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NATURAL SYSTEMS FOR PREVENTING CONTAMINATION AND GROWTH OF MICROORGANISMS IN FOODS

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Abstract

Food-borne illness is a vast and costly worldwide problem. Although complex, it may be divided into two major types of animal and human disease: intoxication caused by toxic substances in food and secondly, infection, caused by microorganisms. Toxic substances may be already present in foods, or they may be produced by microorganisms through contamination and proliferation in food. Prevention of the invasion of foods by microorganisms may be achieved by effective use of intrinsic factors found in plants and animals. These factors include pH, moisture content, oxidation-reduction potential, nutrient content, biological structures, and antimicrobial constituents. A second type of natural protection is that of microbial antagonism. Research work discussed includes that done in the author's laboratories involving tannic acid and microbial antagonism against *Listeria monocytogenes* in milk. The safety of all natural mechanisms of defense is important economically and in public health. We predict that the use of antimicrobials occurring naturally in foods and safe microbial antagonism will receive more attention in future food safety research.

Key Words: Food safety, food-borne diseases, intrinsic factors, extrinsic factors, antimicrobials occurring naturally in foods, microbial antagonism, natural mechanisms, tannic acid.

Introduction

Food-borne illness is a serious worldwide problem in both developed and developing countries. The cost is enormous. For example, in Scotland, outbreaks of poultry-borne salmonellosis are estimated to cost between 200,000 and 900,000 English pounds per year (186). In the U.S.A. food-borne diarrhea alone cost between $5 billion and $17 billion each year (6). However, the cost in terms of health and life cannot be fully measured. Today, there are more than 6.5 million cases of foodborne illness each year in the United States alone (119). Over 9,000 resulted in death in 1990 (111).

This article reviews major types of food-borne diseases and discusses the natural preservation systems which we believe have the most immediate potential for application to the food products industry. The methods discussed will help control contamination and growth of food-borne microorganisms.

Types of Food-Borne Diseases

There are two basic types of food-borne diseases: a. intoxication; and b. infectious.

Food-Borne Intoxication

Food-borne intoxication may be caused by toxic substances already present in certain foodstuffs, toxins produced by microorganisms which contaminate the food, or substances added to food. Examples of food-borne intoxication are numerous (139, 153). One example is mycotoxins produced by molds. These are aflatoxin, zearalenone, zearlenol, trichothecene, ochratoxin, citrinin, penicillic acid, patulin, sterigmatocystin, alternariol methyl ether, mycophenolic acid, panitrem A, and "PR" toxin produced by three genera, i.e., *Aspergillus* sp., *Penicillium* sp., and *Fusarium* species (170). Three of these mycotoxins, i.e., aflatoxin B<sub>1</sub>, sterigmatocystin and ochratoxin A toxins were reported to be carcinogens for animals and possibly for human beings (92). Bacterial toxins are also important source of food-borne intoxication. Enterotoxins produced by *Staphylococcus aureus* (132, 173), and neurotoxins produced by *Clostridium botulinum* (1, 89, 106, 133, 138) are two examples.
additives are often added to food to improve their appearance, flavor or as preservatives. For example, there are currently nine approved food colorants used in food industries of the U.S.A. Five of these colorants are azo dyes (142). There are three thousand other azo dyes which are used in textile, paper, and leather industries (122), which can contaminate water supplies. Many azo dyes such as Butter Yellow and Ponceau 3R can be anaerobically converted into aromatic amines by intestinal microflora (44-46, 87). Many of these aromatic amines are active mutagens and are very likely, important carcinogens (43, 51). They also could be related to our present high incidence of colon cancer (14, 15, 85, 185). It is worth mentioning that although more than 2,000 food additives exist, most of them probably do not cause any food-borne intoxication. However, the chronic and residual type of effects with mutagenic and carcinogenic potential cannot be completely ignored.

Food-Borne Infection

Food-borne infection is caused by ingestion of food containing viable bacteria which then grow and establish themselves in the host, resulting in illness. Some of these pathogens occur in the gastrointestinal tracts of normal healthy animals and humans. Other microorganisms are ubiquitous in nature, occurring on soil and vegetables, in animal wastes, and on animal carcasses. Human skin surfaces and nasal passages also harbor such bacteria as *Staphylococcus* species. Water supplies may be contaminated with fecal materials which contain some of these pathogens. Food and food utensils, air and dust also can be carriers for spread of these pathogens. It is extremely difficult to prevent such pathogens from entering raw foods.

The major food-borne pathogens are: *Salmonella* species, *Listeria monocytogenes*, *Shigella* species, *Campylobacter jejuni*, *Clostridium perfringens*, enteropathogenic *Escherichia coli*, *Bacillus cereus*, *Vibrio* species, *Yersinia enterocolitica*, *Plesiomonas shigelloides* and *Aeromonas hydrophila*. Their major characteristics, the types of food-borne diseases caused and their association with foods are listed in Table 1. *Bacillus cereus* and *Clostridium perfringens* also produce enterotoxins; however, a large number of viable cells must be consumed, which implies the release of toxins in vivo, rather than in the food. In this review, we consider them as causing food-borne infection rather than intoxication.

**Why Is Food-Borne Infection Serious Today?**

Food-borne infection has long been considered a "traditional" or even "common" problem. Why is it becoming even more serious today? There are many reasons such as the following: 1. Greater variety of foods; 2. Mass production and international distribution of foods, allowing for low-level contaminated foods to be distributed to large numbers of people; 3. More meals eaten away from home; 4. More emphasis in these establishments on salads, cold foods, and raw fruits and vegetables that require extensive handling (119).

The current work force of nine million food handlers is poorly paid and poorly educated (119). The relationship between low education levels, low socioeconomic status, and increased frequency of enteric infection is well recognized (119). Many establishments are poorly supervised by health regulating authorities and they often lack proper sanitary facilities. These factors all contribute to the recent increases of incidence of food-borne diseases.

Antibiotics are used either for the prevention of animal diseases or as an additive to stimulate animal growth (69, 91). As a result, many food-borne pathogens have become antibiotic-resistant. Evidence shows that antibiotic-resistant Salmonella is the cause of many salmonellosis outbreaks in the United States (91). The prevalence of antibiotic-resistant food-borne pathogens may also be a factor in enhancing the seriousness of food-borne diseases today.

Prevention of Contamination and Growth of Microorganism in Foods

The "best medicine" is always prevention. Numerous methods were available to minimize microbial contamination of foods. Many of these simply stress available "physical" approaches (3, 54). But they require recognition and implementation.

Several aspects of the storage environment affect both the food and microorganisms. Examples are temperature, relative humidity, and presence and concentration of different gases in the environment. These factors are referred to as "extrinsic" factors (98). By controlling "extrinsic" factors, one can extend the shelf-life of foods. However, this usually requires expensive facilities and operations; and, it may not be feasible in some areas where capital, personnel and equipment are limited.

Plants and animals that serve as food sources have evolved mechanisms of defense against the invasion and proliferation of microorganisms and some remain active in fresh food (98). These inherent food plant or animal tissue elements are referred to as "intrinsic" factors (properties). Among them are pH, moisture content, oxidation-reduction potential, nutrient content, biological structures and antimicrobial constituents (98). These factors serve as natural defense mechanisms against microbial contamination and proliferation during the development stages of food plants and animals. If we can make effective use of these factors, we can further enhance the capability of preventing the contamination and growth of microorganisms in foods. Major "intrinsic" factors are briefly reviewed below.

**pH:**

Most microorganisms normally grow at a pH value around 7, while few grow below 4.0. The pH of
Preventing Contamination and Growth of Microorganisms in Foods

**TABLE 1. Characteristics of Important Food Borne Pathogens**

<table>
<thead>
<tr>
<th>Name of Pathogens</th>
<th>Type of Food-Borne Disease</th>
<th>Gram Stain</th>
<th>$A_w$</th>
<th>Major type of Food Associated</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em> species</td>
<td>Food-borne infection (Salmonellosis)</td>
<td>-</td>
<td>0.95-</td>
<td>Beef, pork, turkey, chicken, ice cream, milk</td>
<td>5, 32, 66, 71, 88, 126</td>
</tr>
<tr>
<td><em>Listeria</em> species</td>
<td>Food-borne infection (Listeriosis)</td>
<td>+</td>
<td>0.99</td>
<td>milk, cole slaw, cheese</td>
<td>27, 29, 40, 62, 80, 116, 120, 143, 145, 157, 158, 159, 160</td>
</tr>
<tr>
<td><em>Shigella</em> species</td>
<td>Food-borne infection (Shigellosis)</td>
<td>-</td>
<td></td>
<td>salads, seafood</td>
<td>31, 163, 167, 174, 181</td>
</tr>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>Food-borne infection (Campylobacteriosis)</td>
<td>-</td>
<td></td>
<td>poultry, pork, beef, lamb, clams, cakes, raw milk</td>
<td>61, 63, 72, 168</td>
</tr>
<tr>
<td>Enteropathogenic <em>Escherichia coli</em></td>
<td>Food-borne infection (Gastroenteritis)</td>
<td>-</td>
<td>0.96</td>
<td>cheese, sandwiches, hamburgers</td>
<td>39, 73, 149</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Food-borne intoxication (Gastroenteritis)</td>
<td>+</td>
<td>0.86</td>
<td>milk, cream saucers, salads, puddings, 131, 176 dings, custards, bakery products</td>
<td></td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>Food-borne infection (Gastroenteritis)</td>
<td>+</td>
<td></td>
<td>meat, poultry</td>
<td>77, 110</td>
</tr>
<tr>
<td><em>Clostridium botulinum</em></td>
<td>Food-borne intoxication (Gastroenteritis)</td>
<td>+</td>
<td>0.94</td>
<td>meat, fruit, vegetables, honey, syrups</td>
<td>1, 89, 106, 133, 138</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>Food-borne infection (Gastroenteritis)</td>
<td>+</td>
<td></td>
<td>cereal, corn, cornstarch, mashed potatoes, vegetables, meat products, puddings, soups, sauces, macaroni, cheese</td>
<td>4, 47, 65, 99, 121, 127, 137</td>
</tr>
<tr>
<td><em>Vibrio</em> species</td>
<td>Food-borne infection (Gastroenteritis)</td>
<td>-</td>
<td>0.94</td>
<td>seafood, oysters, crabs, eggs, asparagus, potatoes, crawfish, shrimp, clams</td>
<td>25, 41, 42, 67, 82, 83, 125, 135</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>Food-borne infection (Gastroenteritis)</td>
<td>-</td>
<td></td>
<td>cakes, meats, seafood, vegetables, beef, lamb, pork, milk</td>
<td>64, 84, 112, 128</td>
</tr>
<tr>
<td><em>Plesiomonas shigelloides</em></td>
<td>Food-borne infection (Gastroenteritis)</td>
<td>-</td>
<td>0.97</td>
<td>fish, shellfish</td>
<td>123</td>
</tr>
<tr>
<td><em>Aeromonas hydrophila</em></td>
<td>Food-borne infection (Gastroenteritis)</td>
<td>-</td>
<td></td>
<td>fish</td>
<td>9</td>
</tr>
</tbody>
</table>

$A_w$: water activity (minimum value for growth)
many foods such as fruits, drinks, vinegar and wine (fermented products), fall below the level at which bacteria normally grow. Proper pH is excellent for maintaining protection from microbial invasion. Artificial adjustment of pH of certain foods may also be a way to protect them.

Moisture Content

Drying or desiccation is one of the oldest methods of preserving foods. Available water is usually defined in terms of water activity (aw). In foods, the aw is defined by the ratio of vapor pressure of food substrate to the vapor pressure of pure water at the same temperature, i.e., aw = p/p0, where p = vapor pressure of solution, and p0 = vapor pressure of solvent (usually water). The addition of salt (NaCl) or sugar lowers aw. For example, a NaCl solution of 22%, has an aw of 0.86. Most spoilage bacteria do not grow below aw = 0.91, while some fungi can grow as low as aw = 0.70. With respect to food-poisoning bacteria, *Staphylococcus aureus* can grow at an aw = 0.86; *Clostridium botulinum* Type E, 0.97; *Escherichia coli*, 0.96; *Clostridium botulinum* Type A and B, 0.94; and *Vibrio parahaemolyticus*, 0.93 (98).

The aw of most fresh food is above 0.99; some foods have lower values, for example: the aw for liver sausage is 0.96; salami 0.82-0.85; dry fruit, 0.72-0.80; honey 0.75 and marmalade, 0.82-0.92. (12).

Oxidation-Reduction Potential (O/R; Eh)

The oxidation-reduction potential (O/R or Eh) of a substrate is defined as the ease with which the substrate loses or gain electrons. When an electron or compound loses an electron, it is said to be oxidized; whereas a substrate that gains an electron becomes reduced. When electrons are transferred from one compound to another, a potential difference is created between the two. This difference is measured by an appropriate instrument and expressed in the term of millivolts (mV). A highly oxidized substrate would have a positive Eh while a reduced substrate a negative Eh.

Microorganisms display varying degrees of sensitivity to the oxidation-reduction potential of their growth medium (98). Some bacteria, such as *Clostridium* require reduced conditions for growth. They are called anaerobes. Others, such as *Bacillus* species require positive Eh for growth and are regarded as aerobes. Some such as lactobacilli and streptococci, actually grow better under slightly reduced conditions, and are regarded as microaerophilic. Some bacteria have the capacity to grow under either aerobic (positive Eh) or anaerobic (negative Eh) conditions, and are called facultative anaerobes. Most molds and yeasts associated with food are aerobic, but a few tend to be facultative anaerobes.

Plant foods, especially plant juices, have Eh values from +300 to +400 mv. Solid meats have an Eh value of -200 mv while cheeses have Eh values of -20 to -200 mv. Different Eh values of foods may influence the types of micro-organisms in those foods (98).

Nutrient Content

Many foods have a variety of energy sources, nitrogen sources, minerals, vitamins and related growth factors required for microbial growth. For energy sources, food-borne microorganisms may utilize sugars, alcohols and amino acids. Some are able to use complicated carbohydrates such as starch, cellulose, pectin, hemicellulose as a source of energy. A few of them can use fat as an energy source. The primary source of nitrogen for food-borne microorganisms are amino acids. Some microbes can utilize nucleotides, peptides or proteins. Growth factors such as Vitamin B are required by many food-borne Gram-positive bacteria. Foods lacking these growth factors may limit the growth of these type of bacteria (98).

Protective Biological Structures

The natural covering of some foods provides limited protection against microbial contamination and spoilage. Examples are: testa of seeds, outer coverings of fruits, the shell of nuts, the hide of animals and shells of eggs (98).

Antimicrobials Occurring Naturally in Foods

Many naturally occurring substances in the food plants and animals have been shown to have antimicrobial activity. These antimicrobial substances contribute greatly to the stability of some foods against attack by microorganisms. A recently detailed review (20) of these antimicrobials occurring in plants and animals showed that there are several categories of these antimicrobials in foods:

**Enzymes and Proteins:** Conalbumin and avidin in the egg white are inhibitory to microorganisms. Conalbumin, which binds with iron, makes it unavailable for use by microorganisms. It is inhibitory to both Gram-negative and Gram-positive bacteria. *Micrococcus* and *Bacillus* species are particularly sensitive to conalbumin (26). Avidin can bind biotin and thus can inhibit the growth of microorganisms which have a strict requirement for biotin.

Another iron binding protein, lactoferrin in bovine milk, is inhibitory to the growth of *Bacillus subtilis*, *B. steatherophilus* and *Escherichia coli* (136, 147). The casein fraction of milk also has been demonstrated to inhibit *B. steatherophilus* (7). Part of this inhibitory action is due to lactoferrin present in the casein fraction.

Lysozyme in both milk and eggs is inhibitory to several organisms including *Listeria monocytogenes*, *Campylobacter jejuni*, *Salmonella typhimurium*, *Bacillus cereus*, and *Clostridium botulinum* (95). The mechanism of action of this enzyme involves hydrolysis of β-1,4-linkage between N-acetylmuramic acid and N-acetylglucosamine layer of the bacterial cell wall. Usually Gram-negative bacteria are resistant to lysozyme, but susceptibility can be induced following sodium chloride and ethylenediamine tetraacetate treatments which weaken the outer membrane (178). Lysozyme has received more
attention recently and is considered to have a good potential as a food preservative because it is specific for bacteria and harmless to humans.

Lactoperoxidase in bovine milk is an effective antimicrobial enzyme when combined with thiocyanate (SCN) and hydrogen peroxide (147). This is called the lactoperoxidase system. Thiocyanate is widely distributed in animal tissues and secretions (148). Hydrogen peroxide is readily produced by catalase-negative bacteria such as Lactobacillus or can be added exogenously. The lactoperoxidase system is active against H₂O₂-producing bacteria such as Lactobacillus and Streptococcus spp., and also Gram-negative catalase positive organisms including Pseudomonas spp. and Escherichia coli (24). It was recently demonstrated (162) that the lactoperoxidase system delayed but did not prevent the onset of the exponential growth of Listeria monocytogenes Scott A culture.

The lactoperoxidase system can form various oxidizing products such as hypohiostycnate, cyanosulfuric acid and cyanosulfurous acid, all of which show antimicrobial activity (148). Several possible mechanisms of inhibition have been postulated including reduced oxygen uptake, reduced lactate production by fermentation organisms, inhibition of key metabolic enzymes such as hexokinase, glyceraldehyde-3-phosphate-dehydrogenase and D-lactate dehydrogenase, inhibition of glucose uptake, cytoplasmic damage with leakage of ions and ultraviolet absorbing materials and inhibition of nucleic acid and protein syntheses (162).

**Organic acids**: Organic acid, whether present in foods naturally, or occurring as a result of fermentation, also have antimicrobial properties (Table 2). Examples are citric acid, succinic acid, malic acid, tartaric acid, benzoic acid, lactic acid and propionic acid. Mechanisms of action involve direct pH reduction of the substrate, depression of the internal cellular pH by ionization of the undissociated acid molecules, or disruption of substrate transport by alteration of cell membrane permeability, or inhibition of nicotinamide adenine dinucleotide (reduced form) [NADH] oxidation (20, 74). Generally the undissociated form of organic acid molecules is responsible for the antimicrobial activity.

**Medium-chain fatty acids**: Medium-chain fatty acids, both saturated and unsaturated, occur in plant and animal fats. Seed fats contain low levels of saturated fatty acids, while marine oils are high in unsaturated fatty acid (58). Medium-chain fatty acids, containing 12 to 18 carbon atoms are effective antimicrobial agents. It has been reported (103) that lauric acid, myristic and palmitic acid (C₁₂, C₁₄, and C₁₆, respectively) are effective inhibitors of bacteria and both capric acid (C₁₀) and lauric acid are active against yeast.

Chain length, degree of saturation and geometric configuration are all important factors affecting antimicrobial activity of fatty acids. For Gram-positive bacteria, saturated fatty acids with 12 carbons as well as the monounsaturated palmitoleic acid (C₁₆:₁) and linoleic acid are most effective (102). Addition of one cis double bond to C₁₄, C₁₆ and C₁₈ fatty acids, increases antimicrobial activity. Generally, Gram-negative bacteria are less susceptible than Gram-positive bacteria to the inhibitory effects of fatty acids. This was possibly due to the fact that the lipopolysaccharide layer of Gram-negative bacteria was resistant to fatty acids penetration into cells and thereby conferred resistance (161). As with organic acid, the undissociated form of fatty acid molecules is responsible for the antimicrobial activity. Therefore, the antimicrobial activity of fatty acid is favored by a reduction in pH.

The mechanisms for the antimicrobial activity of fatty acids have been studied by a number of investigators. Fatty acid caused a reduction of oxygen uptake and induced leakage of amino acids (76). Fatty acid also altered cell membrane permeability or uncoupled the electron transport chain of specific protein responsible for ATP (adenosine triphosphate) generation and nutrient transport to the cell (161). It was suggested (75) that fatty acid caused the inhibition of membrane transport, resulting in nutrient deprivation. It was also demonstrated (81) that inhibition of Staphylococcus aureus was due to increased membrane permeability.

There are problems with using medium-chain fatty acid as food preservatives. For example, solubility is one of the main difficulties. There are also antagonists in food, such as albumin, starch, and cholesterol, which interfere with the antimicrobial activity of fatty acids (132). However, fatty acids may still have potential as microbial inhibitors for slightly acid foods and some foods which do not have suitable preservatives. It was proposed (103) that concurrent use of multiple approved food additives is a good way for food preservation. Fatty acids, will therefore, be used more often as antimicrobial food agents.

The naturally occurring fatty acid esters of sucrose and other polyhydric alcohols have also been reported to possess antimicrobial properties (19, 52, 104, 105, 115). Glycerol monolaurate, for example, was demonstrated to be inhibitory to Vibrio parahaemolyticus (19), Aspergillus niger, Penicillium citrinum, Candida

| **TABLE 2. Sources of Organic Acids in Foods** (from reference 20) |
|--------------------------|--------------------------|
| **Organic Acids**        | **Food Sources**          |
| Citric Acid              | Citrus Fruits            |
| Succinic Acid            | Asparagus, Broccoli, Sugar Beets, Rhubarb, Fermented Cheese and Vegetables |
| Malic Acid               | Fruits and Vegetables Apples |
| Tartaric Acid            | Grapes and Pineapples    |
| Benzoic Acid             | Cranberries, Raspberries, Plums, Prunes, Cinnamon, Cloves |
| Lactic Acid              | Sauerkraut, Pickles, Olives, Meats, Cheeses |
| Propionic Acid           | Swiss cheeses            |

...
2 CH₂ = CH-CH₂-SO-CH₂ -CHNH₂-COOH + H₂O \[ \text{Allinase} \]
CH₂ = CH-CH₂-SOS-CH₂CH = CH₂ + 2 CH₂ COCOOH + 2NH₃

Allin

Pyruvic Acid

Ammonia

**Figure 1.** Enzymatic Conversion of Allin to Allicin.

**Figure 2.** Hydrolysis of Oleuropein.

**Plant Essential Oil Components:** Extracts from plants and plant parts used as flavoring agents in foods and beverages possess antimicrobial activity. Typical examples are the *Allium* species: *A. sativum* (garlic), *A. cepa* (onion) and *A. porrum* (leek) (20). Effects of extracts from garlic and onion on food-borne bacterial pathogens have been reported (59, 100, 114, 155, 176). Several species of food spoilage yeasts and molds were also inhibited by garlic (53, 124, 172). The principal antimicrobial component of garlic has been demonstrated to be allicin (2-propenyl-2-propenethiol sulfinate) (169). The intact garlic bulb does not contain allicin, but rather alliin (S-allyl-L-cysteine-S-oxide), which is hydrolyzed by allinase to yield allicin, pyruvic acid and ammonia (Figure 1).

Other plants used as herbs and spices in food include: achiote, allspice (pimenta), almond (bitter), angelica, basil (sweet), bay (laurel), bergamot, calamus, cananga, caraway, cardamom, celery, cinnamon, citronella, clove, coriander, dill, elecampane, fennel, garlic, ginger, lemon, licorice, lime, mace, mandarin, marjoram, musky bugle, mustard, nutmeg, orange, orange-gano, paprika, parsley, pennyroyal, pepper, peppermint, rosemary, sage, sassafras, spearmint, star anise, tarragon (estragon), thyme, turmeric, verbena and wintergreen (20). Certain antimicrobial compounds in these plants which have been identified are thymol [5-methyl-2-(1-methylethyl)-phenol], cinnamic aldehyde (3-phenyl-2-propenal), and eugenol [2-methoxy-4-(2-propenal) phenol]. These antimicrobial compounds are often in the essential oil fraction (20).

**Pigments and Related Compounds:** It has been shown (117, 118) that proanthocyanidin in cranberries is inhibitory to *Saccharomyces bovis*, and that pelargonidin-3-monoglucoside and its degradation products are inhibitory to *E. coli* and *Staphylococcus aureus* (86). Anthocyanins were shown to be inhibitory to microbes (140, 187). Also demonstrated was that monoglucosides of cyanidin, pelargonidin and delphinidin were inhibitory to certain bacteria (141). The mechanism of inhibitory action of anthocyanins is not fully understood. One possibility is that anthocyanin has chelating ability, which makes metal ion unavailable to enzymes. The addition of magnesium and calcium could reverse bacterial inhibition by malvidin-3-monoglucosides (166). It has also been reported (38, 183) that anthocyanins had an inhibitory effect on certain bacterial enzymes.

Chlorophyllide a, a degradation product of chlorophyll a, has also been shown to inhibit the growth of *Bacillus subtilis*, *E. coli* and *Pseudomonas fluorescens* (17).

**Humulones and Lupulones:** Certain major constituents of hop resin such as humulones (humulone, cohumulone, adhumulone) and lupulones (lupulone, colupulone and adlupulone) were reported to exhibit antimicrobial activities (20). The mechanism of inhibition is not known at the present.

**Oleuropein:** The phenolic components of ethyl acetate extracts of the green olive including oleuropein and aglycone of oleuropein were shown to be inhibitory to *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, and fungi *Geotrichum candidum* and *Rhizopus* spp. (101). In studying the antimicrobial properties of oleuropein and products of its hydrolysis (70), it was discovered that the crude oleuropein extract per se was inhibitory only to 3 of 17 species screened, i.e., *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas solanacearum*, but the acid hydrolysate product of the extract was inhibitory to 11 bacteria including *Lactobacillus plantarum*, *WSO*, *L. brevis* 50, *Pediococcus cerevisiae* 39, *Leuconostoc mesenteroides* 42, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhimurium*, *Pseudomonas solanacearum*, *P. lachrymans*, *Erwinia carotovora*, *Xanthomonas vesicatoria*. Using lactic acid producers, i.e., *L. plantarum*, *L. brevis*, *Pediococcus cerevisiae* and *Leuconostoc mesenteroides* for test organisms, it was found that oleuropein was not inhibitory, but two of its hydrolysis products, the aglycone and elenolic acid were inhibitory (Fig. 2). The aglycone of oleuropein and elenolic acid were much more inhibitory when the broth medium contained 5% NaCl (70).

**Caffeine:** Caffeine is present in coffee, tea and cocoa beans, and has been demonstrated to inhibit the growth and mycotoxin production by several *Aspergillus* and *Penicillium* species (33, 34, 113) at 1 mg/ml. Caffeine uncoupled the regulation of glycolysis and
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Figure 3. Hydrolysis of Tannic Acid.

Gallic Acid

Ellagitannin

Gallic Acid + Glucose

Tannic Acid
(R-Digalllic Acid)

The antimicrobial activity of tannic acid is diminished when the plants or fruits mature, tannic acid serves as a natural antimicrobial agent to protect against microbial infection. When the plants or fruits mature, tannic acid is hydrolyzed and releasing gallic acid, ellagic acid and glucose. Glucose gives the sweet taste of the fruit and the antimicrobial activity of tannic acid is diminished. This is a good natural mechanism against microbial contamination of foods. If we can manipulate this system properly, we may be able to extend the shelf life of certain food products. For example, the hydrolysis of tannic acid can be manipulated to match the commercial readiness of fruit consumption, or it could be added to certain food products to extend shelf-life.
It is worth noting that tannins, ellagic acid, gallic acid and methyl gallate have been used as antioxidants (130, 171). Many beneficial effects of ellagic acid and tannic acid have been reported (8, 50, 93, 94, 146). Tannic acid is categorized as a "Generally Recognized as Safe" (GRAS) food additive from the code of federal regulation (60). It is allowed to a maximum of 400 ppm in frozen dessert/mixed soft candies; 150 ppm in beverages, non-alcoholic beverages and basal gelatins, pudding and fillings; 130 ppm in hard candies; 100 ppm in baked goods, baking mixes; and 10 ppm in meat products (60). The antimicrobial and antioxidant properties of tannic acid, the antimutagenic and anticarcinogenic potential of tannic acid and their hydrolyzed products, will undoubtedly be well received by consumers when its efficiency and safety are known. The potential for utilization of this natural product to defend against microbial spoilage in food systems is therefore very promising.

Additional Plant Antimicrobials: There are many other plant components which also exhibit antimicrobial activity. For example, magnalol and honokiol, the components of Magnoliae Cortex, showed antibacterial activities against Gram-positive bacteria including Staphylococcus aureus and Lactobacillus spp. (129). Sorbic acid from the berries of the mountain ash tree (Rowanberry) are antimicrobial (57). Sorbic acid and its potassium, calcium or sodium salts are collectively known as sorbates. Sorbates have been used in foods as effective inhibitors of fungi, including those that produce mycotoxins, and certain bacteria (13, 28, 151, 164, 165). Extracts from the Chinese Nutgall showed antimicrobial activity (184). Chinese medicinal plants such as Tin Men Chu, Siu Mao Heung, and Sey Lau Pai inhibit the growth of many food-borne bacteria (48). The chemical compositions of these extracts are not totally understood. Medicinal plants which have been used as folkloric medicine include licorice, ginger, paeony root, gassia and many others, all of which may contain antimicrobial components (90). More thorough survey and additional studies will certainly be necessary to disclose basic components of such antimicrobial agents and their modes of action against microorganisms involved in food-borne diseases.

Microbial Antagonism in Foods

Foods are generally good microbial environments. Usually more than one type of microorganism present in a particular food. Microbial antagonism has been documented in various kinds of foods, especially fermented products (96). For example, the growth of salmonellae and staphylococci were retarded in foods when cultured with lactic streptococci (78). The shelf-life of both refrigerated ground and mechanically deboned poultry meat was extended by 2 days using the resting cells of starter cultures, Pediococcus cerevisiae (Accel) and Lactobacillus plantarum (Lactacel DS) (144). Both of these organisms inhibited the growth of Psedomonas fluorescens and P. putrefaciens in buffered brain heart infusion (BBHI). Also, lactic acid bacteria affected the growth of Staphylococcus aureus, Yersinia enterocolitica, Salmonella typhimurium, Salmonella enteritidis and Bacillus cereus in vacuum packed bologna type sausage (134).

Since Listeria monocytogenes has recently become a major concern to the food industry, attention has been given to antagonism against this organism in foods. The growth of L. monocytogenes was somewhat inhibited in medium in the presence of Streptococcus cremoris (Lactococcus lactis subsp. cremoris) or Streptococcus lactis (Lactococcus lactis subsp. lactis) (182). Also, bacteriocin-producing Pediococcus species were inhibitory to L. monocytogenes during the manufacture of fermented semidry sausage (16).

More work has been done on lactic acid bacteria such as Lactobacillus, Lactococcus (group N Streptococcus), Leuconostoc and Pediococcus. This is partly because lactic acid fermentation has been a good method of food preservation. We now learn that lactic acid bacteria are capable of producing inhibitory substances, including hydrogen peroxide, diacytel, and bacteriocins (55, 107). One bacteriocin designated as pediocin AcH was isolated (21) from Pediococcus acidilactici strain H. It is a small peptide with a molecular weight of 2,700. Pediocin AcH inhibited the growth of several food-spoilage bacteria including Aeromonas hydrophila, Bacillus cereus, Brothothrix thermosphaeta, Lactobacillus leichmanni, L. plantarum, L. viridescens, Leuconostoc mesenteroides and Pseudomonas putida as well as the food-borne pathogens, Staphylococcus aureus, Clostridium perfringens and Listeria monocytogenes. The possible mode of action of pediocin AcH was associated with non-specific binding to the non-specific receptor, lipoteichoic acid and attachment to specific receptors. This causes loss of membrane integrity and resulting loss of cytoplasmic materials and dividing capability (22). Another bacteriocin, nisin, produced by Lactococcus lactis has been well characterized and is approved for some uses as a food preservative (68, 97). The primary target of nisin was reported to be the cytoplasmic membrane (152, 154). A number of other bacteriocins such as Lactacin B is produced by Lactobacillus acidophilus (10); Lactocin 27 by Lactobacillus helveticen LP 27 (177) and Pediocin PA-1 by Pediococcus acidilactici PAC 1.0 (79, 143) have also been reported. Studies along this line seem to be promising since no ill effects on human health by foods properly fermented with lactic acid bacteria has generally been reported.

We have also been interested in studying the interaction of L. monocytogenes with other bacteria, particularly in milk. We isolated a Gram-positive coccus from Bulgarian style cultured milk and found that growth of L. monocytogenes was significantly inhibited in the presence of this unidentified culture (Figure 4). Data show that this inhibition is not due to pH exclusively, nor due to the nutrient depletion caused by this organism. It is probably due to some factor or factors produced by this organism (49).
then will we be able to make full and proper use of these compounds. An example is tannic acid. If we can add tannic acid in proper amounts to food products, we may be able to extend the shelf-life of many products. When a product is ready for consumption, a hydrolytic enzyme (tannase) can be added to release the ellagic acid and glucose, which will give a sweet taste and avoid the astringency of tannins.

Microbial antagonisms also deserve further in-depth studies. Although some work has been done with lactic acid bacteria and a number of bacteriocins are known, further research is needed to develop molecular biological techniques to produce large amount of these bacteriocins on an industrial scale. A better understanding of this kind of microbial interaction in food systems may lead to better methods of food preservation. Development of natural means of protection will have the advantages of better product quality, greater economy and will build public confidence. The natural mechanisms discussed in this article, especially the use of the antimicrobials occurring naturally in foods and safe microbial antagonisms, we predict, will receive more attention in future food safety research.

References

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Editor's Note: All of the reviewer's concerns were appropriately addressed by text changes, hence there is no Discussion with Reviewers.