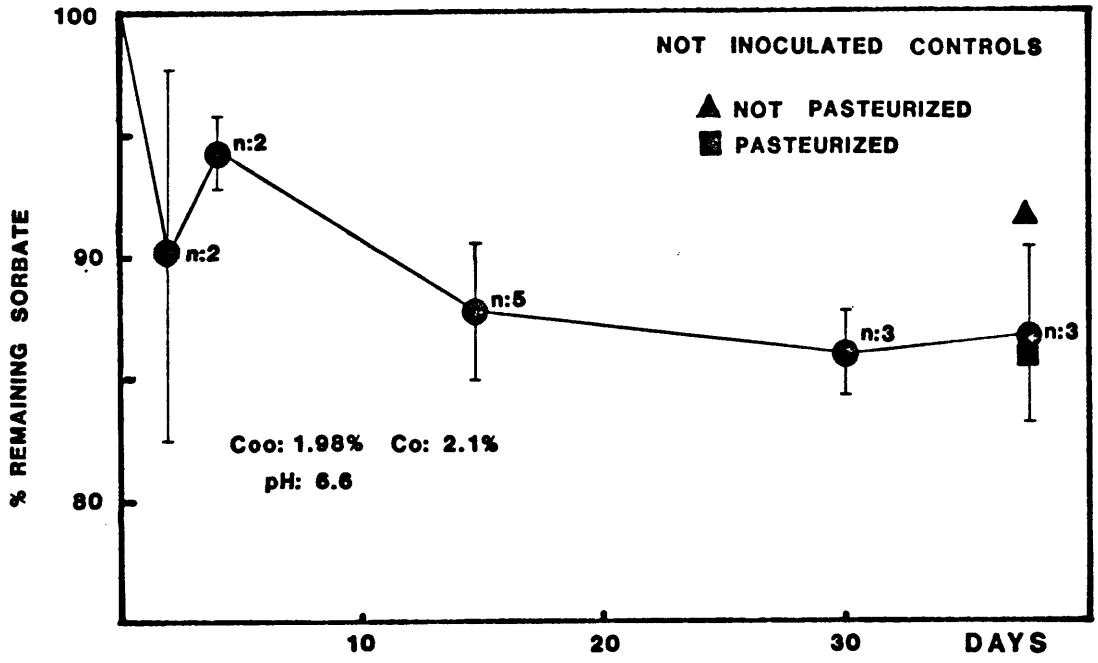
material were as follows. 10 g amylose and 56.7 g dimethyl sulfoxide were mixed for about 2 hours at room temperature. Thereafter 0.6 g of triethyl amine and 0.6 g of acetic anhydride were added. Mixing was continued for another 2 hours at room temperature. The mixture was then filtered and washed three times with ethanol. Supernatant was discarded while solids were vacuum dried (50C, 18 hours). A 10% solution in water was heated to boiling point and used to form films on the cellulose support.

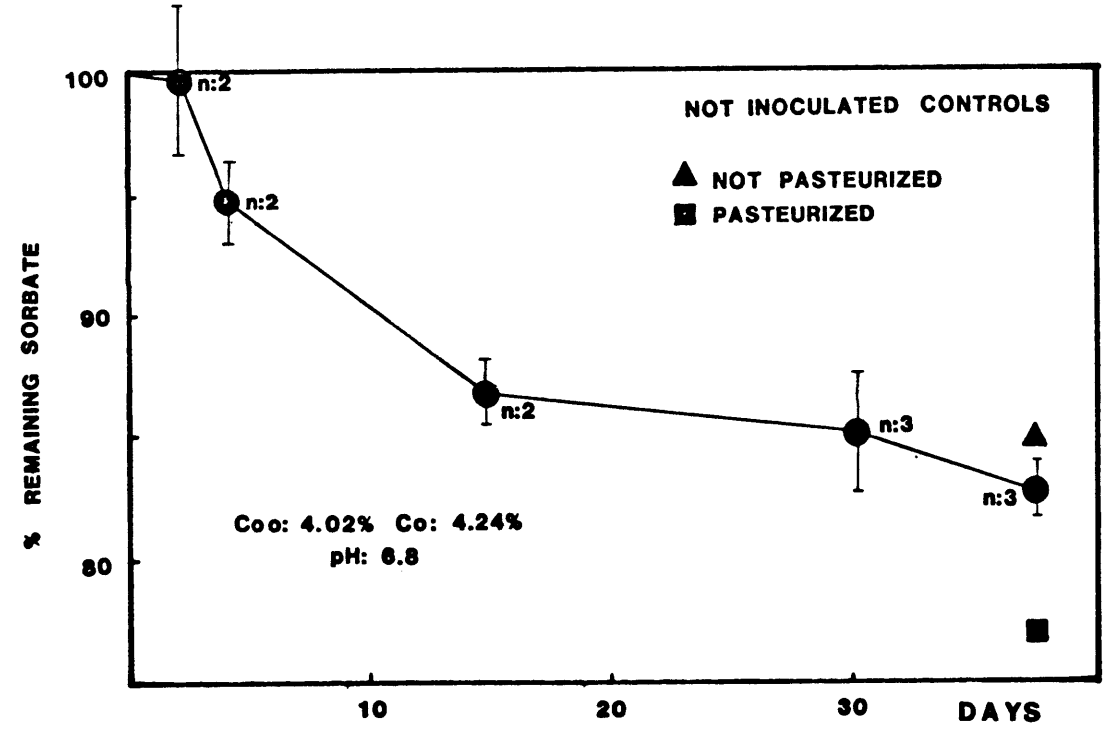
## APPENDIX F

Sorbic acid as percentage of initial concentration.




100
90
80
% REMAINING SORBATE
NOT INOCULATED CONTROLS
NOT PASTEURIZED
PASTEURIZED
n:2
n:2
n:5
n:3
n:3
Coo: 1.98% Co: 2.1%
pH: 6.6
10
20
30
DAYS


100
90
80
% REMAINING SORBATE
NOT INOCULATED CONTROLS
NOT PASTEURIZED
PASTEURIZED
n:2
n:2
n:2
n:3
n:3
Coo: 4.02% Co: 4.24%
pH: 6.8
10
20
30
DAYS

# APPENDIX G

## Sorbic acid surface application

Sorbic acid spraying for a surface application equivalent to 0.2% sample weight was determined experimentally as follows. Several samples were coated with zein and then sprayed with 10% sorbic acid in ethanol 190 proof.

Sorbic acid determinations have been represented in Figure G-1 as a function of spraying time.

The following additional information was used:

sample weight average = 6.2 g

desired % sorbic acid on surface = 0.2

calculated average sorbic acid/sample piece = 12.4 mg

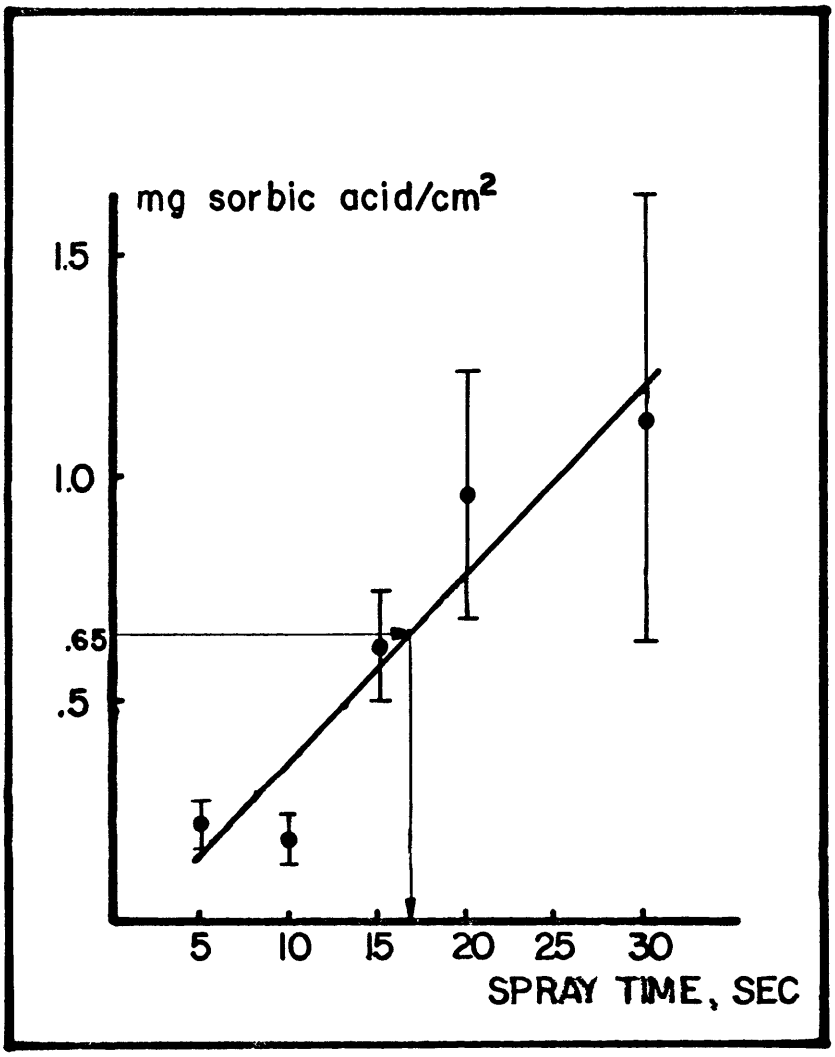
calculated sorbic acid /$cm^2$ = 0.65

When this last value was used in conjunction with Figure G-1 an average spraying time of 17 sec was obtained.

Figure G-1 shows also a large variation between samples: large standard deviations and a relatively low correlation coefficient, r = 0.81.

Figure G-1

DETERMINATION OF SPRAYING CONDITIONS



## APPENDIX H

### Determination of apparent sorbic acid diffusion coefficients from experimentally measured sorbic acid distributions

Sorbic acid apparent diffusion coefficients in our IMF model and in zein coatings can be evaluated using data from Figure 32. The following assumptions were necessary:

1. Experimentally determined average core concentrations were assumed to represent the concentration in the center of the food piece. This is valid when the core sample is very small. In our case it was 1/5 of the food piece, or about 1g.

2. Graphs and equations obtained for unidimensional diffusion for the case of an infinite slab were assumed valid for the analysis of our disk shaped samples (r = 1.3 cm, h = 1 cm) with diffusion occurring from every surface. This simplification was possible because edge effects were eliminated by cutting food pieces as shown in Figure 20, i.e. obtaining a core piece shaped as a smaller disk (r = 0.8 cm, h = 0.5 cm).

Center conditions can be evaluated using Gurney-Lurie graphs. (Adams, 1954, p.36). Therefore we defined:

a. Y = an unaccomplished core concentration change.

$$Y = (C - \bar{C})/\bar{C}$$

with C = core concentration

$\bar{C}$ = average concentration for total food piece

b. X = a relative time

$X = D_f t/r_f^2$ with

$r_f$ = half thickness = 0.5 cm.

t = time, seconds

$D_f$ = food apparent sorbic acid diffusion constant, $cm^2/sec$

c. m = resistance ratio

$$m = (D_f\ r_f)/(D_c\ r_c)$$

with

$r_c$ = coating thickness = 0.0012 cm (1x); 0.0038 cm (3x)

and

$D_c$ = coating apparent sorbic acid diffusion constant, $cm^2/sec$.

The following values were obtained for uncoated food, m=0:

| time h | Y | X | $D_f \times 10^6$ $cm^2/sec$ |
|---|---|---|---|
| 10 | 0.84 | 0.15 | 0.9 |
| 20 | 0.61 | 0.26 | 0.9 |
| 40 | 0.37 | 0.50 | 1.1 |
| 60 | 0.12 | 0.95 | 1.1 |
| | | | $1.0 \pm 0.1$ |

The following values were obtained for 1x coated food. The value obtained for $D_f$ was used to evaluate X which allowed us to determine m.

| time h | Y | X | m | $D_f \times 10^6$ $cm^2/sec$ |
|---|---|---|---|---|
| 60 | 0.46 | 0.85 | 0.55 | 4.4 |
| 80 | 0.39 | 1.14 | 0.70 | 3.4 |
| 100 | 0.35 | 1.42 | 0.75 | 3.2 |
| 120 | 0.31 | 1.71 | 1.00 | 2.4 |
| 140 | 0.18 | 1.99 | 0.75 | 3.2 |
| | | | | $3.3 \pm 0.7$ |

Similarly for samples coated 3 times:

| time h | Y | X | m | $D_f \times 10^6$ $cm^2/sec$ |
|---|---|---|---|---|
| 60 | 0.60 | 0.85 | 1.00 | 7.6 |
| 80 | 0.47 | 1.14 | 0.95 | 8.0 |
| 100 | 0.43 | 1.42 | 1.10 | 6.9 |
| 120 | 0.40 | 1.71 | 1.35 | 5.6 |
| 140 | 0.32 | 1.99 | 1.30 | 5.8 |
| 160 | 0.23 | 2.28 | 1.10 | 6.9 |
| | | | | $6.8 \pm 0.9$ |