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Paper Title: FOOD ADDITIVES
Module – 16: Acidity/pH regulators for the food industry

1. Introduction

The pH of a food is the measure of that product's acidity or alkalinity. The pH-scale ranges from 0 to 14. A pH less than 7 is acidic, a pH of 7 is neutral and a pH greater than 7 is alkaline or basic. Our sense of taste can recognize only major differences in the pH within complex food systems. An acid product would taste sour, while an alkaline product would taste bitter. Acidity regulators (Acidulants) are used to alter and control the acidity or alkalinity on a specific level important for processing, taste and food safety. Inadequate control of the pH can result in the growth of undesirable bacteria in the product that could be a potential health hazard.

Acidulants contribute a variety of functional properties that enhance the quality of food. The proper selection of an acid depends on the property or combination of properties of the desired acid as well as cost. Acids are most commonly used for flavor and tartness, and the buffering ability of the salts of some acids can modify and smooth out these characteristics. Acidulants all share a characteristic sour flavor; however the degree of flavor profile varies significantly depending on the final pH of the product. These differences can be used in the formulation of foods with specific sensory qualities that may be unrelated to any antimicrobial effects of the individual acids. In their synergistic capacity, acids used in conjunction with antioxidants prevent rancidity or complex with heavy metals that might initiate oxidation or browning reactions. Acids stabilize color, reduce turbidity, change melt characteristics, prevent splattering, or enhance gelling. They can also act as leavening or inversion agents, emulsifiers, nutrients, and supplements. A major use of acidulants is as an antimicrobial agent and this use is receiving much attention today in a desire to achieve safer food products. With many foods, the incorporation of acids into the product at sufficiently high levels ensures a commercially sterile product. The target levels necessary for this purpose, however, can overwhelm the sensory properties of the food and thereby prevent the use of an acid. When used with other preservation processes, such as refrigeration or heating, acids extend shelf-life for almost indefinite periods from an antimicrobial standpoint.

1.1 Acidulants and their applications

Food acidulants find their application, for the most part, in beverages and in fruit and vegetable processing. Apart from pH lowering, acidulants provide buffer capacity, impair sourness and tartness, and enhance the effect of preservatives. A well-known, slow-release acidulant is glucono-delta-lactone (GDL), which is used in bakery products, dairy products, and in particular, meat products. The use of GDL in the maturation of dry sausages is well known. During preparation of these sausages, GDL standardizes acidification, strongly reduces the risk of contamination, and improves the quality. GDL gradually lowers the pH to 5.4, and after filling the sausage casings, the temperature is lowered to 0 to 4°C for some hours. During this period the growth of starters and undesirable bacteria is inhibited. Then the temperature is increased so that fermentation can take place normally.

Citric acid (E330) enhances the activity of many antioxidants, but is no antioxidant by itself. It is mainly used as an acidity regulator as well as aroma compound. In addition it increases gel consistency in marmalades and decreases enzymatic browning in fruits and fruit products.

Calcium acetate (E263) has several functions. It is used in some foods as a thickening agent (cake mixtures, puddings, pie fillings), but can act as a buffer in controlling the pH of food during processing, as a preservative to prevent microbial growth, and as a calcium supplement in pet products.

Fumaric acid (E297) is added to foods as an acidity regulator and flavouring agent. They are used in bread, fruit drinks, pie fillings, poultry, wine, jams, jelly.

E-Number	Substance	Some foodstuffs in which they are used
E260	Acetic acid	fish fingers, butter, margarine, processed cheese, curry powder, cooking oil.
E263	Calcium acetate	packet desserts, pie fillings
E270	Lactic acid	cheese, milk, meat and poultry, salads, sauces and beverages
E296	Malic acid	tinned fruit, vegetables and pulses, jams, jelly, frozen vegetables
E297	Fumaric acid	bread, fruit drinks, pie fillings, poultry, wine, jams, jelly
E330	Citric acid	fruits and vegetables (lemons and limes), soft drinks
E334	Tartaric acid	bakery, candies, jams, juices and wine

1.2 Legislation

Acidity regulators are subject, just like any other food additive, to stringent EU legislation governing authorisation, use and labelling, Directive 95/2/EC of the European Parliament and the Council of 20 February 1995 on Food Additives other than Colours and Sweeteners. This legislation requires all added acidity regulators, as all food additives, to be declared on food packaging by their category with either their name or E-number.

1.3 CHEMICAL ANALYSIS AND ASSAY

A variety of factors dictates the effective use of acidulants not only in the food product, but also by the physical properties of the acidulant.

The *Official Methods of Analysis* of the *Association of Official Analytical Chemists International* are used as the basis for conducting many assays for the various acids in foods. Assays for organic acids in foodstuffs are generally carried out for three reasons: (1) the organic acid is a natural component of the product and therefore the assay is a quantitative or qualitative measure of wholesomeness or lack of adulteration or is a confirmation of the standard of identity for that product; (2) the acid is not normally present in the food product or is present at levels lower than that normally detected in a standard assay and serves as a measure of adulteration either through addition of the acid or fermentation of the substrate to form acidic byproducts; or (3) the organic acid is added to achieve a desired effect as regulated by good manufacturing practice.

Enzymatic analysis for acetic, citric, dehydroacetic acid, isocitric, L- and D-lactic, L-malic, and succinic acids can be conducted to identify the acid. Acids can also be quantitated using thin layer chromatography, HPLC and titrimetric or colorimetric methods.

2. Mode of action of acids as antimicrobial agents

After years of research work carried out regarding mode of action of acid as antimicrobial agents, it was concluded that besides hydrogen ion concentration (pH), inhibition is apparently also dependent upon the undissociated molecule. This could be the reason why any two acids equilibrated to the identical pH achieved different inhibitory effects.

During 1970s, considerable research on the action of organic acids on the microbial cell focused at the molecular level. It was known that the mode of action of an acid was related to the undissociated portion of the molecule. This action was thought to be more important than any external change in pH brought about by the addition of acids. Fatty acids incorporated into the plasma membrane at a physiological pH of the microorganism would be expected to be almost completely dissociated due to the dissociation constants of the acid. Hunter and Segel (1973) hypothesized that membrane transport processes were inhibited and the proton gradient was somehow affected. The undissociated form of a weak acid penetrates rapidly to the interior of the cell because of its lipid solubility and could discharge the gradient by diffusing through the plasma membrane and dissociate internally. Dissociated forms of weak acids, on the other hand, could not be absorbed by microorganisms to any great extent.

In the year 1972, it was discovered that acetic acid acts as an uncoupler of amino acid carrier proteins from the electron transport system to which they are coupled by protein interaction or cation gradient. The uncoupling effect inhibits amino acid transport noncompetitively. This may come about by acetic acid reacting with the cellular membrane or its proteins by altering the membrane structure or uncoupling electron transport from the proteins responsible for ATP regeneration or transport. Reynolds (1975) proposed that the undissociated portion of the molecule penetrates the cell membrane, dissociates owing to the pH differential, and then causes general protein denaturation. Freese and Levine (1978) found that acids act as proton shuttles that effectively uncouple the trans-membrane proton gradients from energy-coupling circuits. This action was reversible. In practical situations, however, prolonged contact of an acid with a microorganism can lead to decreased viability and death. In addition, some microorganisms can utilize acids in anabolic reactions that lower their effectiveness and account, at least partially, for some differences among microbial species in their sensitivity to acids.

3. Organic Acids

Numerous studies compare several organic acids for their antimicrobial effect. Some of the important organic acids are described below:

Sr. No.	**Name of acid**	**Microbial function**	**Regulatory use in foods**	**Toxicology**
1	Acetic acid	One of the primary functional uses of acetic acid in food has been that of an acidulant. The microcidal activity has been attributed to a lowering of the pH below that needed for optimal growth. In addition, acetic acid is an excellent antimicrobial additive that has bactericidal and fungicidal properties. As with all acids, different organisms can display varying tolerances dependent upon the type, concentration, and pH of the acid when formulated in either a broth or foodstuff system. Lactic, acetic, propionic, and butyric acid–producing bacteria are among the most tolerant of the bacterial groups to the growth inhibitory aspects of acetic acid or other organic acids. Acetic acid has been used primarily to limit bacterial and yeast growth rather than mold growth,	Acetic acid is generally recognized as safe when used in accordance with good manufacturing practice. It can be used as a curing and pickling agent, a pH control agent, flavor enhancer, flavoring agent and adjuvant, solvent, and vehicle. Acetic acid can be commonly found in such products as marinades, vinaigrettes, mustard, catsup, salad dressings, sauces, canned fruits, and mayonnaise. Commercial vinegar contains not less than 4%acetic acid. When acetic acid is coupled with sodium bicarbonate, carbon dioxide is released as a leavening agent.	Acetic acid causes a variety of adverse reactions in humans ranging from allergic-type symptoms such as mouth sores, cold sensitivities, and epidermal reactions to death. Persons consuming acetic acid in high concentrations at a low pH have exhibited burned lips, stomach, and intestinal mucosa, corroded lung tissue, and subsequent pneumonia resulting from inhalation of vapors. Acidosis and renal failure, reduction of

		however some molds are sensitive to this acidulant e.g. Acetic acid has been shown to be an effective antifungal agent at pH 3.5 against the black bread molds, particularly *Aspergillus niger* and *Rhizopus nigrificans.*		clotting efficiency, and interference in blood coagulation are also reported
2	Acetate salts	Derivatives of acetic acid in the form of the calcium, sodium, or potassium salts are substituted for acetic acid in certain formulations. These salts, sometimes used interchangeably with the free acid form, have the same antimicrobial properties at the same pH value.	Calcium and sodium acetates are generally recognized as safe when used in accordance with good manufacturing practice. Calcium acetate can be used as a firming agent, pH control agent, processing aid, sequestrant, texturizer, stabilizer, and thickener	Acetate is a normal intermediate metabolite in humans. No toxicological data were available for calcium acetate nor are reports of acute toxicity to sodium acetate in humans available
3	Dehydroacetic acid	Dehydroacetic acid (DHA) is unique in that, because of its high dissociation constant, it is a more effective inhibitory agent in high pH ranges than other acids. DHA was inhibitory to *Saccharomyces cerevisiae*, *Enterobacter aerogenes*, and *Lactobacillus plantarum* at levels of 0.025, 0.3, and 0.1%, respectively, whereas the sodium salt was inhibitory at twice the levels for the same species	Dehydroacetic acid and its sodium salt are generally recognized as safe when used in accordance with good manufacturing practice. They may be used as a preservative for cut or peeled squash only.	No Toxicological effect was observed by ingesting dehydroacetic acid as reported by several studies.
4	Diacetate salts	Sodium diacetate has been used primarily as a fungistat to retard mold growth in cheese spreads at levels of 0.1–2.0% and in malt syrups at the 0.5% level. Butter wrappers may also be treated with sodium diacetate at the same concentrations to prevent surface mold growth. At levels of 0.05–0.4% at pH 3.5. The same strains and *Mucor pusillus* were inhibited at levels of 0.15–0.5% at pH 4.5. Sodium diacetate was also shown to be effective in limiting growth of *Listeria monocytogenes*, *Escherichia*	Sodium diacetate is generally recognized as safe when used in accordance with good manufacturing practice. Calcium diacetate can be used as a sequestrant in edible oils. Sodium diacetate can be used also as antimicrobial agent, pH control agent, flavoring agent, and adjuvant	No toxicity data are available for the diacetates, although it is assumed that they would be metabolized in a fashion similar to that of acetic acid

		coli, *Pseudomonas fluorescens*, *Salmonella enteritidis*, *Shewanella putrefaciens*, *Pseudomonas fragi*, *Yersinia enterocolitica*, and *Enterococcus fecalis*; while *Lactobacillus fermentus* and *Staphylococcus aureus* were not inhibited. Sodium diacetate was more effective than acetic acid in the pH range of 5.0–6.0, effect increased with decreasing temperature.		
5	Adipic acid	Adipic acid does not have any unusual antimicrobial properties other than a pH effect. Microorganisms susceptible to low pH levels would most likely be affected by this acid.	Adipic acid is generally recognized as safe when used in accordance with good manufacturing practice. It can be used as flavoring, leavening, and pH control agents.	Low levels of adipic acid was reported to show no toxicological effect. According to FAO/WHO, 1966, adipic acid is limited conditionally for humans to a level of 5 mg/kg body weight daily on a free acid basis
6	Ascorbic acid	One of the most important antimicrobial agent. Advantages of ascorbic acid or its salts is its use in conjunction with nitrite to inhibit *Clostridium botulinum* growth in cured meats.	Ascorbic acid and its calcium and sodium salts are generally recognized as safe when used in accordance with good manufacturing practice. Ascorbic acid can be used as a dough acidulant, and color preservative to retard dark spots on shrimp; to preserve color in canned apricots, fruit cocktail, peaches, and asparagus; and to preserve color and flavor in juice and wine. Ascorbic or erythorbic acid can be used as an antioxidant preservative not to exceed 150 ppm in canned. Ascorbic acid can also be used as a preservative in artificially sweetened fruit jelly, preserves, and jams at levels not to exceed 0.1% by weight of finished food	Ascorbic acid exists in nature in its reduced form or as L-dehydroascorbic acid in its readily oxidized form, with biological activity confined to the L isomer. Plants and all mammals except humans, monkeys, and guinea pigs can synthesize ascorbic acid, and these three animals require external sources such as citrus fruits and vegetables in their daily diet.
7	Caprylic acid	Caprylic acid has been ineffective as an antimicrobial	Caprylic acid is generally recognized as safe when	Since caprylic acid occurs

		compound against many gram positive and gram negative bacteria or yeasts in concentrations as high as 7.8 mol/ml. However some results indicate that *Escherichia coli* can be inhibited with only 0.3% caprylic acid when grown in a chemically defined medium. This concentration was far more effective than acetic acid at 1.5% and propionic acid at 1.8%, which did not significantly reduce the population. Caprylic acid was also more inhibitory to *Shigella* spp. than acetic or propionic acids.	used in accordance with good manufacturing practice. It can be used as a flavoring agent and adjuvant in many food products.	normally in various foods and is metabolized as a fatty acid, no limit has been set on the acceptable daily intake for humans.
8	Citric acid	Citric acid and its salts have been investigated for their effects on inhibition of bacteria, yeasts, and molds. One of the reports suggests that on an equal molar basis, citric acid was more inhibitory than lactic acid, followed by acetic acid. Citric acid was particularly found to be inhibitory to flat-sour organisms isolated from tomato juice, and this inhibition appeared to be related to the inherent pH of the product. Citric acid, rather than acetic or lactic acids, was also shown to have an effect on the inhibition of thermophilic bacteria, *Salmonella typhimurium*, lactic acid bacteria such as *Streptococcus agalactiae*, and *S. anatum* and *S. oranienburg*. As little as 0.3% citric acid has been shown to be particularly effective in decreasing native levels of salmonellae on poultry carcasses. In addition to the pH-lowering effects of citric acid, a secondary inhibitory effect is attributed to the chelation of essential minerals. Citric acid was also	Citric acid is generally recognized as safe with no limitations when used in accordance with good manufacturing practice. Citric acid can be used as an acidifying agent in dairy products. Citric acid is one of the major acidulants in carbonated beverages, imparting a refreshing, tangy citrus flavor that enhances fruit flavors, such as lemon, lime, orange, and berry. It is used commercially as a synergist for antioxidants and retardant of browning reactions.	No toxicological effect of citrate has been proved. Citric acid is found in all animal tissues as an intermediate in the Krebs cycle, and therefore no limit has been set on the acceptable daily intake for humans for either the acid or salt.

		effective in reducing mesophilic and thermophilic spoilage in canned mushrooms		
9	Fumaric acid	Fumaric acid has been used as an antimicrobial agent in the prevention of malolactic fermentation in wines and for adding acidity to wines. Esters of fumaric acid have also been found to be effective in the prevention of mold growth on bread. Fumaric acid at a concentration of 0.5% was superior to acetic, citric, malic, or tartaric acids in inactivating *Talaromyces flavus* during heating at 80°C. Lethality also increased as the pH was decreased from 5.0 to 2.5	Fumaric acid can be used to acidify milk, to enhance flavor in canned fruits and, and in artificially sweetened fruit jelly, preserves, and jams. Fumaric acid can be used to accelerate color fixing in cured, comminuted meat and poultry products at a level of 0.065% of the weight of the product before processing. Sodium stearyl fumarate can be used as a dough conditioner in yeast-leavened bakery products, as a conditioning agent in dehydrated potatoes and processed cereals for cooking, in starch- or flour-thickened foods, and as a stabilizing agent in non–yeast-leavened bakery products. Fumaric acid imparts a sour taste to food products and is one of the most acidic of the solid acids. Its low solubility in cold water, however, is a limiting factor in its widespread use.	Fumaric acid is found as an intermediate in the Krebs cycle. It is limited unconditionally for humans to a level of 6 mg/kg body weight daily and conditionally to 6–10 mg/kg daily.
10	Lactic acid	Lactic acid has been used more extensively for its sensory qualities than its antimicrobial properties, although lactate compounds are showing promise as effective rinses for muscle foods to remove microbial loads. Lactic acid was effective in decreasing the number of *Enterobacteriaceae* as well as other gram negative mesophilic and psychrotrophic spoilage organisms. Psychrotrophic, gram negative bacteria were the most sensitive to lactic	Lactic acid is generally recognized as safe when used in accordance with good manufacturing practice. It can be used as antimicrobial and pH control agents, curing and pickling agent, flavor enhancer, flavoring agent and adjuvant. Lactic acid can be used as an acidifying agent in dairy products, except in infant foods and formulas and in grape wines. It also aids in the emulsification of hydroxylated lecithin.	Lactic acid has been found to be lethal in infants who consumed milk that had been Acidified with various quantities of lactic acid. Hemorrhaging, gangrenous gastritis, and esophageal burning resulted in these cases mainly with use of isomeric forms. Since lactic acid

		acid, followed by mesophilic *Enterobacteriaceae*, psychrotrophic gram positive bacteria, lactobacilli and yeasts were the least sensitive. Sodium lactate is purported to lower water activity, and thereby some of its antimicrobial activity may be attributed to a combined acid/low water activity synergy. The inhibiting effect appears to be temperature dependent with lower temperatures being more antibacterial.		is a normal constituent of food and an intermediate metabolite in humans, there is no set limit on the acceptable daily intake for humans. There is also no ADI for calcium lactate. Neither D (-) nor DL lactic acid should be used in infant food.
11	Malic acid	No unusual antimicrobial action is attributed to malic acid other than that associated with pH effects.	Malic acid is generally recognized as safe when used in accordance with good manufacturing practice. It can be used as a flavor enhancer in canned fruits; flavoring agent and adjuvant and pH control agent, and to increase effectiveness of antioxidants	Long-term studies involving rats fed up to 5000 ppm malic acid in their diet showed no change in growth rate, histology, or blood and urine chemistries. The L(-) isomer of malic acid occurs naturally in foods, while the DL form does not and is found as an intermediate in the Krebs cycle. DL-Malic acid is limited to 100 mg/kg body weight as an acceptable daily intake for humans.
12	Propionic acid	Most research using propionic acid and its salts as microbial inhibitors has been directed against fungi although some bacterial species have also been studied. The addition of propionic acid at levels of 8–12% retarded mold growth on the surface of cheese and butter. Fungistatic pH effects were also noted with yeasts and molds. The sodium salts of propionic acid have also shown antimicrobial	Propionic acid and its calcium and sodium salts are generally recognized as safe when used in accordance with good manufacturing. The salts are classified as antimycotics when migrating from food packaging material and as an antimicrobial agent and flavoring agent. Derivatives of propionates can be used as flavoring agents in foods.	Propionic acid is a normal constituent of food and an intermediary metabolite in humans and ruminants, and therefore no limit has been set for the acid or salt forms of propionate on the acceptable daily intake for human

		properties. Sodium propionate retarded growth of *Staphylococcus aureus*, *Sarcina lutea*, *Proteus vulgaris*, *Lactobacillus plantarum*, *Torula* spp., and *Saccharomyces ellipsoideus* for 5 days at levels of 0.1–5.0% when incorporated into food products.		
13	Succinic acid	Like malic acid, succinic acid has not been used extensively in food products for its antimicrobial activity. succinic acid at levels of 3 or 5% at 60°C are as effective as acetic acid in decreasing the microbial load on chicken carcasses. Despite the antimicrobial nature of the compound, however, this concentration adversely affected the appearance of the product.	Succinic acid is generally recognized as safe when used in accordance with good manufacturing practice. It can be used as both a flavor enhancer and a pH control agent in acidified milk. Derivatives of succinic acid can be used as flavoring agents or in combination with paraffin as a protective coating for selected fruits and vegetables.	Succinic acid occurs naturally in small amounts in fruits and as an intermediate in the Krebs cycle. Therefore, no limit has been set on the acceptable daily intake for humans.
14	Tartaric acid	The antimicrobial use of tartaric acid is limited.	Tartaric acid can be used as a firming, flavoring, and pH control agent, flavor enhancer, and humectant. Tartaric acid is an acidifying agent in acidified, artificially sweetened fruit jelly, preserves, and jams and grape wines. Tartaric acid has a strong, tart taste and is the most soluble of all acidulants.	Tartaric acid as the L(-) form is limited unconditionally for humans up to a level of 30 mg/kg body weight.

4. Conclusion

Generally acidulants are used as acidity regulators or pH controlling agents. There are a number of acidulants and their salts that can be added within regulated concentration to get desired effects in various kinds of foods mainly as antimicrobial agents, conditioners in some foods, flavour enhancers, flavouring agents (acidic) or as pH control agents. However their affectivity is dependent on the type of food to which it is added, the solubility of acidulants and their behaviour under different conditions of food system.