The role of lipids in controlling microbial growth

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INTRODUCTION

Many foods are, or contain, emulsions. Growth of microorganisms in emulsions may lead to spoilage by bacteria, yeasts, moulds or food-poisoning bacteria. In biphasic foods (e.g. oil-in-water or water-in-oil emulsions), food structure may influence both rate of growth and conditions under which growth is initiated. The site of occupancy of microorganisms is the aqueous phase. Therefore the chemical composition of this phase is what has a direct influence on the survival and growth of microorganisms. This paper describes the chemical effects of organic acids used as preservatives in oil-in-water (acetic and lactic acids) and water-in-oil (sorbic and benzoic acids) emulsions as well as the influence of their structures on the food stability.

KEY-WORDS: Emulsions — Food (stability) — Microbial growth — Preservatives.

Growth of micro-organisms in emulsions may lead to spoilage due to the production of gas or taints by bacteria or yeasts, or to visible colonial growth of bacteria, yeasts or moulds on the surface of the product. Some food emulsions can support the growth of food-poisoning bacteria and although their growth is often accompanied by the growth of spoilage micro-organisms, and obvious visible spoilage, this is not always so.

Historically, food microbiology has assumed that, within the food matrix, it is the chemistry alone that controls the growth of micro-organisms. The experimental basis of this paradigm was the growth of micro-organisms in liquid culture medium. The assumption was then made that the organisms would grow similarly in the liquid culture medium as they would in foods of an equivalent chemical composition, but this approach ignores any effect of structure of the food on microbial growth
In biphasic foods, the lipid component may have a controlling influence on microbial growth through its contribution to the structure of the growth domain of the micro-organisms, and its contribution to the redistribution of chemical components between the phases of the foods, and it is these features that are discussed here.

CHEMICAL EFFECTS

The site of occupancy of micro-organisms in emulsions is the aqueous phase (Tuynenburg Muys, 1971; Brocklehurst et al., 1995), and hence it is the chemical composition of this phase that has a direct influence on the survival and growth of bacteria, yeasts and moulds.

Oil-in-water food emulsions such as salad creams and mayonnaise rely heavily for their preservation on the concentration of organic acids, such as acetic acid or lactic acid, which are added to the product partly for organoleptic purposes. These and water-in-oil emulsions such as margarines or low-fat spreads sometimes also contain sorbic acid or benzoic acid, which are added specifically as preservatives. In addition, the concentration of sugars or salts can contribute to preservation, as will be described later. The organic acid components interact with the lipid phase, however, and the effect is a decrease in the preservation of the products.

ORGANIC ACIDS

Acetic, lactic and sorbic acid (and its salts) (Figure 1) are amongst the most widely used antimicrobial food additives. They may be added to foods deliberately, although acetic and lactic acids are also produced in fermented foods as end-products of microbial metabolism. By virtue of their effect on the pH of the food and the antimicrobial properties of the undissociated form of the molecule, these acids inhibit the growth and metabolism of a wide range of pathogenic and spoilage micro-organisms. The target concentration of acetic and lactic acids found in foods is a balance between the effects of the acids on the organoleptic properties of the food, and the concentration of acids required for their antimicrobial activity. Sorbic acid has a neutral taste and odour and the acid and its salts are permitted widely in foods in the USA, where they have «Generally Recognised As Safe» (GRAS) status, although their use in the UK is restricted by statute (Anon, 1979).

The antimicrobial efficacy of organic acids is affected by the fundamental thermodynamic characteristics of dissociation and partition. However, these characteristics of the acids are also key to an understanding of the controlling effect of the lipid phase of emulsions on the growth of micro-organisms, and are described below.

DISSOCIATION

This is the characteristic of a chemical compound to separate into certain component parts. In the case of weak acids, such as acetic and sorbic acids, it is an ionization reaction.

A general equation for compounds that dissociate by ionization in this way is:

\[
AH \rightleftharpoons A^- + H^+
\]

For example, acetic acid dissociates according to the equation:

\[
CH_3COOH \rightleftharpoons CH_3COO^- + H^+
\]

The dissociation is an equilibrium, and the concentration of each side of the equilibrium equation is dictated by the dissociation constant (or Ka) that is a characteristic of the compound, thus:

\[
K_a = \frac{[anion][H^+]}{[undissociated acid]}
\]

So, in the case of acetic acid it is the ratio, thus:
Equation 2

\[ K_a = \frac{[\text{acetate}][H^+]}{[\text{acetic acid}]} \]

The values of \( K_a \) for acetic acid and sorbic acid at 25°C are identical at 1.76 \( \times \) 10\(^{-5} \) moles/litre (Windholz, 1983), and the \( K_a \) of lactic acid is 1.37 \( \times \) 10\(^{-4} \) (Windholz, 1983) (Table II).

The dissociation constant is a cumbersome small value, and, for convenience, is often expressed in the form of its negative logarithm and given the term \( pK_a \).

Equation 3

\[ K_a = -\log(K_a) \]

so that a \( K_a \) of 1.76 \( \times \) 10\(^{-5} \) moles/litre (acetic acid and sorbic acid) becomes a \( pK_a \) of 4.76, and a \( K_a \) of 1.37 \( \times \) 10\(^{-4} \) (lactic acid) becomes a \( pK_a \) of 3.86.

Table II

<table>
<thead>
<tr>
<th>Organic acid</th>
<th>Dissociation constant at 25°C (moles litre(^{-1} ))</th>
<th>( pK_a ) at 25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic</td>
<td>1.76 ( \times ) 10(^{-5} )</td>
<td>4.76</td>
</tr>
<tr>
<td>Lactic</td>
<td>1.37 ( \times ) 10(^{-4} )</td>
<td>3.86</td>
</tr>
<tr>
<td>Benzoic</td>
<td>6.46 ( \times ) 10(^{-5} )</td>
<td>4.20</td>
</tr>
<tr>
<td>Sorbic</td>
<td>1.73 ( \times ) 10(^{-5} )</td>
<td>4.76</td>
</tr>
</tbody>
</table>

The \( pK_a \) varies with temperature, but only slightly, and an empirical equation which describes this variation for a number of weak acids is:

Equation 4

\[ pK_a = \left( \frac{A}{T} \right) - B + (CT) \]

Where \( T \) is the temperature in degrees Kelvin (°K), and \( A, B \) and \( C \) are shown in Table III.

Dissociation is important in the preservation of emulsion foods because the preservatives that undergo these reactions are usually more active in one form than the other. It is the undissociated form that has the predominant antimicrobial effect in foods (Baird-Parker, 1980; Sofos and Busta, 1981; Eklund, 1983), and hence it is the concentration of this form that is important in the formulation of foods that rely on these acids as the preservative.

For any given concentration of these acids in foods a proportion exists as the acidic undissociated form, and a proportion as the dissociated anionic form (See Equation 1 and Equation 2). When the \( \text{pH} \) of a food is equivalent to the \( pK_a \), the proportions of the acid and its salt are equal. If the \( \text{pH} \) of the food is decreased, then the concentration of the undissociated form is increased. If the \( \text{pH} \) of the food increases, however, the concentration of the undissociated form declines.

Increasing Acidity  
(low \( \text{pH} \))

\[
\begin{align*}
\text{CH}_3\text{COOH} & \rightarrow \text{CH}_3\text{COO}^- + H^+ \\
\end{align*}
\]

Decreasing Acidity  
(high \( \text{pH} \))

The Henderson-Hasselbalch equation (Equation 5) derives directly from the definition of \( pK_a \), and relates the \( \text{pH} \) of the food to the \( pK_a \) and the relative proportions of dissociated and undissociated acid, thus:

Equation 5

\[ \text{pH} = pK_a + \log_{10} \left( \frac{[\text{dissociated acid}]}{[\text{undissociated acid}]} \right) \]
This may be rearranged to give the concentration of weak acid in its undissociated (i.e. microbiologically active) form, \([HA]_{aq}\), given the pH, \(pK_a\), and total weak acid concentration, \([HA]_T\), as follows:

\[
[HA]_{aq} = \frac{[HA]_T}{1+10^{(pH-pK_a)}}
\]

Using this equation and a \(pK_a\) of 4.76 (25°C) in the case of acetic acid, the effect of pH on the proportion of the dissociated and undissociated antimicrobial forms is shown in Figure 2.

For example, in a food where the total concentration of acetic acid or lactic acid is 1.5%(w/v), the actual concentration of the antimicrobial undissociated form changes as shown in Table IV.

**Table IV**

<table>
<thead>
<tr>
<th>pH</th>
<th>Concentration (%w/v) of undissociated acetic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>1.47</td>
</tr>
<tr>
<td>3.5</td>
<td>1.42</td>
</tr>
<tr>
<td>4.0</td>
<td>1.28</td>
</tr>
<tr>
<td>4.2</td>
<td>1.18</td>
</tr>
<tr>
<td>4.4</td>
<td>1.04</td>
</tr>
<tr>
<td>4.6</td>
<td>0.89</td>
</tr>
<tr>
<td>4.76</td>
<td>0.75</td>
</tr>
<tr>
<td>4.8</td>
<td>0.72</td>
</tr>
<tr>
<td>5.0</td>
<td>0.55</td>
</tr>
<tr>
<td>5.2</td>
<td>0.40</td>
</tr>
<tr>
<td>5.4</td>
<td>0.28</td>
</tr>
<tr>
<td>5.6</td>
<td>0.19</td>
</tr>
<tr>
<td>6.0</td>
<td>0.08</td>
</tr>
<tr>
<td>6.5</td>
<td>0.03</td>
</tr>
<tr>
<td>7.0</td>
<td>0.01</td>
</tr>
</tbody>
</table>

PARTITION

The lipid component of biphasic foods is key in controlling the concentration of undissociated antimicrobial compounds in the aqueous phase. This is because the antimicrobial undissociated form of organic acids is lipophilic, partitions between the aqueous and lipid components of foods (Gordon and Reid, 1922; Bodansky, 1928; von Schelhorn, 1964; Leo et al., 1971), and hence decreases the concentration of undissociated acid in the aqueous phase. The aqueous phase of many foods, however, contains a range of solutes, often added for organoleptic purposes, or, as in the case of NaCl and sucrose, to decrease the water activity of the food in order to increase its preservation. However, the movement of undissociated organic acids into the lipid phase of biphasic foods can be increased by the addition of solutes to the aqueous phase (Goody et al., 1955; Sofos and Busta, 1981). This further decreases the concentration of the effective form of the antimicrobial in the aqueous phase. In many foods the concentration of acetic, lactic and sorbic acid is poised to the minimum required for conferring microbiological stability and so any decrease in this concentration due to partition into the lipid phase can allow micro-organisms to grow.

The oil:water partition coefficient (\(K_p\)) is the ratio of the concentration of a compound in the lipid phase to its concentration in the aqueous phase. So, for the case of the undissociated weak acid moiety, \(HA\), we have:

\[
K_p = \frac{[HA]_{oil}}{[HA]_{aq}}
\]
The concentration of weak acid remaining in the aqueous phase, and in its undissociated (i.e. microbiologically active) form may be calculated for a biphasic system as follows:

**Equation 8**

\[
[\text{HA}]_{aq} = \frac{[\text{HA}]_T}{1 + K_p \left( \phi \frac{1}{1-\phi} \right) + 10^{(pH - pK_a)}}
\]

where \( K_p \) is the apparent partition coefficient between the aqueous phase and the oil of interest, and \( \phi \) is the volume fraction of oil in the dispersion, given by:

**Equation 9**

\[
\phi = \frac{V_{oil}}{V_{aqueous} + V_{oil}}
\]

where \( V_{oil} \) and \( V_{aq} \) are the volumes of oil and aqueous phases respectively.

### Table V

**Published values for partition coefficients of organic acids commonly used to preserve food emulsions.**

<table>
<thead>
<tr>
<th>Organic acid</th>
<th>Lipid: water partition coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic</td>
<td>0.03 - 0.07</td>
</tr>
<tr>
<td>Benzoic</td>
<td>6 - 13</td>
</tr>
<tr>
<td>Sorbic</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Part of the value of acetic acid as an antimicrobial agent in multiphase foods is due to its low partition coefficient. This means that only a very small proportion of it is dissolved in the lipid phase, and it is therefore predominantly available in the water phase as a preservative. This is not the case for the deliberately-added preservatives such as sorbic acid and benzoic acid, however, where the controlling effect of the lipid phase on their distribution is greater. These acids have a relatively large partition coefficient, and so a large proportion of their total concentration in the emulsion is in the lipid phase, and hence unavailable in the aqueous phase.

Prediction of partition based on published data is difficult, however. Many partition coefficients are determined between model alkanes (e.g. octane) and water (Table V), and comparison of empirically determined partition coefficients between a lipid phase and water show that partition coefficients determined in an alkane (hexadecane):water system are often a poor guide to the value of partition coefficients in a food lipid (sunflower oil):water system (Table VI).

### Table VI

**The partition coefficients of acids in a hexadecane: water system and in a sunflower oil: water system**

<table>
<thead>
<tr>
<th>Acid</th>
<th>Hexadecane:water</th>
<th>Sunflower oil:water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic</td>
<td>0.03 - 0.08</td>
<td>0.02</td>
</tr>
<tr>
<td>Sorbic</td>
<td>0.08</td>
<td>2.15</td>
</tr>
<tr>
<td>Lactic</td>
<td>0.0</td>
<td>0.033</td>
</tr>
</tbody>
</table>

The hexadecane:water partition coefficient and the sunflower oil:water partition coefficient for these preservatives increased when solutes were added to the aqueous phase (Table VII), at concentrations typical of those found in acid-preserved foods, such as mayonnaise (Brocklehurst and Lund, 1984).

### Table VII

**The effect of solutes in the water phase on the partition coefficient in a two-phase system.**

<table>
<thead>
<tr>
<th>Non-polar phase</th>
<th>Acid</th>
<th>No solutes</th>
<th>10% (w/v) NaCl</th>
<th>40% (w/v) Sucrose</th>
<th>Both</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexadecane</td>
<td>Acetic</td>
<td>0.02</td>
<td>0.10</td>
<td>0.62</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>Sorbic</td>
<td>0.08</td>
<td>0.47</td>
<td>0.47</td>
<td>0.88</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>Acetic</td>
<td>0.02</td>
<td>0.08</td>
<td>1.15</td>
<td>1.36</td>
</tr>
<tr>
<td></td>
<td>Sorbic</td>
<td>2.15</td>
<td>4.99</td>
<td>2.87</td>
<td>5.84</td>
</tr>
</tbody>
</table>
(Strictly, if the partition coefficient is defined in terms of the thermodynamic activity of the weak acid rather than the more easily measured concentration, then there would be no effect of additional dissolved solutes on the partition coefficient. The effect observed on the concentration of weak acid is merely a reflection of the dissolved solutes changing the activity of the weak acids.)

Typically, the methodology for determination of partition coefficients involves poising the pH of the system sufficiently low to ensure that the acids are present in the undissociated form, and measuring the residual aqueous phase concentration after equilibrating with a known amount of the oil of interest.

The pH of biphasic foods, however, is typically in a region where weak organic acids are present in both the undissociated and the dissociated form. This makes calculation of the residual concentration of the undissociated form of the acid following partition difficult, as the concentration itself is subject not only to the effects of partition, but to the equilibrium of dissociation based on the new pH of the system and the new residual concentration of the undissociated acid. Such calculations require a more complex approach coupling the equations for dissociation and partition with the buffering behaviour of the complex systems encountered in foods. For a fuller discussion of these issues, see Wilson et al., 2000.

THE EFFECTS OF STRUCTURE

Oil-in-water emulsions

As well as the dairy emulsions such as milk or cream, these also include the «manufactured» emulsions such as salad creams and mayonnaises, where the manufacturer has some control over formulation of the product.

In addition to the effects of the lipid component on the composition of the aqueous phase as described above, the lipid phase imposes structure on these emulsions, and this can affect the form of growth of bacteria, their rate of growth, and the habitat domain in which growth occurs.

In oil-in-water food emulsions the oil phase is present as polydisperse droplets that typically have a mean diameter of between 1 and 8 micrometers (Table I), which means that, in concentrated emulsions, the space of the interstices between the droplets is of the same order of size. This is also the same order of size as many bacteria, which varies between approximately 0.4-1.5 × 0.5-5 micrometers in the case of rod-shaped bacteria or approximately 0.5-2 micrometers in diameter in the case of cocci. In model emulsions where the concentration of lipid phase was low (30% v/v) the growth of bacteria was as free-living (or planktonic) cells. An increase in the concentration of lipid had no effect on the form of growth of bacteria until the lipid content increased to 83% (v/v), when the bacteria became immobilised between the oil droplets. The structure imposed by the lipid phase caused the bacteria to grow not as planktonic cells, but as discrete colonies. Visualisation of micro-organisms within emulsions is difficult due to the opacity of the droplets. However, using a chloroform:methanol solvent system Brocklehurst et al. (1995) and Parker et al. (1995) examined these colonies in situ (Figure 3), and showed that as the colonies formed from a single bacterium they expanded by displacing the emulsion droplets, although eventually the constraint of crowding of the lipid phase caused the colony to grow around some of the droplets (Figure 3).

Water-in-oil emulsions

These include margarines and low-fat spreads. The emulsions consist of an internal aqueous phase
concentration of end-products of metabolism, normally, micro-organisms cease to grow when the number density within a droplet is extremely high. Water-in-oil food emulsions can be contaminated with micro-organisms at the point of manufacture (Verrips and Zaalberg, 1980), and these will be isolated within the droplets of the aqueous phase. The proportion of droplets occupied by micro-organisms is small, and is a function of the initial contamination, and the numbers of droplets exceeding the minimum size for occupancy (Verrips and Zaalberg, 1980; Charteris, 1995).

The micro-organisms metabolise the carbon sources within the droplets, and classical theories to describe microbial growth rely on the droplets remaining as discrete compartmentalised structures, which limit the availability of water, space and nutrients for growth. The microbiological stability of these emulsions is enhanced, therefore, when the droplet size is small, and the controlling effect of lipid in these emulsions is to maintain a distribution of discrete droplets separated from one another, such that if microbial growth occurs in one droplet, it is limited in its extent, and the majority of the aqueous phase remains unaffected in sterile droplets. Based on these assumptions, the models of Verrips and Zaalberg (1980) and Verrips et al. (1980) can be used mechanistically to predict the potential for growth of bacteria within discrete droplets of known dimensions when the growth and energy demands of the contaminating bacteria are known (Ter Steeg et al., 1995). These models show that bacterial growth and survival can be limited when the emulsion structure remains intact, and when coalescence of the droplets does not occur.

In the new generation of low fat spreads, a large volume of dispersed aqueous phase is contained within a small volume of lipid phase, and instability due to coalescence is a key challenge to the manufacture of these products. Coalescence may be spontaneous in inherently unstable emulsions, but can occur in parallel with microbial growth in otherwise stable emulsions, and experiments in model experimental water-in-oil emulsions, which had a fluid lipid phase, showed that an increase in numbers of bacteria was always accompanied by coalescence of the droplets of aqueous phase (Brocklehurst et al., 1993). This is an example of where the controlling effect of a solidified lipid phase was removed.

Although the bacteria in the droplets can grow well, their numbers remain small when expressed per unit volume of emulsion, although their local number density within a droplet is extremely high. Normally, micro-organisms cease to grow when the concentration of end-products of metabolism, typically organic acids, produced during growth becomes toxic or if a requirement for growth, such as oxygen or a carbon source, is exhausted. However, the lipid phase of emulsions could decrease the concentration of organic acid within the aqueous phase by partition, as described above, and this may contribute significantly to high local numbers of bacteria within droplets.

REFERENCES


