Microbiological Spoilage
Of A Moderate Acid Food System Using A Dairy-Based
Salad Dressing Model

A Thesis
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by

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* * * * *

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To My Husband and Parents
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INTRODUCTION

Increasing the acidity of food has been used for many years to deter microbial spoilage of foods. Mayonnaise and traditional pourable salad dressing are two microbiologically stable food products primarily due to a high acid concentration (Minor and Marth 1972). Acetic acid is the predominantly used acidulant in salad dressings and mayonnaises which have a characteristic tart acidic taste. The pH range for these products is traditionally from 3.2 to 3.9.

Within this pH range, microbial spoilage of these food products is largely due to only a few groups of microorganisms surviving or growing at low pH. The most common spoilage organisms include the lactic acid bacteria and yeast. Pathogenic bacteria are not considered to grow in salad dressings and mayonnaise production and cause problems (Smittle 1977).

In recent years United States consumption trends have moved toward mild creamy varieties of salad dressings and away from traditional tart, acidic products as evidenced by the popularity of "ranch style" and
"creamy" salad dressings. Dairy products are among the dominant ingredients in these "creamy" dressings. Various ingredients such as vegetables and meat are also included in current dressing formulations. These ingredients act as buffers, a source of contamination and provide nutrients allowing the growth of fastidious organisms (Simmons 1979). Due to the buffering capacity of these ingredients, the pH of dressings may rise above the traditional pH range of 3.2 to 3.9 for salad dressings and it is conceivable that number and type of spoilage organisms will increase in these formulations.

This investigation has evaluated the growth of two common spoilage organisms and a pathogen in a pourable salad dressing model at different pH values. A model salad dressing was developed to represent current creamy formulations. Nonfat dry milk was included in the model to simulate the buffering activity of the commonly added ingredients in today's "ranch style" dressings. No traditional preservatives which might affect microbial growth behavior were included in the formulations.

Acetic acid or vinegar and gluconic acid were the two organic acidulants used in this investigation. Acetic is the most commonly used organic acid in mayonnaise and pourable salad dressings. Gluconic acid is recognized as GRAS by the FDA and has a mild sweet flavor. Its use
in food products remains largely unexplored. In contrast to acetic acid which at higher concentrations imparts a harsh acidic taste to dressings, gluconic acid imparts little flavor penalty even at higher concentrations. In this investigation the effect of acetic and gluconic acids on the growth behavior of the test organisms was determined over the pH range of 3.0 to 4.5.

By increasing the concentration of acetic acid the growth of yeast can normally be inhibited; however, higher concentrations of acetic acid are necessary to prevent spoilage of dressings by lactobacilli species. Usually the taste penalty incurred at higher acetic acid concentrations makes inhibition of lactic acid bacteria in this manner undesirable. Preservatives such as benzoate or sorbate can be added to achieve acceptable microbiological shelf life. Their addition is limited by the pH of the dressing and FDA concentration regulations. Also, many spoilage bacteria and yeast can become resistant to benzoate and sorbate (Emard and Vaughn 1952). Therefore, controlling microbial stability in pourable salad dressings presents a challenge.

In addition to exploring the growth of the common spoilage organisms and a pathogenic bacterial strain in a dairy-based salad dressing model, a salad dressing
formulation that is microbiologically stable to yeast and lactobacilli was developed. This stability was achieved by the addition of gluconic and acetic acids to the formulations. The model dressing contains no traditional preservatives (benzoate or sorbate) and has a mild flavor. The pH and acid concentration parameters to obtain this microbiologically stable salad dressing are defined.
LITERATURE REVIEW

I. Fates of Microorganisms in a Food Product

Microorganisms are an intrinsic part of life. They have colonized practically every possible niche on our planet and few places remain truly sterile or without the presence of microorganisms. Food products grown in soil, from animal origin or containing ingredients from these two sources harbor many microorganisms and provide nutrients for their growth, reproduction or survival. In fact, many food products such as pickles, cheese or yogurt are fermented by microorganisms to aid in preservation. These products are produced by the metabolic activity of specific microorganisms which contribute to distinctive organoleptic or physical properties of the foods.

As previously mentioned, the raw ingredients of a food product provide a source of microorganisms in the product; likewise, the processing environment, handling and storage of the product and the packaging material can also act as sources of microbial contamination. The chemical and physical nature of each food product
defines which organisms may survive in the product. Once introduced to a food product, microorganisms have three fates: they may grow and reproduce, survive without growth or die. Over the shelf life of a product all three options may be encountered. For example, a bacterial contaminant may grow rapidly in a food product producing toxic waste products which accumulate to sufficiently high levels causing the death of that species in the product; in turn, another species may begin to now grow in the product. Another scenario is that microorganisms may survive in a product as it is held at refrigerated temperatures and then begin to exponentially grow if the product is stored at room temperature. The number of alternative situations is great.

Microorganisms in food may act as spoilage organisms, pathogens, favorable actors in a food process or they may have no effect in a food product. It is advantageous to inhibit the growth of spoilage organisms and pathogens to extend the shelf life of a product and to prevent human illness and potential morbidity or mortality. Whether an organism grows, survives or dies in a food product is dependent on several variables affecting the microbial growth behavior in food. These
variables include temperature, water activity, pH, oxidation/reduction potential and substrate availability. The effect of these variables on microbial growth is largely due to the effect on microbial enzyme systems (Banwart 1979).

II. Variables Affecting Microbial Growth in Food

Temperature

Each microorganism has a specific temperature range with a maximum, optimum and minimum temperature at which the organisms will grow. This temperature range is linked to the temperature range of microbial metabolic enzyme activity. Above and below certain temperatures, microbial enzyme activity will cease ultimately causing death of the microorganisms.

Organisms growing in the range 0-20°C are defined as psychrophiles. Obligate psychrophiles will not grow well above approximately 20°C, while facultative psychrophiles and psychrotrophs usually have a maximum temperature near 40°C. Psychrotrophs are considered to multiply as low as 5°C. Those organisms growing optimally in the range of 25-45°C are termed mesophiles. Thermophiles are defined as having an optimum growth temperature of 45°C with obligate thermophiles unable to
grow below 37°C. The differences in growth temperature ranges can be attributed to the differences in temperatures of maximum enzyme activity, the microbial cell membrane chemical content, and the thermal stability of protein synthesis machinery or organular membranes.

Thermophiles, mesophiles, and psychrotrophs are all considered of importance in food products. The large percentage of the food supply stored at refrigerated temperatures makes psychrotrophs particularly important as spoilage organisms. The temperature range of microbial growth also reflects the requirement of water for growth. Therefore, the growth temperature range corresponds to temperatures when water is in the liquid phase.

**Water Activity (Aw)**

Microorganisms require an aqueous solution for growth and to conduct normal metabolism. In dried conditions, survival of organisms can occur, but growth cannot. Water acts as a solvent carrying nutrients and waste products in and out of the cell and as a substrate in hydrolysis and other chemical reactions within the cell. A food product must contain enough available water to support microbial growth. The moisture content of foods
is composed of "bound" and "free" water. The "free" water is used for chemical reactions and microbial metabolic activity. It is measured as the water activity (Aw) and is defined as:

\[ Aw = \frac{P}{P_0} \]

where \( P \) = vapor pressure of water above a material and \( P_0 \) = vapor pressure of pure water at the same temperature (Banwart 1979).

Water activity is related to the equilibrium relative humidity (ERH) and can be expressed as:

\[ Aw = \frac{ERH}{100}. \]

In a food product, water activity is also affected by temperature. It will also be affected by the atmosphere in which the product is contained and the conditions of shelf life.

Microorganisms have a range of water activities for growth (maximum, minimum, and optimum). The maximum water activity for growth is slightly less than 1.00. For most bacteria, the minimum water activity is 0.90. Yeast have a lower water activity growth range than bacteria with a minimum range of 0.87 to 0.94. Most molds have a minimum growth range of 0.70 to 0.80 and xerophilic molds may multiply at values as low as 0.60 to 0.70.
Certain species of bacteria such as *Staphylococcus aureus* and the halophilic bacteria have lower minimum water activity growth ranges of 0.86 and 0.75 respectively (Banwart 1979).

The minimum water activity that organisms may grow is affected by the specific solute lowering the activity (Banwart 1979) and influences the way microorganisms will react to storage times. A lower water activity will increase resistance to heating and increase survival for longer storage times. In a food product the water activity is reduced in three ways: dehydration, addition of solutes such as sugar or salt, or freezing. The water activity of the food will select which microorganisms will dominate. With a high water activity food the prominent spoilage organisms will be bacteria and in a low water activity food, mold and yeast may dominate.

**pH**

pH as a measure of acidity is an important factor in the growth of microorganisms. It can be defined as the negative logarithm of the hydrogen ion (proton) concentration. In practice it is more correct to describe pH as the hydrogen ion activity present in the aqueous fraction of the sample. As for water activity
and temperature, microorganisms have a minimum, optimum and maximum pH for growth. A pH of near 7.0 is the optimum growth pH for most bacteria and is the pH of the microbial cell interior (Banwart 1979). The minimum and maximum values are 4.5 and 9.0 respectively for most bacteria, 1.5 and 11.0 for molds, and 1.5 and 8.5 for yeast (Baird-Parker 1980a). Different species or strains have a wide range of variation in their pH growth range. The pH growth range for a particular organisms or strain may also be affected by storage temperature, type of growth medium, heat processing, oxygen tension and competing microorganisms (Baird-Parker 1980a).

The pH range for survival, toxin production and growth may or may not overlap. The type of substrate and acid or base used to adjust the pH may vary the pH growth range (Banwart 1979). The pH of a food product may alter the availability of metallic ions needed for growth or influence cell permeability. At low pH a saturation of the microbial cell membrane with hydrogen ions can occur limiting passage of essential cations. At high pH, hydroxyl ions can saturate cell membranes inhibiting essential anion passage.
Foods may be categorized by their pH. Foods may be classified as high acid, acid, medium acid or low acid foods (Table 1). Unprocessed meats, fish and vegetables normally have pH values of 5.5 to 6.8; fruits, 3.0 to 4.6 and at the other end of the pH spectrum, egg whites at greater than pH 7.0 (Wagner and Moberg 1989). The pH of the food is affected by the buffering capacity of its ingredients. For example, vegetables have a lower buffering capacity that foods containing protein and hence, less addition of acid to vegetables is required to lower pH.

Substrate Availability

Substrate used as a source of energy must be available within a food to support microbial growth. Although different organisms utilize different substrates for metabolism, there are common microbial growth requirements: a carbon and nitrogen source, water and growth factors such as vitamins and minerals. Heterotrophs or organotrophs obtain carbon for biosynthesis from organic compounds. Autotrophs acquire carbon from carbon dioxide and can live in the absence of complex organic substrates.

Within a food, the spoilage microorganisms and pathogens of most concern are heterotrophs which use
Table 1  Food Acid Categories

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<td>&lt; 3.7</td>
</tr>
<tr>
<td>Acid</td>
<td>3.7 to 4.6</td>
</tr>
<tr>
<td>Medium Acid</td>
<td>4.6 to 5.3</td>
</tr>
<tr>
<td>Low Acid</td>
<td>&gt; 5.3</td>
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organic compounds as carbon and energy sources. Carbohydrate, fats, minerals, proteins, vitamins and water, the necessities for microbial growth are present in most forms of foods. The difference in composition will determine which types of microorganisms may grow in the food.

**Oxidation/Reduction (Redox) Potential**

Simultaneous redox reactions are the source of metabolic activity in microbial cells. Electron transfer is the basis of these redox reactions. In oxidation, a reactant gives up an electron and becomes oxidized. In reduction, another reactant accepts this electron and becomes reduced.

The oxidation/reduction potential of a substance is a measure of its tendency to give up electrons and become oxidized. Foods have a redox potential dependent on the redox potential of the constituents, the oxygen tension of the atmosphere and access of the atmosphere to the food.

In microbial metabolism organic compounds are converted into energy and waste products through redox reactions. Aerobic organisms utilize respiration for metabolism where carbon dioxide is the endproduct and molecular oxygen is the terminal electron acceptor in a
series of redox reactions. Fermentative organisms produce various organic endproducts and oxidation/reduction occurs without an added electron acceptor. Anaerobes utilize carbon dioxide, nitrate, sulfate, nitrite or fumarate as terminal electron acceptors and produce carbon dioxide as an endproduct. Anaerobes lack the terminal cytochromes necessary to transfer electrons to oxygen (Brock 1979). Facultative anaerobes transfer electrons to oxygen or nitrate if it is present or utilize fermentative pathways for energy production.

Foods with a higher redox potential usually have shorter shelf-lives. Vacuum packaging is used to change the atmosphere the food, to decrease oxygen content and to increase shelf life. Obligate aerobes require oxygen for growth due to their inability to generate energy by fermentation and will not grow in a reducing environment. Microaerophilic organisms require oxygen for growth, but grow best at low oxygen pressures (0.2 atmospheres). They will grow in oxidizing and reducing environments if levels of oxygen are not prohibitive.

Some facultative organisms grow in either reducing or oxidizing environments due to their ability to carry out respiration or fermentation. Aerotolerant anaerobes do
not use oxygen but are not harmed by its presence. Obligate anaerobes are drastically harmed by oxygen and will die in an oxidizing environment due to their lack of superoxide dismutase (an enzyme destroying superoxide, a toxic intermediate formed in respiration). Instead these organisms may grow in foods with a low redox potential.

III. Methods of Food Preservation

Food preservation methods include removing microorganisms present, destructing microbial contaminants, restricting the growth of microorganisms or preventing microbial contamination. It is usually not practical to remove microorganisms from food products. Therefore, prevention of contamination, microbial destruction and restriction of microbial growth are the foremost methods of food preservation. Also, the majority of preservative methods currently used focus on slowing or inhibiting growth of microorganisms verses killing them (Gould et al. 1983).

The prevention of contamination is carried out by sanitation measures such as proper cleaning of collection, processing and storage equipment. Also of importance is maintenance of these environments to decrease the number of organisms present by techniques
such as positive air flow ventilation systems and air filtering. Choosing raw material of high quality with low microbial loads will decrease the numbers of microorganisms present in the food product and help minimize microbial contamination. Aseptic packaging and/or processing also plays an important role in preventing recontamination in certain food products.

The numbers of microorganisms in a food may be reduced by removing them from the product. This can be accomplished by washing foods before processing. Centrifugation or filtration can also be used to remove organisms or spores from a liquid food. Decontamination of packaging materials by use of chemicals, heat or irradiation also aids in lowering the number of microorganisms in the final product (Gould et al. 1983).

Food products may be preserved by controlling the variables of temperature, water activity, pH, redox potential or substrate availability. By manipulating these variables microbial growth in a food may be restricted or destruction of the organisms may be achieved.

Temperature

Controlling the storage temperature or process temperature is widely used to affect the growth of
microorganisms. As previously discussed the microbial growth and enzyme activity is linked to the environmental temperature. To inhibit and in some instances cause cell destruction, the environmental temperature can be raised above or lowered below the growth or survival temperature range depending on the particular food product and contaminants to be removed. Different types of foods may be destroyed by heating or cooling treatment making the use of this preservation method not practical.

Refrigeration (0-5°C), freezing (-10-0°C), and rapid cooling are examples of lowering temperature to control microbial growth. Even when food is frozen, microorganisms may only be reduced in number, not destroyed (Banwart 1979). Spores and toxins resistant to freezing are not destroyed. Whether microorganisms survive or die during freezer storage is dependent on several factors: the specific organism, age and population density of cells, cooling rate, minimum temperature, type and pH of suspension medium, length of storage and rate of thawing (Banwart 1979).

Heat treatment of a food product is the most frequently used method to kill microorganisms and increase storage life. Heating the food product to
certain temperatures may result in protein denaturation or inactivation of microbial enzymes and enzymes contained in the product further lengthening storage (Banwart 1979). Mild heat treatment is often used in conjunction with refrigeration, freezing and other preservation methods.

Cooking, scalding, pasteurizing, blanching, canning, evaporation, drying, distillation and concentration are processes involving the application of heat. Foods with low pH and high water activity require less heat treatment to reduce cell numbers than foods with higher pH and lower water activity.

**Water Activity**

By changing the water activity of a food product to ranges outside that for growth, growth can be restricted. This is usually accomplished by reducing the water activity of food products through drying, concentration, or raising the solids content of the product. Drying reduces the amount of available water for microbial growth and has been used for centuries as a preservative method. In addition to prevention of microbial growth, drying acts to prevent chemical reactions supported by moisture, reduce transportation costs, recover waste products and bring
water content to a preferred level (Banwart 1979).

Hot air drying, spray drying, vacuum drying and freeze drying are the four principal methods of drying used today. After drying the food product is not sterile; the extent of drying lethality is dependent on the organism, temperature, suspension medium, time, rate and method. For example, although stressing the organisms, freeze drying is used as a method for microbial culture preservation. Care must be taken upon rehydration of dried foods to prevent growth of spoilage or pathogenic microorganisms.

The water activity of a food can also be lowered by concentrating the product or by adding solutes such as sugar or salt to increase the solids level. The water activity of foods must be lowered to 0.60 or less to be considered essentially free from potential microbial growth (Banwart 1979). Intermediate moisture foods utilize this lowering of water activity to 0.60 to 0.90 and often the addition of preservatives to limit growth. Potassium sorbate, calcium propionate or propylene glycol and/or reduced oxygen packaging may be required to limit the growth of yeast and mold. Jams and jellies are examples of intermediate moisture foods where sugar is added for flavor and to reduce water activity.
**Modified Atmosphere**

Controlling the atmosphere of a food product is another method to preserve food products. Growth of mold and other aerobic spoilage organisms can be retarded by eliminating oxygen or lowering the oxygen concentration in packaging. This can be accomplished by vacuum packaging; the oxygen in a food package is evacuated and replaced with carbon dioxide or nitrogen.

Daniels *et al.* in a 1985 review article noted that high concentrations of carbon dioxide are effective in inhibiting the growth of microorganisms in many foods, although the exact mechanism is not known. The lag phase and generation time of the spoilage organisms is increased in the presence of high carbon dioxide concentrations; however, in concentrations slightly above normal, an increase in growth is observed. The exclusion and replacement of oxygen by carbon dioxide are thought to provide a small bacteriostatic effect. The high permeability of carbon dioxide through the cell membrane and the rapid acidification of intracellular pH upon arrival of the carbon dioxide to the cytoplasm appear to have an inhibitory role. In addition, carbon dioxide exerts an inhibitory effect on certain enzyme systems in some microorganisms. Nitrogen itself does not directly
inhibit growth, instead it acts by replacing oxygen needed for aerobic organisms for growth and survival (Wagner and Moberg 1989).

The food industry has also explored the use of carbon monoxide, ethylene oxide, propylene oxide, sulfur dioxide and ozone as antimicrobial gases. Sulfur dioxide is widely used to inhibit growth of yeast, mold and bacteria in fruit, fruit juices, wines, seafood and fermented vegetable products (Wagner and Moberg 1989). Similarly ethylene oxide has been used in dry foods and packaging materials.

**pH**

Adjusting the pH of foods is a very effective preservation method. As previously discussed there is a pH range of growth (minimum, optimum, and maximum) for each organism varying from organism, species and strain. For centuries the pH of foods has been lowered (artificially or naturally) to increase the shelf life of foods. Raising the pH above the maximum pH values for microbial growth is usually not used due to negative alterations of the chemical, physical and flavor properties of foods. One example of negative alterations is the bitter taste associated with alkaline foods. Lowering the pH of a food will inhibit the growth of certain organisms while
selecting for the growth of others.

Three types of acid preservatives exist: strong inorganic acids, weak lipophilic acids and acid potentiated ions (Baird-Parker 1980a). The microbial intracellular pH is affected by the pH of the environment (media or food) in which the cell resides. Strong inorganic acids dissociate completely in solution producing a high external proton concentration. This high external proton concentration affects the intracellular pH as protons leak into the cell cytoplasm. These protons must be removed, an energy demanding process, to maintain a near neutral intracellular pH and prevent acidification (Gould et al. 1983). As energy is expended to maintain intracellular pH, less energy is available for biosynthesis and growth. When energy generation fails to expel protons from the cytoplasm, the cell interior is acidified causing the destruction of acid labile cell components such as DNA and ATP and cell death occurs (Brock 1979). Most foods do not have a sufficiently low pH to achieve this effect.

Weak lipophilic acids dissociate into protons and dissociated anions in aqueous solution. The extent of this dissociation is affected by the pH of the solution.
In the undissociated form, weak lipophilic acids can freely diffuse through the cell membrane and dissociate in the more alkaline cytoplasm into protons and the dissociated forms (anion) of the acids (Herrero et al. 1985). In the rate limiting step of proton transfer across the cell membrane, the negatively charged anion form is returned to the outside, in order that it might pick up a proton and begin the transfer cycle again (Freese and Levin 1978). A dimer form of the anion can be formed which is more lipophilic than the anion and facilitate passage through the cell membrane (Freese and Levin 1978). As this dimer is excluded from the cell, a proton is left behind lowering the intracellular pH. This can inhibit substrate transport and oxidative phosphorylation (Baird-Parker 1980a).

With the resultant increase in intracellular protons, the lipophilic acid acts as an uncoupler of the membrane pH gradient and membrane potential (proton motive force). The acid diffuses through the cell membrane shuttling protons into the cytoplasm disrupting the proton motive force and interfering with the active transport of substrates requiring a proton motive force for entry (Freese and Levin 1983, Herrero et al. 1985). However, certain substrates do not require this proton
motive force for active transport and their transport can generate a proton gradient across the cell membrane. This can act to offset the loss of proton motive force due to presence of lipophilic acids. For example, in the presence of glucose, which does not require the proton motive force for entry, a proton motive force is generated and microbial growth may take place even in the presence of low concentrations of lipophilic acids.

For lipophilic acids to be effective microbial growth inhibitors, in the presence of such carbon sources, more protons or as least as many protons as are exported by the electron transport system must be imported (Freese and Levin 1978). Also in terms of preservative efficiency, acids that can be easily metabolized by microbial metabolism will not have as great a preservative effect as unmetabolizable sugars. Different organisms react differently to specific weak lipophilic acids due to different anion transport mechanisms, metabolisms and membrane permeability. Weak organic acids effect the intracellular pH more greatly than strong inorganic acids (Baird-Parker 1980a).

As most bacteria grow well between pH 5 and 8, lowering the pH to below 5 will limit the growth of most bacterial organisms and pathogens. Certain bacteria
such as the lactobacilli and acetic acid bacteria are able to tolerate lower pH and are of importance in the production and spoilage of high acid foods. At low pH the resistance of spores and vegetative cells to heat is lessened (Baird-Parker 1980a). Therefore, acidification of foods can also prevent spoilage by sporeforming bacteria. The antimicrobial activity of an acid preservative, influenced by the concentration of undissociated molecules, is increased as the pH of the food is lowered.

Yeast and mold are usually acid tolerant and require additional methods of preservation other than lowering the pH to inhibit their growth. Growth of molds which are aerobic organisms is retarded by reducing the oxygen content with vacuum packaging. Addition of fungistats is also helpful in certain instances to control yeast and mold growth.

The type of acid used to lower pH is critical in limiting the growth of pathogens and yeast as certain acids are more antimicrobial than others. Branched chain fatty acids and unsaturated fatty acids are more antimicrobial than their straight chain or saturated counterparts (Banwart 1979). With increasing chain length, antimicrobial activity increases up to a length
of ten or eleven carbon atoms above which water solubility becomes a problem (Kabara 1981). Due to differences in cell membrane, long chain fatty acids are more effective antimicrobial agents against gram positive organisms than gram negative organisms. This is due to the inability of the long chain acids to penetrate the lipopolysaccharide layer of the gram negative bacteria and enter the cytoplasm. For high molecular weight fatty acids to inhibit the growth of gram negative organisms, the fatty acid must be used at a much higher concentration than used to inhibit gram positive organisms (Freese and Levin 1978).

The optimum chain length of fatty acids for antimicrobial activity against gram positive bacteria is twelve (Kabara 1981). Short chain fatty acids with chain lengths of two to six are effective against molds and gram positive and negative organisms. Most chemical preservatives with an antimicrobial activity are short chain organic acids; further discussion of the fatty acid properties will be included under the heading of chemical preservatives.
Chemical Preservatives

Chemical preservatives can be added to foods to prevent the chemical and biological decomposition of the food products. Microbial inhibitors, antioxidants, acidulants and sequestrants are included in the preservative category.

The antimicrobial activity of preservatives is affected by the type and concentration of the preservative, the type and number of organisms, the pH and composition of the food and the storage temperature (Banwart 1979). For example, spores are more resistant to preservatives than vegetative cells and older cells are more resistant than younger cells. Preservatives may act as bacteriostats or fungistats retarding growth or as fungicides and bacteriocides causing the destruction of cells. They may work in a variety of ways including interfering with genetic systems and cell membranes, inhibiting enzyme activity or binding essential nutrients (Banwart 1979).

Preservatives can be added directly to the food product or incorporated in the wash water or wrapping materials. Care must be taken in the use of preservatives to insure the levels of microorganisms present in the food is fairly low. In a survey of
preservative effectiveness against yeast, Jermini and Schmidt-Lorenz (1987) determined that preservatives are not effective if the contamination levels exceed 1000--10,000 cells/g.

Most preservatives used in foods for an antimicrobial effect are short chain organic acids such as acetic, benzoic, propionic, sorbic and other acids. This is due to their water solubility, taste and low toxicity (Baird-Parker 1980a). Their usefulness in foods is determined by the interrelationship between the pH of the food and pKa of the acid (90%). The pKa of the acid is the pH at which fifty percent of the acid is in the undissociated (antimicrobial) form. These antimicrobials will be effective against microbiological growth in foods with pH plus or minus one pH unit of the pK of that acid. Most acid preservatives are effective only in foods with a pH of less than 5.5 (Baird-Parker 1980a). The efficiency of their use is also limited by the presence of resistant organisms and organisms capable of using the organic acid as a metabolizable carbon source. For further discussion of the exact mechanism of the antimicrobial activity of organic acids, see the previous "pH" heading.
Acetic acid is widely used in the food industry due to its water solubility, low toxicity, low cost, GRAS FDA standing, and effectiveness against a broad spectrum of microorganisms. Only Acetobacter species, some lactobacilli, yeast and molds are resistant to acetic acid. Acetic acid with a pK of 4.76 is most frequently used in moderate to high acid food systems such as pickled meat, fish, catsup, mayonnaise and pickles. Acetic acid in foods functions as an antimicrobial agent and a flavorant. Sodium diacetate, dehydroacetic acid and its sodium salt are used as fungistats in alcoholic beverages and baked goods.

Citric and lactic acids are used primarily in low pH foods due to their pK of 3.1. Propionic acid is used extensively in baked goods to prevent the growth of mold and Bacillus mesentericus, the causative organism of "rope in bread". Propionic acid does not have any effect on yeast fermentation (Kabara 1981). Propionates have little antimicrobial activity against bacteria other than B. mesentericus. Propionic acid is water soluble, GRAS, and has a pK of 4.87. Its calcium and sodium salts are also widely used in the same food products as propionic acid.
Sorbid acid is the only FDA permitted unsaturated organic acid acting as a food preservative. It has a pK of 4.8 and is used in foods with pH below 6.0. Fruit juices and soft drinks are just two of the many products which contain sorbic acid. Its salt forms are more effective against yeast and molds than bacteria and are more water soluble than sorbic acid. Lactic acid bacteria and other catalase negative bacteria are generally not affected by sorbic acid (Emard and Vaughn 1952). Therefore, sorbic acid can be used in lactic fermentations to control yeast and mold.

Benzoic acid, naturally found in cranberries, prunes, cinnamon and cloves, is effective in controlling yeast and mold growth and, to some extent, bacterial growth. However, many spoilage bacteria are resistant to benzoate and therefore its use is limited (Chichester and Tanner 1972). The pK of benzoic acid is 4.2. In addition to benzoic acid, sodium benzoate and the para-hydroxyl esters (parabens) are also used in food applications. The parabens with a pK of 8.5 have the advantage of effective use in high pH foods (Kabara 1981). Similarly to benzoic acid, parabens are more effective antimicrobial agents against mold and yeast than gram negative bacteria. Their antimicrobial
activity is directly linked to chain length increasing with alkyl chain length.

Kabara (1981, 1982) has also determined the antimicrobial activity of some chemical preservatives that are not organic acids. Kabara (1981, 1982) determined that monoesters of glycerin such as monolaurin, diesters of sucrose and polyglycerol ester have antimicrobial activity against some microorganisms. Noting the antimicrobial activity of antioxidants such as BHA and BHT, Kabara (1981, 1982) proposed a combination preservative system using monolaurin, a food grade phenolic (paraben, BHA or BHT) and a chelator. This combination system is more effective antimicrobially than the individual constituents, has a wider spectrum of activity, and utilizes the multiple functions of food additives.

EDTA (ethylene diamine tetraacetic acid) is a chemical preservative, which is not a short chain organic acid. It is added to foods for its chelating action. The primary action of EDTA is to bind metal ions which can accept and donate electrons in the free radical mechanisms of lipid oxidation. By binding metal ions, free radical formation and propagation is reduced along with the off-flavors produced by these oxidation
reactions. Citrates, pyrophosphates, and polyphosphates are also used as chelators in foods. EDTA and these compounds have an effect on the growth of microorganisms by chelation of trace metals essential for growth and metabolism of certain organisms.

EDTA and other chelators when used with other preservatives appear to make the microbial membranes more sensitive to the bacteriocidal substances (Kabara 1981). For example, EDTA appears to cause portals in cell membranes in the presence of lipophilic acids. Due to this effect, high molecular weight (long chain) fatty acids are effective preservatives against gram negative bacteria such as *E. coli* in the presence of EDTA (Freese and Levin 1978). Therefore, EDTA and other chelators, although having little antimicrobial activity by themselves, are considered potentiaters of antimicrobial activity.

Propylene glycol, another non-organic acid preservative, is utilized for not only its water binding activity in intermediate moisture foods, but also as an antimicrobial.
IV. Salad Dressing

Types and Composition of Salad Dressings

Salad dressings are composed of "oil in water" emulsions where water is the continuous phase and oil is the discontinuous phase. Three types of food products falling into the category of salad dressings are mayonnaise, "pourable", and "spoonable" salad dressings. Mayonnaise, "pourable", and "spoonable" salad dressings differ in the amount of oil present, the seasonings incorporated and the viscosity of the final product. Salad dressing was first introduced in the 1930's as a low cost substitute for mayonnaise (Smittle 1977).

The U.S. Food Drug Administration (FDA) defines the standard of identity of mayonnaise as an emulsified semisolid food prepared from vegetable oil (at least 65%), vinegar, lemon or lime juice and egg yolk containing ingredients (Anon 1979b). Optional ingredients that may be included are salt, sweeteners, spices, monosodium glutamate, sequestrants, citric acid and/or malic and crystallization inhibitors. The preservatives, sodium benzoate and/or potassium sorbate, are often incorporated as preservatives (Smittle and Flowers 1984).
In addition, the pH of mayonnaise must be below 4.1 and 1.4% acetic acid or more must be incorporated in the aqueous phase if raw eggs or raw egg yolk containing ingredients are used (Anon 1979b). In general, a typical formula for mayonnaise contains 9-11% salt, 7-10% sugar, 70-80% vegetable oil and 0.3-0.5% acetic acid producing a creamy pale yellow and mild tasting product with a pH 3.6 to 4.0. Mayonnaise has a titratable acidity range of 0.30 to 0.38% acetic acid (Fabian and Wethington 1950).

In contrast, salad dressings may contain a heated starch paste, more acid (double) and sugar (triple), and less salt (one-third) and vegetable oil (one-half) than mayonnaise. The FDA defines salad dressing as an semisolid emulsified food with the same ingredients and optional ingredients as mayonnaise with the exception of the inclusion of a cooked or partially cooked starch paste. Likewise, the pH of 4.1 and below and 1.4% acetic acid are required if raw egg ingredients are used. A typical salad dressing formulation includes 0.9 to 1.2% acetic acid, 3-4% salt, 20-30% sugar and at least 30% vegetable oil. The pH range is from 3.2 to 3.9 and the finished product has a tart taste. Both mayonnaise and salad dressings finished products are required to be
held 72 hours before being made available to the consumer (Anon 1979b and 1979c).

"Pourable" or "spoonable" salad dressings have no FDA standard of identity with the exception of French dressing (Anon 1979a). They vary greatly in flavor and chemical and physical properties (primarily viscosity). Pourable dressing may be composed of two phases separating into oil and water requiring shaking before use or as a homogeneous phase. Composed of the same general ingredients as mayonnaise and salad dressing, these products traditionally have a pH range of 3.5 to 3.9. Acetic acid and other organic acids (sodium benzoate and/or potassium sorbate) are the primary preservative agents in pourable or spoonable salad dressings (Smittle and Flowers 1982). They have titratable acidity ranges of 0.82 to 1.41% acetic acid (Fabian and Wethington 1950). "Spoonable" dressing and some pourable dressing contain less acid than mayonnaise or salad dressing and may require refrigeration to maintain microbial stability.

Preparation of Salad Dressings and Mayonnaise

Colloid mills, homogenizers, and liquid whistles are used in the production of mayonnaise and salad dressings. A colloid mill mixes the ingredients of the
mayonnaise and salad dressings intimately by utilizing the shear and turbulence created by passage of liquids between two surfaces that are closely spaced, the stator and the rotor (Desrosier 1977). Colloid mills are suited to the mixing of higher viscosity materials and pressure homogenizers are used with lower viscosity materials. Homogenizers pass fluids thorough a thin orifice at high pressures and speeds to produce a very thorough mixture of the ingredients of the fluid (Farrall 1976). Liquid whistles use high energy ultrasonic waves to tense and compress liquids resulting in emulsification (Desrosier 1977).

In the production of mayonnaise the ingredients are blended in refrigerated tanks with high-speed beaters. The salt is mixed with the egg components and refrigerated. Next, the spices, sugar and vinegar are added to the refrigerated salt and egg mixture and then the oil is gradually incorporated. After a coarse emulsification a colloid mill is utilized to homogenize the product prior to packaging (Desrosier 1977).

In the production of salad dressing, the starch, water, salt, and vinegar are mixed and heated to approximately 90°C to form a starch paste. After cooling, the eggs, sugar, spices and oil are then
incorporated into the paste. Passage through a colloid mill, liquid whistle or homogenizer takes place before packaging (Baird-Parker 1980b).

The exact addition of the ingredients in mayonnaise and salad dressing varies as the ingredients themselves may vary. For example, vinegar can be added before or after the addition of oil. This is particularly true of pourable or spoonable salad dressings. These dressings may contain egg in which is lecithin, a natural emulsifier, or the addition of emulsifiers such as Polysorbate 60 is made to these dressings. Gums such as xanthan or tragacanth are commonly incorporated to act as stabilizers and viscosity enhancers (Desrosier 1977). High fructose corn syrup is commonly used in the food industry as the primary sweetener in salad dressings.

In pourable dressings with an oil and aqueous phase, the solid components (for example, salt and seasonings) are dry mixed. The solid components are then mixed with water and this mixture added to the oil. The dressing is then packaged. In spoonable or pourable dressings appearing as one phase, an emulsifier and/or gum are used and with mixing in a colloid mill before homogenizing. If used, the gum is hydrated in the water. The dry mixed solids and any liquid components
(high fructose corn syrup) are then mixed into the gum and water mixture. If an emulsifier is used, it is normally distributed in the oil prior to mixing with the aqueous portion. The acid (vinegar) can be incorporated after or before the addition of the oil (Smittle and Cirilgiano in press). The dressing is emulsified by a colloid mill or homogenizer and packaged.

**Spoilage of Salad Dressings**

Smittle in a 1977 review article noted that two sources of quality assurance problems exist in the manufacture of salad dressings; chemical and biological. The chemical aspects include emulsion separation and oil oxidation and hydrolysis. The oxidative rancidity of the oil results in flavor deterioration. The biological spoilage results in gas and off-flavor production. A loss of viscosity in the product also may accompany the microbial spoilage (Baird-Parker 1980b).

However, mayonnaise and salad dressings are resistant to spoilage by most microorganisms. This protection against growth of most microorganisms in these products is due primarily to their low pH (high acetic acid content) and high salt and sugar concentrations. The acid content contributes the most to the antimicrobial effect (Smittle 1977). This preservative effect is due
not only to the low pH produced by incorporation of the acetic acid, but also to the antimicrobial effect of the undissociated acetic acid molecule. The high concentration of dissolved solids (salt and sugar) in salad dressings act to bind free water making it unavailable for microbial growth. Mayonnaise and salad dressings typically have water activity values of 0.93 and below (Smittle 1977). In these products the control of microbial growth is due to the interaction of all these factors: pH, specific acid and water activity. This interaction is evidenced by the requirement of a higher minimum water activity for microbial growth at lower pH.

Strict microbiological control of raw ingredient and adherence to good manufacturing procedures also play an important role in the protection of salad dressings from spoilage. The air, contaminated raw ingredients, manufacturing equipment and faulty sanitation procedures are the most frequent contributors to microbiological spoilage. The acid in a product can overcome a small number of spoilage organisms in the dressing; however a large insult will result in microbiological instability (Kurtzman 1971).
Only organisms able to tolerate the high acid, sugar/salt environmental conditions will succeed at growth and spoilage of salad dressing. Lactobacilli and yeast, the normal flora of spices, vegetables and other ingredients, are the major spoilage organisms of salad dressings (Smittle and Cirilgiano in press). These acid tolerant organisms will be selected for growth in these low pH finished products.

The Lactobacillaceae are gram positive asporogenous nonmotile rods (Holt 1984). They are anaerobic or facultatively anaerobic. The lactobacilli have complex organic nutritional requirements. Two types of metabolic pathways are utilized-homofermentative and heterofermentative. For homofermenters lactic acid is the major product of glucose metabolism with little gas production. Heterofermenters produce lactic acid, carbon dioxide, acetic acid and ethanol as endproducts from glucose. Lactobacillus fructivorans is a major spoilage organisms of high acid foods which was overlooked in initial studies due to its requirement for fructose in isolation medium (Kurtzman 1971). Lactobacillus planatarum has also been isolated from spoiled dressings in the 1971 survey by Kurtzman et al.
Zygosaccharomyces bailii has also been determined to be a major spoilage organism of high acid food. Kurtzman et al. (1971) reported that Z. bailii was responsible for the spoilage of 13 of 17 spoiled dressings they evaluated. Z. bailii has high tolerance to acid, osmophilic conditions and preservatives. This resistance to preservatives is due to an inducible intracellular preservative elimination system (Warth 1977). Z. bailii utilizes an active transport system requiring high energy input. This preservative pump has a broad specificity and will eliminate benzoic, sorbic, acetic, butyric and other lipophilic acids of similar molecular weight from the cell interior.

Sucrose when incorporated into the dressing will hydrolyze into glucose and fructose over time. Sucrose is slowly fermented by L. fructivorans and most isolates of Z. bailii (Kurtzman 1971). With the slow hydrolysis of sucrose, spoilage of these dressings may be delayed and escape initial detection. High fructose corn syrup is currently widely used in the manufacture of salad dressings and encourages the rapid growth of L. fructivorans and Z. bailii.
In improperly packaged dressings, surface mold and yeast may be present due to airborne contamination. In properly packaged salad dressing and mayonnaise, yeast, mold and lactobacilli should be present at levels less than ten per gram. As numbers of these organisms can increase rapidly in these products, higher levels are an indication of a sanitation problem (Smittle and Flowers 1984).

Growth of Pathogens in Salad Dressings

As stated in Smittle's 1977 review article, it is well-documented that pathogens do not grow and survive in mayonnaise and salad dressings. This is primarily due to low pH and in some instances the lower water activity. Properly prepared products (pH 4.1 and below, equal or greater than 1.5% acetic acid concentration and held for 72 hours) will not support the growth of Salmonella, Staphylococcus aureus, Clostridium botulinum, Clostridium perfringens, Streptococcus viridans, Shigella flexneri or Bacillus cereus (Smittle 1977). The 72 hour holding period insures the death of any pathogens that might have been present in the ingredients. In improperly prepared or homemade salad dressings (higher pH), spoilage organisms or pathogens could potentially grow.
It has long been a consumer opinion that spoilage and foodborne illness will result from the addition of mayonnaise and salad dressing to salad and sandwiches. However, properly prepared mayonnaise and salad dressings retard the growth of pathogens and spoilage organisms when added to salads or sandwiches. When refrigeration of these products follows preparation, an antimicrobial effect is observed (Smittle 1977).
NEW PRODUCT DEVELOPMENT TRENDS

Changes in the United States demographic and social trends over the past forty years are shaping the way Americans eat, the products they purchase and the food products produced. Major demographic trends include the ageing of America, the increase of Hispanics and other minorities and the population migration to the U.S. sunbelt states. By 2000, the median age of the U.S. population will be 36 (Evans 1987). Increasingly more Americans will be in the over 65 age category. Older people and minorities have different food preferences than younger or traditional white Americans. Development of new products must take into consideration these differences in taste. Also, food packaging must be designed for easy access by ageing fingers.

Another social trend with great consequences on American food consumption is the increase of working women in the U.S. population and the increase in numbers of single parent households. The number of women employed outside the home has increased greatly in the
past twenty years. Sixty percent of women ages twenty to forty-four are now employed outside the home (Collin 1988). Working women and single parents have less time to spend in food preparation and cleanup. Easy to prepare meals and those requiring less cleanup are sought by such consumers. In two income families, more income is available for more expensive prepared foods and luxury food items (Heller 1988). The food industry has met this market demand by developing and marketing prepared meal packages, frozen dinners and easy to cook meals.

In the 1970's and 1980's, Americans have become health and weight conscious. "Eating healthy" and "watching your weight" are two popular themes; even more desirable is "eating healthy while cutting calories". The food industry has responded to this trend by producing low-calorie varieties of many food products.

Americans are also spending less money for food than ever before. In 1987 the U.S. consumer spent 14.3% of their disposable personal income for food verses 16.3% ten years ago (Collin 1988). This is due to lower inflation rates, increases in inflation-adjusted disposable incomes, and lower food production costs. Of this 14.3% disposable income, 9.6% was used to purchase
food for home consumption and 4.7% for purchases of food consumed away from home.

Food sales growth in 1988-1989 is predicted to be an average of 4.3% (Collin 1988). Salad dressing sales account for $635.31 million dollars in sales and an average retail gross profit of 28%. Salad dressings and sauces are among the most frequently used supermarket items. A 1988 article in Standard and Poor's Industry Surveys discussed salad dressing consumers and current U.S. salad dressing consumption trends (Anon 1988). This article cited 84.8% of the U.S. consumers as users of mayonnaise and mayonnaise-type salad dressings and 71.4% as users of pourable prepared salad dressings. Dry mix salad dressings are used by 30.3% of U.S. consumers (Anon. 1988).

Approximately 28% of these pourable prepared salad dressings are considered heavy-users, consuming two or more bottles in the last thirty days. Households in the $30,000 per year income range and 25-54 age range are more likely to be heavy users (Anon 1988). Consumption of salad dressings exhibits seasonal fluctuations, being more popular in the summer than winter months. The 1988 salad dressing industry survey also estimated that 37% of pourable prepared salad dressing users are exclusive
one-brand users.

Liquid prepared salad dressings range from two phase dressings with high acetic acid and oil levels to homogenized one phase creamy dressings (Smittle and Cirigliano in press). Due to the consumer preference trends for low calorie and/or healthy products, there have been two main trends in the salad dressing industry, the production of reduced calorie and mild tasting products containing dairy ingredients.

Low-calorie dressings are produced primarily by reducing the oil content of salad dressings. Creamy, mild tasting dressings are produced by the addition of dairy ingredients. Consumers consider dairy products as wholesome and nutritious and are more likely to consider dressings containing dairy ingredients as such. These creamy salad dressing formulations contain not only dairy ingredients, but also vegetables, meat, eggs and other such components.

The reduction of oil and/or the incorporation of dairy and other ingredients in the production of mild tasting salad dressings produce challenges to traditional methods of preservation. High acetic acid, high salt and sugar levels, and the use of chemical preservatives comprise the traditional preservation methods for salad
dressings. The acid content of the dressing formulations is altered by the incorporation of dairy ingredients, vegetables and meat.

When ingredients such as eggs, cheese, meat and vegetables are included in salad dressing formulas, a rise in pH of the products is observed due to the buffering capacity of these ingredients. Salad dressing containing chopped hard cooked eggs which neutralize the dressing's acidity may become an excellent growth medium for bacteria (Simmons et al. 1979). Brocklehurst et al. (1984) reported that following the addition of vegetables to coleslaw, the pH of the mayonnaise contained in the coleslaw rose and the concentration of acetic acid decreased due to absorption of the acetic acid by vegetable tissue. This resultant pH rise allowed spoilage by yeasts to occur. Therefore, to inhibit microbial growth in these mild tasting dressings, chemical preservatives are required. Their use is limited by the pH of the product and resistant spoilage organisms. In reduced oil formulations, the water phase is increased to account for reduced oil; acid, sugar, salt and chemical preservative concentrations are decreased and less antimicrobial activity is expressed (Smittle and Cirilgiano in press).
OBJECTIVE

Two of the most significant consumption trends in the salad dressing industry are the demand for creamy mild tasting salad dressing and low calorie products. In this investigation, creamy mild tasting dressings and their preservation were studied. The first objective of this research project was to develop a pourable salad dressing model with a mild taste, creamy texture and viscosity similar to currently marketed pourable dressings.

The second objective was to determine the growth behavior of the test organisms, *Lactobacillus buchneri*, *Zygosaccharomyces bailii* and *Bacillus cereus*, in the model at different sample pH values. The third objective was to incorporate acetic and gluconic acids separately and together into the model and evaluate the growth behavior of the test organisms. From this growth behavior evaluation, the acid concentration parameters and pH needed to achieve microbiological stability to challenge by the test organisms was defined.
As previously discussed today's pourable salad dressings commonly contain various ingredients: vegetables, meat and dairy products such as buttermilk, cheese and milk solids. When the buffering effects of these ingredients are coupled with the trend towards reduced oil and low acid dressings to produce low calorie and mild tasting dressings, problems in producing a microbiological stable salad dressing are encountered.

In order to achieve stable microbiological shelf life, product developers are faced with increasing the acid (resulting in a tart-tasting product) or adding preservatives like benzoate or sorbate. There are several limitations to the addition of benzoate or sorbate. First, antimicrobial activity of these preservatives is influenced by the concentration of the undissociated form of the acid. It is this species which is primarily responsible for the antimicrobial activity. At pH 5 and higher and current FDA concentration regulations, less than 30% of the benzoate or sorbate concentration is undissociated; therefore, less antimicrobial activity remains. Secondly, many spoilage bacteria and yeasts are resistant to benzoate (Chichester and Tanner 1972). Lactobacilli may also become
resistant to sorbate (Emard and Vaughn 1952). Also, high concentrations of sorbate and/or benzoate can impart undesirable flavors to food products.

In response to the difficulties of controlling microbial stability in creamy salad dressings, the fourth objective was to develop a model dressing formulation that was inhibitory to growth of yeast, lactobacilli and pathogens.
MATERIALS AND METHODS

I. Salad Dressing Model and Preparation

A model salad dressing was developed to simulate current creamy style salad dressings. The model used in this investigation contained the following ingredients: water; soybean oil, sodium chloride, instant nonfat dry milk (NFDM) (local store, Columbus, OH); high fructose corn syrup (HFCS) (Cargill Incorporated, Dayton, OH); organic acids (acetic and gluconic) and polyoxyethylene-sorbitan monostearate (Sigma Chemical Company, St. Louis, MO); and xanthan gum (Advanced Food Systems Incorporated, Somerset, NJ). The formulations used in this investigation are presented in Table 2.

The salt, HFCS, NFDM, and one-third of the water were combined using a magnetic stir plate. Xanthan gum was hydrated in the remaining two-thirds of the water using a blender and then added to the previously mixed aqueous portion.
Table 2  
Salad Dressing Formulations

<table>
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<th>Ingredient</th>
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<th>B</th>
<th>C</th>
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<td>47.5</td>
<td>47.5</td>
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</tr>
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</table>

a  HFCS contains 17.7% water  
b  NFDM contains 4.0% water  
c  Poly 60 contains 3.0% water

Note:
Formula F for Combination 5% Gluconic Acid and 0.5% Acetic Acid is the same as Formula D with 0.5% Acetic Acid added.

Formula G for Combination 10% Gluconic Acid and 0.5% Acetic Acid is the same as Formula E with 0.5% Acetic Acid added.
Acetic and gluconic acids were used in the model formulations. Acetic acid was added at 0.5 and 1.0\%(w/w) concentration levels; gluconic acid at 5 and 10\%(w/w) levels. The acids were then mixed with the aqueous phase separately or together after the addition of hydrated gum. The pH of resultant mixture was adjusted with 36\% hydrochloric acid (HCL) or 50\% sodium hydroxide (NaOH). Formulations included samples within the pH range 2.0 to 4.5. The appropriate weight of oil was added to the aqueous phase and mixed for two min in a Waring Blender.

After blending, the dressing formulations were heated for 20 min. at 100°C in closed glass vessels. This mild heat treatment eliminated the microbial contamination (primarily mold and yeast) introduced by the ingredients.

II. Microorganisms and Cultural Conditions

*Lactobacillus buchneri* (ATCC 4005), *Zygosaccharomyces bailii* (isolated from commerical salad dressings and provided by T.J.Lipton,Inc.,Englewood Cliffs,NJ) and *Bacillus cereus* (BYAC)(obtained from the Ohio State University Food Science and Nutrition Department culture collection, Columbus,OH) were the test organisms used in
this investigation. *L. buchneri* was maintained on MRS agar (Difco, Detroit, MI) at 4C and *B. cereus* and *Z. bailii* on trypticase soy glucose agar (Difco) at 4C.

The growth behavior of the test organisms at their optimum pH was determined prior to inoculation into the dressing model. Approximately two log cfu of *L. buchneri* were inoculated into MRS broth (pH 6.2) (Difco). Similarly, *Z. bailii* was inoculated into trypticase soy glucose broth (pH 7.0) (Difco) and *B. cereus* into trypticase soy broth (pH 7.0) (Difco). The broth samples were incubated at 25C and the growth behavior of the organisms determined by the duplicate pour plate technique. Peptone diluent (0.1% (w/w) (Difco) was used for all dilutions.

III. Inoculation and Sampling of the Salad Dressing Model

One hundred gram aliquots of the dressing were aseptically placed into sterile plastic bags (Tekmar, Cincinnati, OH). The test organisms, *Lactobacillus buchneri*, *Zygosaccharomyces bailii*, and *Bacillus cereus*, were separately inoculated into the sample bags. Each sample bag was then stomached for one minute (Stomacher 400, Unilever, England). Two inoculum
levels of each organism were used for each formulation. Low (2 log10 cfu/g) and high (4 log10 cfu/g) inoculum levels were separately inoculated into each formulation at various pH and acid concentrations to represent low and high microbial contamination loads and to serve as a duplicate trial. An uninoculated control sample was also evaluated for each challenge experiment.

Using MRS agar incubated at 25°C for 5 days for lactobacilli and tryptase soy agar with 5% dextrose incubated at 25°C for 3 days for the yeast and bacilli containing samples, each salad dressing was sampled at the time of inoculation (time 0) and every seven days thereafter for six weeks or until spoilage occurred. Spoilage was defined as >2 log increase in cfu/g salad dressing. Samples with yeast were also sampled at day 4 to observe a potential growth peak and decline before seven days.

IV. Physical Measurements

The pH of samples after adjustment, during the sampling period, and at the end of the sampling period were determined with a Fisher Scientific Accumet pH Meter Model 815MP. A derivation of the Henderson-Hasselbach Equation was used to calculate the values of undissociated acid and dissociated acid concentrations.
as they vary with pH and the pKₐ of the acid. The equation used is as follows:

\[
\% \text{ Undissociated Acid} = \frac{1}{10^{(pH-pK_a)}} + 1
\]

The titratable acidity of the different formulations at different pH values were determined using the standard titratable acidity method as outlined by Gould (1977). The following equation was used to calculate the percent acid in a sample by the titratable acidity method:

\[
\% \text{ Total Acid in Sample} = 100 \times \frac{(V \times N \times \text{Meq.Wt.})}{Y}
\]

where: 
- \(V\) = volume of ml of NaOH titrated
- \(N\) = normality of NaOH (0.1N)
- \(\text{Meq.Wt.}\) = milliequivalents of acid
- \(Y\) = volume in ml or weight in gram of sample

The water activity values for the dressings were measured using a Decagon Water Activity Meter Model CX-1 (Pullman, Washington, D.C.). The computer program SLIDEWRITE (Advanced Graphics Software Inc., Sunnyvale, CA) was utilized in preparing the graphs subsequently plotted on a HP Colorpro plotter using an IBM compatible microcomputer. Each value (mean log10 cfu/g) is the average of duplicate plates and the mean of duplicate trials. Bars on the graphs are an indication of the
standard error of the means.

Taste panels were conducted to determine if differences in the type and level of organic acid added were detectable by the panelists. Thirty-two untrained panelists participated in the ranking study to determine the level of tartness of four samples. The pH values of all formulation samples were adjusted to pH 3.5. The four formulations included 0.5%(w/w) acetic acid (Formula B), 1.0%(w/w) acetic acid (Formula C), 10%(w/w) gluconic acid (Formula E) and 0.5% (w/w) acetic and 10% (w/w) gluconic acids (Formula G) (Table 2). The significance of the rankings at the 5% confidence level was determined by a table constructed by Kahan et al. (1973).
RESULTS

I. Growth Behavior in Broth

The growth behavior of the three test organisms (Lactobacillus buchneri, Zygosaccharomyces bailii, and Bacillus cereus) was determined under optimal growth conditions (pH and growth medium). L. buchneri was inoculated into MRS broth pH 6.2, Z. bailii into trypticase soy glucose broth pH 7.0 and B. cereus into trypticase soy broth pH 7.0. All organisms were incubated at 25°C. The duplicate pour plate technique was used to determine the growth curves for each organism. The growth behavior curves for L. buchneri, Z. bailii and B. cereus are shown in Figures 1 and 2. From these data, the number of organisms per ml at a given time of incubation was used to estimate the appropriate dilution of the culture prior to inoculation into the salad dressing.
Figure 1  Growth Behavior of *L. buchneri* and *Z. bailii* in Broth
Figure 2  Growth Behavior of *B. cereus* in Broth
II. Growth Behavior in Salad Dressing Model

Source of Contamination Due To Ingredients

To pinpoint sources of laboratory mold contamination in samples not containing acetic acid, a survey of the microbial load of the ingredients was conducted. By enumerating the microorganisms present in the raw ingredients used in the model salad dressing, the ingredients representing the largest source of microbial contamination were determined. As presented in Table 3 the xanthan gum and nonfat dry milk contain the largest number of contaminants (mold and yeast) upon sampling. By heating the dressings at 100°C for 20 min, this microbial contamination was minimized.

Effect of pH on Growth Behavior

The effect of pH alone on the growth behavior of the test organisms was evaluated. By inoculating the test organisms separately into the model dressing formulation containing no acetic or gluconic acid, the effect of pH alone was studied. The pH of these samples were adjusted with completely dissociating HCL or NaOH.

At pH 3.5 and 4.5 L. buchneri present in model increased in number rapidly. Spoilage as defined as >2 log increase in colony forming units (cfu)/g occurred in
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Type of Contaminant</th>
<th>Mean Log_{10} cfu/g*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>none</td>
<td>&lt;1</td>
</tr>
<tr>
<td>High Fructose Corn Syrup</td>
<td>none</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Nonfat Dry Milk</td>
<td>yeast, mold</td>
<td>2</td>
</tr>
<tr>
<td>Salt</td>
<td>none</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Soybean Oil</td>
<td>none</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Gluconic Acid</td>
<td>mold</td>
<td>2</td>
</tr>
<tr>
<td>Acetic Acid</td>
<td>none</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Poly 60</td>
<td>mold</td>
<td>1</td>
</tr>
<tr>
<td>Xanthan Gum</td>
<td>mold</td>
<td>2</td>
</tr>
</tbody>
</table>

* approximate
less than two weeks. At pH 3.0 and below no growth of *L. buchneri* was observed in the model. Data collected concerning the growth behavior of *L. buchneri* in model samples over the pH range 2.5 to 4.5 are presented in Figure 3. At pH 2.5, 3.0, 3.5 and 4.5, *Z. bailii* readily grew. Only at pH 2.0 and below was *Z. bailii* growth inhibited (Figure 3). A preliminary investigation on the growth behavior of *B. cereus* in TSB broth at different pH values detected growth in the pH range 4.4 and above.

Effect of Addition of Acetic Acid

Model formulations containing 0.5 and 1.0 % (w/w) acetic acid and at pH values of 3.0, 3.5 and 4.5 were challenged with each test organism independently. Spoilage occurred at pH 3.5 and 4.5 and acetic acid levels of 0.5 and 1.0% (w/w) for the dressings inoculated with *L. buchneri* (Figure 4). At pH 3.0 and 0% acetic acid concentration, no growth of *L. buchneri* was observed (Figure 3). Growth of *Z. bailii* was inhibited by 0.5 and 1.0 % (w/w) acetic acid at pH 3.0, 3.5 and 4.5 (Figures 5 and 6). *B. cereus* failed to increase in number at these acetic acid concentrations at model pH values of 3.5 and 4.5.
Figure 3  Growth Behavior of *L. buchneri* and *Z. bailii* in Salad Dressing Model at Various pH Values
Figure 4  Growth Behavior of *L. buchneri* in Salad Dressing Models Formulated with Acetic Acid at pH 3.5 and 4.5
Figure 5  Growth Behavior of Z. bailii in Salad Dressing Models Formulated with Acetic Acid at pH 3.0 and 3.5
Figure 6  Growth Behavior of *Z. bailii* in Salad Dressing Model Formulated with Acetic Acid at pH 4.5
Effect of Addition of Gluconic Acid

Salad dressing formulations with 5 and 10 % (w/w) gluconic acid were inoculated with *L. buchneri* and *Z. bailii*. The growth behaviors of the two organisms in response to these concentrations of gluconic acid are presented in Figures 7 and 8. The addition of 5 % (w/w) gluconic acid to the model at pH 4.5 failed to inhibit *L. buchneri* growth, but at pH 3.5 the level of *L. buchneri* numbers remained stationary. The addition of 10 % (w/w) gluconic acid to the model was sufficient to inhibit *L. buchneri* growth at pH 4.0 and below. *Z. bailii* grew rapidly at pH 4.0 and below with 5 and 10 % (w/w) gluconic acid. In a dressing model containing 5 % (w/w) gluconic acid with a pH value of 4.5, no growth of *B. cereus* was observed.

Effect of the Combination of Acetic and Gluconic Acids

A salad dressing containing 0.5 % (w/w) acetic acid and 5 % (w/w) gluconic acid was prepared at pH 3.5 and 4.3. At pH 3.5 and 4.3 this model was inhibitory to yeast growth; however the lactobacilli were not prevented from spoiling the model (Figure 9) even though the growth rate was reduced. 10 % (w/w) gluconic acid was
Figure 7  Growth Behavior of *L. buchneri* in Salad Dressing Models Formulated with Gluconic Acid at pH 3.5 and 4.0
Figure 8  Growth Behavior of *Z. bailii* in Salad Dressing Models Formulated with Gluconic Acid
Figure 9
Growth Behavior of *L. buchneri* and *Z. bailii*
in Models Containing 0.5% (w/w) Acetic Acid and
5% (w/w) Gluconic Acid at pH 3.6 and 4.3
combined with 0.5%(w/w) acetic acid in another model formulation at pH 3.6 and 4.3. *L. buchneri* and *Z. bailii* were prevented from spoiling the product during the storage period of 8 weeks (Figure 10). *B. cereus* was unable to grow in the 10%(w/w) gluconic acid and 0.5%(w/w) acetic acid model at pH 4.5, but viable cells were obtained from sampling the model throughout the testing period.

**Effect of Absence of Oil on Growth Behavior**

Salad dressings containing no oil or emulsifier were prepared as described in the Materials and Methods section. The test organisms were inoculated into these oilless dressings. There was no observed difference in the growth behavior of *L. buchneri* when inoculated into formulations of pH 3.3 with no organic acid, pH 3.5 containing 0.5%(w/w) acetic acid, pH 3.3 containing 5%(w/w) gluconic acid, and pH 4.0 containing 10%(w/w) gluconic acid as compared with similar formulas containing oil. Similarly, *Z. bailii* growth behavior was not altered in dressings containing no oil at pH 3.0 with no organic acid, pH 4.0 containing 0.5%(w/w) acetic acid, and pH 2.5 containing 5%(w/w) gluconic acid.
Figure 10  Growth Behavior of *L. buchneri* and *Z. bailii* in Models containing 0.5%(w/w) Acetic Acid and 10%(w/w) Gluconic Acid at pH 3.6 and 4.3
III. Physical Measurements

The effect of storage time on the pH of the formulations was observed to determine if any buffering activity took place during storage. Results of a 14 day study to determine the pH stability of five dressing formulations (no organic acid, 0.5%(w/w) acetic acid and 0.5 and 1.0%(w/w) gluconic acid are presented in Table 4. The pH values of all the formulations remained stable over the 14 day period. Also, included in Table 4 are the average pH values of inoculated and uninoculated salad dressings models over the sampling periods. These pH values remain stable over storage periods ranging from 37 to 52 days. The effect of heating at 100°C for 20 min on the model pH was also evaluated. Heating appears to slightly reduce the pH of the samples containing organic acids and has no effect on those not containing organic acids.

The water activity of the different formulations was measured and data presented in Table 5. Currently marketed creamy salad dressings were also sampled to determine water activity(Table 6). The average water activity of the model dressing was 0.95 with a standard deviation of ±0.02. Likewise for commercially prepared
Table 4  pH Stability of Various Salad Dressing Models During Storage at 25°C

<table>
<thead>
<tr>
<th>Days</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>B1</th>
<th>B2</th>
<th>D1</th>
<th>D2</th>
<th>E1</th>
<th>E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.4</td>
<td>3.7</td>
<td>4.2</td>
<td>4.3</td>
<td>3.8</td>
<td>2.5</td>
<td>4.1</td>
<td>3.7</td>
<td>4.1</td>
</tr>
<tr>
<td>1</td>
<td>na</td>
<td>3.9</td>
<td>4.5</td>
<td>4.5</td>
<td>4.0</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
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<td>na</td>
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<td>4.4</td>
<td>4.3</td>
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<td>na</td>
<td>na</td>
<td>na</td>
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<td>na</td>
<td>3.8</td>
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<td>na</td>
<td>3.7</td>
<td>4.3</td>
<td>4.5</td>
<td>3.9</td>
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<td>4.2</td>
<td>3.6</td>
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<td>na</td>
<td>3.7</td>
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<td>35</td>
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<td>3.6</td>
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<td>na</td>
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<td>na</td>
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<tr>
<td>52</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>4.1</td>
<td>na</td>
<td>4.1</td>
</tr>
</tbody>
</table>

* Average values obtained from salad dressing models
na= not applicable

Compositions of formulas are presented in Table 1

Formula A = No organic acid in formula
B = 0.5%(w/w) acetic acid in formula
D = 5%(w/w) gluconic acid in formula
E = 10%(w/w) gluconic acid in formula
Table 5 Physical Property Values for Salad Dressing Formulations

<table>
<thead>
<tr>
<th>Formula</th>
<th>pH&lt;sup&gt;a&lt;/sup&gt; Initial pH&lt;sup&gt;b&lt;/sup&gt;</th>
<th>%TA&lt;sup&gt;c&lt;/sup&gt;</th>
<th>s.d.&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Aw&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>No organic acid</td>
<td>3.5</td>
<td>6.0</td>
<td>0.69</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td></td>
<td>0.56</td>
<td>0.01</td>
</tr>
<tr>
<td>Acetic Acid</td>
<td>0.5%(w/w)</td>
<td>3.5</td>
<td>4.6</td>
<td>1.09</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td></td>
<td>0.73</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>3.5</td>
<td>4.2</td>
<td>1.46</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
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<td>1.00</td>
<td>0.01</td>
</tr>
<tr>
<td>Gluconic Acid</td>
<td>5%(w/w)</td>
<td>3.5</td>
<td>2.8</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td></td>
<td>0.52</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>10%(w/w)</td>
<td>3.5</td>
<td>2.6</td>
<td>1.57</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td></td>
<td>0.69</td>
<td>0.05</td>
</tr>
<tr>
<td>Acetic &amp; Gluconic Acids</td>
<td>.5% Acetic &amp; 5% Glc</td>
<td>3.5</td>
<td>3.1</td>
<td>1.31</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td></td>
<td>0.70</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>.5% Acetic &amp; 10% Glc</td>
<td>3.5</td>
<td>2.7</td>
<td>1.95</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td></td>
<td>0.65</td>
<td>0.10</td>
</tr>
</tbody>
</table>

<sup>a</sup> pH after adjustment with HCL of NaOH  
<sup>b</sup> approximate pH before adjustment with HCL or NaOH  
<sup>c</sup> mean titratable acidity (n=3) calculated as %acetic acid  
<sup>d</sup> ± one standard deviation of TA  
<sup>e</sup> water activity at temperature 25°C ± 3°C
### Table 6 Physical Property Values for Currently Marketed Salad Dressings

<table>
<thead>
<tr>
<th>Formula</th>
<th>pH&lt;sup&gt;a&lt;/sup&gt;</th>
<th>%TA&lt;sup&gt;b&lt;/sup&gt;</th>
<th>s.d.&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Aw&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seven Seas Ranch</td>
<td>3.7</td>
<td>0.927</td>
<td>0.01</td>
<td>0.93</td>
</tr>
<tr>
<td>Kraft Ranch</td>
<td>3.9</td>
<td>0.725</td>
<td>0.02</td>
<td>0.97</td>
</tr>
<tr>
<td>Kraft Bacon &amp; Tomato</td>
<td>3.7</td>
<td>1.00</td>
<td>0.08</td>
<td>0.96</td>
</tr>
<tr>
<td>Kroger Lite Bacon &amp; Tomato</td>
<td>3.5</td>
<td>1.10</td>
<td>0.05</td>
<td>0.95</td>
</tr>
<tr>
<td>Hidden Valley Ranch</td>
<td>3.6</td>
<td>1.44</td>
<td>0.08</td>
<td>0.97</td>
</tr>
</tbody>
</table>

<sup>a</sup> mean pH n=3  
<sup>b</sup> mean titratable acidity n=3 calculated as % acetic acid  
<sup>c</sup> ± one standard deviation of TA  
<sup>d</sup> water activity at temperature 25C ± 3C
dressings, the average was 0.96 ±0.02. It is evident from these data that acid type, acid concentration and pH have no measurable effect on the water activities of the model formulations used in this investigation.

The titratable acidity for the model at different acid concentrations and pH was measured (Table 5). Similar results are contained in Table 6 for commercial pourable salad dressings. Comparable results were obtained for each set of dressings. The effect of pH on the undissociated and dissociated acid concentrations of gluconic and acetic acids is presented in Table 7.

Preliminary taste panels were conducted by tasting the dressing models as they were prepared for microbial inoculation. The dressings containing gluconic acid are the least tart in taste. In addition, 32 untrained panelists participated in a taste panel to rank four dressing samples on basis of tartness. The four sample formulations included 0.5% (w/w) acetic acid, 1.0%(w/w) acetic acid, 10% (w/w) gluconic acid and a combination dressing containing 0.5%(w/w) acetic and 10%(w/w) gluconic acids. The pH values of all four samples were adjusted to pH 3.5. Using a table prepared by Kahan et al. (1973) for determining the significance of differences for ranked data, it was determined that at
Table 7  Calculated Undissociated and Dissociated Acid Concentrations with Varying pH in Salad Dressing Model

<table>
<thead>
<tr>
<th>Formula</th>
<th>% Acid(^a) Added</th>
<th>% Undiss(^b) Added</th>
<th>%Acid. in H(_2)O phase</th>
<th>%Undiss. in H(_2)O phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5% Acetic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 3.0</td>
<td>0.5</td>
<td>0.49</td>
<td>1.05</td>
<td>1.03</td>
</tr>
<tr>
<td>pH 3.5</td>
<td>0.5</td>
<td>0.47</td>
<td>1.05</td>
<td>0.99</td>
</tr>
<tr>
<td>pH 4.5</td>
<td>0.5</td>
<td>0.32</td>
<td>1.05</td>
<td>0.67</td>
</tr>
<tr>
<td>1.0% Acetic</td>
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<tr>
<td>pH 3.0</td>
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<td>pH 4.5</td>
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<td>0.65</td>
<td>2.11</td>
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<tr>
<td>5% Gluconic</td>
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<td>pH 3.0</td>
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<td>10% Gluconic</td>
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<td>7.99</td>
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<td>pH 3.5</td>
<td>10.0</td>
<td>5.57</td>
<td>23.0</td>
<td>12.8</td>
</tr>
<tr>
<td>pH 4.5</td>
<td>10.0</td>
<td>1.10</td>
<td>23.0</td>
<td>2.5</td>
</tr>
</tbody>
</table>

\(^a\) all % Acid indicate \%(w/w)  
\(^b\) all % Undiss. indicate \%(w/w) Undissociated Acid
the 5% level the sample containing 0.5%(w/w) acetic and
the sample containing 10%(w/w) gluconic acid were
significantly less tart than the other samples and the
combination acid sample containing 0.5%(w/w) acetic and
10%(w/w) gluconic acids was significantly more tart than
the other samples. In Table 8 is presented a scoring
sheet given to the taste panelists to rank the tartness
of the samples.
Table 8  Taste Panel Scoring Chart Given to Panelists

Please rank the four samples 1 through 4 for tartness.

1 = most tart
2 = moderately tart
3 = slightly tart
4 = least tart

<table>
<thead>
<tr>
<th>Samples</th>
<th>854</th>
<th>413</th>
<th>067</th>
<th>529</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rank</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comments about samples (viscosity, overall likability, etc):
DISCUSSION

I. Dressing Model Preparation and Characteristics

A salad dressing model was formulated to simulate currently marketed "ranch style" pourable salad dressings. Based on a review of the literature on salad dressing composition, a model was postulated. The major ingredients were water, soybean oil, HFCS, salt, instant nonfat dry milk, organic acid, emulsifier and xanthan gum. The preparation of the salad dressing model has been outlined previously. In order to obtain the consistency of currently marketed pourable salad dressings, different concentrations of emulsifier, gums and methods of ingredient incorporation were investigated.

The effects of the use of lecithin crystals or polysorbate 60 were compared. Lecithin crystals were largely insoluble in the model making the control of emulsifier concentration difficult. When polysorbate 60 was used alone in the model at concentrations of 0.5% (w/w) and below, separation of the aqueous and oil phases of the dressings resulted and little increase in
viscosity was observed. Addition of xanthan gum to the formulation produced thickening in the salad dressing and a viscosity similar to currently marketed salad dressings was obtained. At higher levels than 0.15%(w/w) xanthan gum, the dressing lost its pourable qualities. As currently marketed formulations often contain xanthan gum and polysorbate 60, both were included in the model formulation. A polysorbate 60 concentration of 0.35%(w/w) and 0.15%(w/w) xanthan gum yielded a dressing with a smooth, creamy texture that was pourable. Instantized nonfat dry milk was incorporated into the model to provide a creamy texture and the buffering capacity found in today's creamy dressings containing milk protein, vegetables, eggs and other similar ingredients.

Studies were also conducted to determine the most effective means of mixing the ingredients. The use of the blender to combine the dry ingredients with water resulted in unacceptable air incorporation and foaming. The use of the stir bar and magnetic stir plate allowed for sufficient mixing of the aqueous ingredients with minimum foaming. The use of a homogenizer and a stomacher to combine the aqueous and oil phases was explored. Dressing prepared with the stomacher
separated after three days and the dressing clogged the
table top homogenizer. Mixing the oil and aqueous
phases for two minutes in a blender at high speed
produced a dressing which exhibited no signs of emulsion
separation or viscosity decrease during a six week
storage period.

In order to control the pH during the experiment, the
pH of the dressings was adjusted with NaOH or HCL. The
pH of the model without organic acid (Formula A) before
adjustment was measured to be 5.9 and the pH of model
with acetic acid (Formula B and C) approximately 4.7.
The pH of the model dressing containing 10% gluconic
acid (Formula E) before adjustment was 2.8. The pH of
the samples were adjusted to 3.0, 3.5 or 4.3 in this
investigation. Addition of different concentrations of
organic acids to the model result in different sample pH
values (Table 5); therefore, the pH values of the
different formulations were adjusted to control the pH
values. The pH values were also adjusted to provide
enough hydrogen ions to insure the desired concentration
of undissociated acid molecules were present in each
model.
Surveys were conducted to determine if time or mild heating had an effect on the pH of the model. Storage of the samples at 25C appeared to have no effect on the pH of the different formulations. Mild heat treatment had no effect on the formulations containing no organic acids and slightly lowered the pH of the formulations containing organic acids by an average of <0.3 units. Denaturation of some of the milk proteins during mild heat treatment is postulated to cause this pH decrease.

Microbial contamination (primarily mold and yeast) was found in the dressing samples over the pH range studied. The commercial ingredients were determined to be the source of this contamination with the dry ingredients, nonfat dry milk, salt, and xanthan gum, being the primary sources (Table 3). To reduce this microbial contamination, the model dressing was heated for 20 min at 100C to destroy with heat the yeast and mold cells introduced into the dressing by the ingredients.

The water activities of the model formulations were measured at different sample pH values. The average water activity for the formulations was 0.96 with a standard deviation of ±0.02. The minimum water activity for growth of most bacteria is 0.90, 0.87 to 0.94 for most yeast and 0.70 for most mold (Baird-Parker 1980).
The water activity values for the model are well within this water activity range for growth for most microorganisms. Currently marketed creamy pourable salad dressing were determined to have water activities ranging from 0.93 to 0.97. These commercial values are comparable with those obtained in this investigation's model dressing. The titratable acidity values for commercial dressings and the model are also similar. Titratable acidity is a measure of the total acid in a food product. It is determined by titration of the sample with a base of known strength. Due to changes in the buffering capacity, titratable acidity is not a measure of the actual concentration of the total acid.

Three test organisms; a lactic acid bacteria, yeast and pathogen were inoculated separately into the model at different pH levels and acid concentrations. Two inoculum levels of each organism were used for each formulation to represent low and high microbial contamination loads that might be encountered during the industrial preparation of salad dressings. After inoculation the salad dressing samples were incubated at 25°C to simulate storage at room temperature on the grocery store shelf.
II. Effect of pH on the growth behavior of microorganisms

The pH or hydrogen ion concentration is an important factor in the spoilage of a food product by microorganisms. The type of acid used to lower the pH of a food product will also determine the microbial tolerance to pH. Certain organisms are able to tolerate certain acids more readily than other organisms due to difference in cell metabolism. The antimicrobial effect of lowering the pH of a food product is due to three factors: the effects of the specific acid, the effect of the hydrogen ion concentration, and the amount of undissociated acid present (Baird-Parker 1980).

Strong acids like hydrochloric acid dissociate completely to hydrogen and chloride ions in aqueous solution. In low pH conditions (high hydrogen ion concentrations), the microbial cell interior can be acidified, acid labile cell components destroyed, cell growth is affected and death may occur (Gould et al. 1983). Weak lipophilic acids like acetic, citric, lactic and gluconic acids dissociate incompletely in aqueous solution. An equilibrium relationship is formed between the hydrogen ions, the dissociated form of the acid and the undissociated form of the acid. For a more complete discussion on the mechanism of weak lipophilic acids,
see "pH" section of the literature review.

The concentration of the undissociated acid largely determines the antimicrobial activity of the food product. The undissociated form of the acid readily passes through the microbial cell membrane into the cell interior. Upon arrival into the cell interior, the undissociated form of the acid may dissociate into hydrogen ion and the dissociated form of the acid due to the near neutral pH of the cell interior (Herrero et al. 1985). Acidification of the cell interior, interference with the cell membrane permeability, uncoupling of the active transport system, inhibition of growth and ultimately cell death may result (Freese and Levin 1978).

The concentration of the undissociated acid in a food product is determined by the pH of the food. As pH is decreased, the concentration of undissociated acid increases and the antimicrobial activity of the food increases (Figure 11) (Baird-Parker 1980).

As discussed previously, consumption trends of salad dressing have moved toward mild creamy varieties of dressings and away from traditional tart tasting acidic products. These creamy dressings often contain dairy products, vegetable, and meat which serve to buffer the dressings, act as inoculum source and a source of
Figure 11  Undissociated Acetic and Gluconic Acid Concentrations at Different pH Values
nutrients for fastidious organisms. This trend has complicated the preservation of salad dressings which are traditionally preserved by a low pH, high salt and sugar concentration, and/or the addition of sorbate or benzoate. The model salad dressing containing 10% (w/w) nonfat dry milk was developed to represent current creamy dressings. This model was used to determine the effect of pH and organic acid concentration on the growth behavior of the test organisms. Two of the test organisms, L. buchneri and Z. bailii, were chosen for study because they are common spoilage organisms in salad dressing. The third, B. cereus, was inoculated into the model to represent the growth behavior of a pathogen in the model. B. cereus and other sporeformers are present in dry ingredients such as nonfat dry milk. Most microorganisms will not grow below pH 5 and do not grow in salad dressings which have a traditional pH range of 3.2 to 3.9. However, lactobacilli have a minimum growth pH near 3.0 and yeast near pH 2.0 (Banwart 1979).

The effect of pH or hydrogen ion concentration alone on microbial growth behavior was studied by inoculation of the test organisms into a dressing model containing no organic acid. The sample pH range was adjusted with
HCL or NaOH to 2.0 to 4.5. Spoilage of the dressings was defined as > 2 log increase in number cfu/g. *L.* buchneri cell numbers increased rapidly in the dressing model at pH 3.5 and 4.5. At pH 3.0 and below no growth of *L.* buchneri was observed. *Z.* bailii readily spoiled samples with pH 2.5 and above. Only at pH 2.4 was *Z.* bailii inhibited by hydrogen ion effect alone. In preliminary investigations involving inoculation of *B.* cereus into different pH TSB broth samples, it was determined that *B.* cereus was able to grow in the broth at pH values of 4.4 and above.

From the data obtained in this pH study, it can be concluded that salad dressings stable to spoilage by lactobacilli can be constructed by adjustment of the pH to 3.0 and below. By adjustment of the pH to 2.0, microbial stability to yeast can be attained; however, this pH is extremely low and would presents physical production problems involving protein denaturation and emulsion stability as well as consumer safety. At the pH values necessary to prevent lactobacilli and yeast spoilage of the dressing model by hydrogen ion effect alone, *B.* cereus and other pathogens would not be able to grow and cause illness in humans. Since organic acids are generally recognized to be more
antimicrobial than inorganic acids such as HCL, the use of these organic acids as acidulants should provide a greater antimicrobial activity when used to lower the pH of the food.

III. **Effect of the Addition of Acetic Acid on Growth Behavior of Test Organisms in Dressing Model**

Acetic Acid (commonly used as vinegar) is the predominantly used acidulant in salad dressing and mayonnaise. It is a highly effective and widely used antimicrobial agent. A short chain organic acid, acetic acid has a pKa of 4.75 is very soluble in aqueous solutions and is recognized as GRAS by the FDA (Baird-Parker 1980). Acetic acid imparts an acidic tart taste to food products.

Acetic acid at concentrations of 0.5 and 1.0%(w/w) was incorporated into the salad dressing model to determine its effect on the growth behavior of the test organisms in the model salad dressing. Levels of greater than 1.0%(w/w) acetic acid were not analyzed due to the taste penalty incurred at higher acetic concentrations.

The test organisms were separately inoculated into samples containing 0.5 and 1.0%(w/w) acetic acid and having a pH of 3.0, 3.5, or 4.5. At pH 3.5 and 4.5 and the above acetic concentrations, spoilage of the samples
by \textit{L. buchneri} occurred. No growth of \textit{L. buchneri} was observed at pH 3.0 as would be expected by the previous observation that this organism failed to grow at pH 3.0 and no organic acid. \textit{Z. bailii} was inhibited by 0.5\%(w/w) acetic acid at pH 4.5 and below. Growth of \textit{B. cereus} failed to be detected in a dressing model containing 0.5\%(w/w) acetic acid at pH 4.5. Therefore, addition of 0.5\%(w/w) acetic acid to model dressings will produce dressings that are microbiologically stable to yeast and bacilli. Acetic acid is not an effective inhibitor of the growth of lactobacilli at concentrations 1.0\%(w/w) and below and in the pH range of this investigation.

IV. \textbf{Effect of the Addition of Gluconic Acid to the Model on the Growth Behavior of the Test Organisms}

Gluconic acid (2,3,4,5,6-Pentahydroxyacaproic Acid) is a carboxylic acid derivative of glucose. It is freely soluble in water, slightly soluble in ethanol and has a pKa of 3.60 (Windholz 1976). Although the use of gluconic acid in food products as an antimicrobial is largely unexplored, it has GRAS status with the FDA (Anon. 1988). Gluconic acid is currently used in the food industry for the introduction of minerals such as calcium, manganese and iron into foods for vitamin and
mineral enrichment. Fligner et al. (1988) reported the successful enrichment of yogurt with calcium gluconate.

Gluconic acid is also employed as an acidulant in the food industry. Heil et al. (1988) have determined that addition of gluconic acid to fruit and fruit syrup will improve color retention. McIntyre and Reed have patented the use of aldonic acids and their lactones such as gluconic acid and its lactones, glucono-delta lactone and glucono-gamma lactone, to lower the pH of low acid food products to pH 4.6 and lower (1988). Lowering the pH of low acid food products lowers the heat requirements for sterilization of these acidified foods; therefore, the product maintains flavor, texture and color closer to the fresh or home-cooked product. The commonly used acidulants such as acetic or citric when added in concentrations sufficient to lower the food product pH to 4.6 and below impart a strong acidic taste penalty. Gluconic acid imparts a very mild to no flavor to foods (McIntyre and Reed 1988). Its presence has been observed to be organoleptically undetected in some food products. McIntyre and Reed have utilized aldonic acids and their lactones to acidify low acid products lowering processing time requirements, improving texture and color and maintaining a mild unacidic
taste(1988).

The lactones of gluconic acid, glucono-delta lactone (GDL) and glucono-gamma lactone, readily hydrolyze in water forming an equilibrium mixture of gluconic acid and the lactones. Glucono-delta lactone (GDL) and its salts are widely added to meats and other food products to the lower pH and stabilize the food color.

Formulations of the model dressing were prepared with 5 and 10 % (w/w) gluconic acid and inoculated with the test organisms to determine the effect of gluconic acid on the growth behavior of the test organisms. L. buchneri was unable to spoil dressings prepared with 5 and 10 % (w/w) gluconic acid at pH 4.3 and below. Gluconic acid fails to have an inhibitory growth effect on Z. bailii at the acid levels and pH range investigated in this research. Growth of B. cereus failed to be detected in a pH 4.5 model containing 5%(w/w) gluconic acid.

Gluconic acid alone is insufficient to prevent spoilage of the model dressing by yeast. With the mild taste imparted to the model dressing by gluconic acid, appropriate amounts of gluconic acid can be added that yield preservative (antimicrobial) activity against
the lactobacilli while maintaining a nonacidic taste. In this way more flavorful salad dressings can be produced. It can be postulated that less flavorants, spices and sweeteners will be required for incorporation into salad dressings due to the mild flavor of gluconic acid. However, gluconic acid must be added at higher levels than many frequently used GRAS acidulants to achieve preservation and could be more costly to use.

V. Effect of the Addition of the Combination of Acetic Acid and Gluconic Acid on the Growth Behavior of the Test Organisms

The effect of including acetic and gluconic acid together in the model formulation on the growth behavior of the test organisms was determined. A salad dressing containing 5 %(w/w) gluconic acid and 0.5%(w/w) acetic acid was prepared at pH 3.5 and 4.3 and inoculated with the test organisms. Yeast growth was inhibited in this model; however _L. buchneri_ was not prevented from eventually spoiling the product (Figures 9 and 10). Using 10%(w/w) gluconic acid and 0.5%(w/w) acetic acid, another combination acid dressing was produced at pH 3.5 and 4.3 that provided microbiological stability against both yeast and lactic acid bacteria. The test organisms, _L. buchneri_, _Z. bailii_ and _B. cereus_, were
prevented from spoiling this combination dressing model. Both dressing formulations containing the combination of acetic and gluconic acid have an acceptable mild flavor and contain no traditional preservatives (sorbate or benzoate).

Many combinations of organic acids have been determined to act synergistically in antimicrobial action. Specific examples of this synergism are lactic and acetic acids (Rubin 1978), phosphoric and acetic acids (Ores 1979), and propionate and sorbate (Preonas et al. 1969). However, in this investigation no evidence exists that acetic and gluconic acids act synergistically. Their antimicrobial mechanisms may be postulated to be mutually exclusive or antagonist. The inhibition of the growth behavior of *Z. bailii* in the formulations containing 5 or 10% (w/w) gluconic and 0.5% (w/w) acetic acids cannot be differentiated from the growth behavior in formulations containing 0.5% (w/w) acetic acid solely. *L. buchneri* growth exhibits rapid decline and cell death in model salad dressings containing 10% (w/w) gluconic acid; however, in the combination dressing with 10% (w/w) gluconic and 0.5% (w/w) acetic acids, stationary cell numbers of *L. buchneri* are observed over the storage period of eight
weeks. Similar results were observed for *B. cereus*. Therefore, some antagonistic activity between gluconic and acetic acid on the growth behavior of *L. buchneri* and *B. cereus* does occur in the model.

Further research is needed to determine and define the exact mechanism of this antagonistic activity and the scope of organisms affected by this activity. Similar antagonist antimicrobial activity for weak lipophilic acids was observed by Warth (1977) in regards to the mechanism of resistance of *Z. bailii* to weak lipophilic acids such as benzoic, sorbic, acetic and butyric. Warth reports that *Z. bailii* is primarily able to grow in the presence of low levels of weak lipophilic acids due to an energy inducible pump. *Z. bailii* utilizes glucose to setup an active transport system or pump expelling preservative anions and protons from the cell. Perhaps, the gluconic acid or its biochemical predecessors act similarly to glucose as an energy source inducing an active transport mechanism in the lactobacilli and bacilli studied allowing them to survive for longer time periods.
VI. Effect of Absence of Oil in Formulations on the Growth Behavior of the Test Organisms

To determine the effect of oil in the formulation of the microbial stability of the model salad dressing formulations, a series of model dressings were prepared without the oil phase (soybean oil and emulsifier) and challenged with the test organisms. The growth behavior of the organisms in the formulations without oil was then compared with the growth behavior in dressings containing the oil phase. This comparison was made with dressings containing no organic acid, containing acetic acid and gluconic acid over the pH range of 2.5 to 4.0. From the data obtained there is no observable effect of the growth behavior of \textit{L. buchneri} or \textit{Z. bailii} when oil is omitted from the formulation regardless of type of acid, acid concentration or pH. No studies were conducted using \textit{B. cereus}; however, no reason exists to suppose that this organism would deviate from the above results.

VII. Further investigations

In light of consumption trends towards low calorie food products including salad dressing, future avenues of investigation should include studies on the microbial stability of these low-calorie dressings. One prominent
way to reduce caloric values in dressing is to reduce the amount of oil in the formulation. This reduction of oil accompanied by the increase of water has several effects on the formulation. First, the acid, preservatives, salt, and other components of the aqueous phase are diluted. Second, as oil concentrations decrease emulsion dynamics are altered, the size of the oil droplets changes, and the amount of free water available for microbial growth increases. Therefore, pertinent investigations would involve determining the effect on microbial stability of reducing the oil and increasing the water and physical studies to determine the emulsion stability and oil droplets dimensions as changes in oil concentration occur. Another area of exploration should be to determine the scope of gluconic acid in food products and against different organisms.
CONCLUSION

The advent of mild tasting ranch-type salad dressings with added ingredients (cheese, meat and dairy products) have challenged traditional methods of preservation. In this investigation a dairy-based model dressing has been developed that is representative of currently marketed "ranch style" dressings. This model dressing was determined to have physical characteristics similar to these commercial dressings. The growth behaviors of two common spoilage organisms, Lactobacillus buchneri and Zygosaccharomyces bailii, and a pathogen, Bacillus cereus, were evaluated in the model dressing. The pH, type of organic acid used as acidulants, and acid concentration in the dressing formulation were varied to provide information about how these parameters affected the growth behavior of the test organisms. With the addition of two organic acids, acetic and gluconic, to the dressing formulation, a dressing was developed that was stable to microbiological spoilage without the incorporation of traditional preservatives such as...
benzoate or sorbate.

To inhibit the growth of lactobacilli, the model pH can be adjusted to pH 3.0 and below with HCL without the addition of organic acids. Similarly, adjustment with HCL to pH 2.0 and below will produce a model dressing stable to spoilage with yeast. *Bacillus cereus* and other pathogens fail to grow in the pH range required to prevent the growth of spoilage organisms by hydrogen ion effect alone.

The growth of yeast can be inhibited by adding 0.5%(w/w) acetic acid into the formulation at pH 4.5 and below. Acetic acid in concentrations of 1.0%(w/w) and below in the pH range of 3.5 to 4.5 is ineffective at preventing the spoilage of the model salad dressing. Spoilage of the model dressing by lactobacilli was prevented by adding 5%(w/w) gluconic acid to dressing formulations and adjusting the sample pH to 3.5 with HCL and by the addition of 10%(w/w) gluconic acid to samples with adjustment of pH to 4.3 and below. From the data collected in this investigation, gluconic acid was not observed to exhibit an inhibitory growth effect against yeast at the acid concentrations and pH range studied.
To prepare a model dressing that was simultaneously stable to challenge by yeast and lactobacilli, a combination organic acid dressing formulation was prepared. This stability to both spoilage organisms and \textit{B. cereus} was achieved by combining 10\%(w/w) gluconic and 0.5\%(w/w) acetic acid over a pH range of 4.3 and below. This creamy dressing has a very mild pleasant taste in keeping with today's consumption trends towards mild tasting creamy dressings and away from harsh acidic tasting products.

Studies investigating the effect on the growth behavior of the test organisms of removing the oil phase from the dressing formulation indicate that there is no discernable differences in growth behavior due to the absence of the oil phase. Further research of importance to the food industry includes investigation on the microbial stability of reduced oil (low calorie) dressings and the application of gluconic acid and the combination gluconic and acetic acids to other moderate food systems. Hopefully, the information and trends obtained from the data collected in this research will provide information about how spoilage organisms and pathogens react in current and future salad dressing formulations and suggest methods of preservation for pH
buffered moderate acid food systems.
REFERENCES


