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Microbial Safety of Pickled Fruits and Vegetables and Hurdle Technology

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Escherichia coli O157:H7 is a member of the enterohemorrhagic group of pathogenic *E. coli* that has emerged as a foodborne pathogen of major public health concern. *E. coli* O157:H7 is highly tolerant of acidic pH and outbreaks attributed to this bacterium have been in many acidic foods which have pH level with similar to those of pickled products. Therefore, pickled vegetables, although acidic, may not safe. In pickling vegetables, the combination of preservation factors (heat, acetic acid, and salt) will contribute to increase the microbial safety. Recently, the concept of combining preservative factors for food preservation was developed, called 'hurdle technology'. In hurdle technology, combination treatments are applied because it is expected that the use of combined preservative factors will have greater effectiveness at inactivating microorganisms than the use of any single factor. However, recent studies show that the combination of preservation factors can have unexpected antimicrobial activity. Therefore, this article includes an overall review of the microbial safety of fruits and vegetables, preservative method including major preservative factors used in pickling technology, concept and mechanism of hurdle technology and *E. coli* O157:H7.

1. Microbial hazards in fruits and vegetables

The quality of food can be adversely affected by physical, chemical, biochemical and microbiological processes. Quality deterioration caused by microorganisms may include a wide range of types of spoilage that are undesirable commercially, because they limit shelf life or lead to quality complaints, but are safe from a public health point of view. More seriously, the presence or growth of infectious or toxinogenic microorganisms (foodborne pathogens) represent the worst forms of quality deterioration, because they threaten the health of the consumer (ICMSF, 1996). Therefore, while the aim of effective food preservation is to control all forms of quality deterioration, the overriding priority is always to minimize the potential for the occurrence and growth of food spoilage and food poisoning microorganisms (ICMSF, 1996).

Despite the extensive scientific progress and technological developments achieved in recent years, food safety problems continue to exist and may actually increase in the future. It is estimated that foodborne pathogens cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths in the United States each year, most due to unknown causative agents (Mead et al., 1999). Among the know pathogens associated with foodborne illnesses, there is an increasing involvement of environmentally resistant and host-adapted species or strains, which may be difficult to inactivate or control with traditional food preservation methods (Alterkruse et al., 1997; Foster, 1997; Tauxe, 1997) Although the incidence of foodborne illnesses linked to fresh produce is low, there is increased awareness that fruits and vegetables can be contaminated with microbiological pathogens. For its microbiological sampling program of certain fresh fruits and vegetables, the U.S. Food and Drug Administration (FDA) conducted surveys of both imported and domestic produce. A 4% (44 of 1003 sampled) contamination rate was reported in published results for imported products (Novak et al., 2003). In the interim report on domestic products, there was a 1.6% contamination rate (Novak et al., 2003). With the shift in diet toward the consumption of more fresh fruits and vegetables and greater distribution distances from new geographic sources, there have been more reported illnesses involving fresh produce (Tauxe, 1997). In the United States from 1988 to 1992, 64 outbreaks of disease were related to the consumption of fruits and vegetables resulting in 9 deaths (Bean et al., 1996). From 1993 to 1997, in 66 outbreaks with 2 deaths, fruits and vegetables were the vehicles of transmission (Sumner and Peters, 1997). Because foodborne infections are sporadic and many go unreported, the exact numbers of cases related to produce is unknown (Tauxe, 1997). Due to changes in the food supply and food consumption patterns in the United States, deaths caused by foodborne diseases are even more difficult to estimate (Mead et al., 1999). These foodborne outbreaks cause gastroenteritis and in severe cases, hospitalization is required. Fruit and vegetable contamination problems can occur in the growing environment. During growth the fruit or vegetable can become contaminated from sources such as soil, water, animals, birds, and insects. Following production, the processes of harvesting, washing, cutting, slicing, packaging, and shipping can create additional conditions where contamination can occur. Because of high water activity

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TABLE 1. Most important hurdles for food preservation (adapted from Leistner, 1995)

Symbol	Parameter	Application
F	High temperature	Heating
T	Low temperature	Chilling, freezing
a_w	Reduced water activity	Drying, curing, conserving
pH	Increased acidity	Acid addition or formation
Eh	Reduced redox potential	Removal of oxygen or addition of ascorbate
Pres.	Preservatives	Sorbate, sulfite, nitrite,
c.f.	Competitive flora	Microbial fermentations

(a_w) and nutrient content, fresh produce can support the growth of a variety of disease-causing microorganisms (Sumner and Peters, 1997). Pathogens frequently associated with minimally processed vegetables include *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Clostridium botulinum*, *Aeromonas hydrophila*, *Salmonella* spp., and *Campylobacter jejuni* (Schuenzel and Harrison, 2002).

2. Food preservation technology

Food preservation in the broad sense of the term refers to all measures taken against any spoilage of food. In its narrower sense, however, food preservation connotes processes directed against food spoilage due to microbial or biochemical action. Preservation technologies are based mainly on the inactivation of microorganisms or on the delay or prevention of microbial growth. Consequently they must operate through those factors that most effectively influence the survival and growth of microorganisms (ICMSF, 1980).

Factors used for food preservation are called '*hurdles*' and there are numerous hurdles that have been applied for food preservation. Potential hurdles for use in the preservation of foods can be divided into physical, physicochemical, microbially derived and miscellaneous hurdles (Leistner and Gorris, 1995). Among these hurdles, the most important have been used for centuries and are as either 'process' or 'additive' hurdles including high temperature, low temperature, water activity , acidity, redox potential (E_h), competitive microorganisms (e.g. lactic acid bacteria) and preservatives (e.g. nitrite, sorbate, sulphite) (Table 1). Recently the underlying principles of these traditional methods have been defined and effective limits of these factors for microbial growth, survival, and death have been established. Recently, about 50 additional hurdles have been used in food preservation. These hurdles include: ultrahigh pressure, mano-thermo-sonication, photodynamic inactivation, modified atmosphere packaging of both non-respiring and respiring products, edible coatings, ethanol, Maillard reaction products and bacteriocins (Table 2).

Hurdles used for food preservation influence the quality as well as the safety of foods. The effects of hurdles on food quality can be positive or negative, depending on their intensity. Some hurdles (e.g. Maillard reaction products) have antimicrobial properties and at the same time improve the flavor of the products; this also applies to nitrite used in the curing of meat. However, some hurdles also provide a negative effect on food quality. For instance, chilling to an unsuitably low temperature will be detrimental to some foods of plant origin ('chilling injury'), whereas moderate chilling is beneficial for shelf-life. Another example is the pH of fermented sausages, which should be low enough to inhibit pathogenic bacteria but now so low as to impair taste. If the level of a particular hurdle in a food is too small, it should be strengthened; if it is detrimental to the food quality, it should be lowered and an additional hurdle considered.

To ensure the stability and safety of foods, each hurdle should be applied in the proper manner. However, the high strength or concentration of any single hurdle could adversely affect food quality such as loss of nutrients, texture, color, etc. Therefore, if manufacturers are to achieve a product that accommodates consumer preferences while remaining safe and stable, they must achieve synergies through the combined use of these or other hurdles which, while they may not prevent the growth of pathogenic or spoilage microorganisms on their own, will do so collectively. And each product may require a different combination of hurdles, depending on a range of factors including the following; (i) the initial microbial load of the product requiring preservation, (ii) how favorable conditions are within the product for microbial growth, (iii) target shelf-life.

An important current trend is toward the use of procedures that deliver food products are less "heavily" preserved, higher in quality, perceived as being more "natural", contain less additives, and are nutritionally healthier. Some new and "emerging" techniques aim to meet some of these objectives. Most of these techniques act by inactivating microbes (e.g., the application of high hydrostatic pressure, high-voltage electric pulses, high-intensity laser and noncoherent light pulses). Many naturally occurring antimicrobials have been explored for used as food additive preservatives (Conner, 1993; Dillon and Board, 1994; Hoover, 2000). But few have yet been widely exploited. Lysozyme has gained a useful market for the destruction of outgrowing cells from spores of *Clostridium tyrobutyricum* in some cheeses. The bacteriocin, nisin, likewise has a well-developed portfolio of uses in cheese, canned and some other foods, and the antimycotic natamycin (pimaricin), is employed to prevent mold growth on cheese and surfaces of salami type products (Leistner and Gould, 2002).

TABLE 2. Examples of hurdles used to preserve foods (adapted from Ohlsson and Bengtsson, 2002)

Type of hurdle	Examples
Physical hurdles	Aseptic packaging, electromagnetic energy (microwave, radio frequency, pulsed magnetic fields, high electric fields), high temperatures (blanching, pasteurization, sterilization, evaporation, extrusion, baking, frying), ionic radiation, low temperature (chilling freezing), modified atmospheres, packaging films (including active packaging, edible coatings), photodynamic inactivation, ultra-high pressures, ultrasonication, ultraviolet radiation
Physico-chemical hurdles	Carbon dioxide, ethanol, lactic acid, lactoperoxidase, low pH, low redox potential, low water activity, Maillard reaction products, organic acids, oxygen, ozone, phenols, phosphates, salt, smoking, sodium nitrite/nitrate, sodium or potassium sulphite, spices and herbs, surface treatment agents
Microbially derived hurdles	Antibiotics, bacteriocins, competitive flora, protective cultures

3. Hurdle technology (combination of hurdles)

a. History and definition

The combined use of several preservation methods, possibly physical and chemical, or a combination of different preservatives is an age-old practice. It has been commonly applied by the food industry to ensure food safety and stability. In smoked products, for example, combination treatment includes heat, reduced moisture content and antimicrobial chemicals deposited from the smoke onto the surface of the food. Some smoked foods may also be dipped or soaked in brine or rubbed with salt before smoking, to impregnate the flesh with salt and thus add a further preservative mechanism. In jam and other fruit preserves, the combined factors are heat, a high solids content (reduced water activity) and high acidity. In vegetable fermentation, the desired product quality and microbial stability are achieved by a combination of factors such as salt, acidification, and so forth (Ohlsson and Bengtsson, 2002).

In recent years, the concept of combining several factors has been developed by Leistner (Leistner, 1995) and others into the '*hurdle effect*'. From an understanding of the hurdle effect, hurdle technology has been derived (Leistner, 1985), which has the goal not just to understand why a certain food is safe and stable, but to improve the microbial quality of the food by an optimization and intelligent modification of the hurdles present. It employs the intelligent combination of different hurdles or preservation techniques to achieve multi-target, mild but reliable preservation effects. Hurdle technology has arisen in response to a number of developments; (i) Consumer demands for healthier foods that retain their original nutritional properties, (ii) The shift to ready-to-eat and convenience foods which require little further processing by consumers, (iii) Consumer preference for more 'natural' food which require less processing and fewer chemical preservatives. Hurdle technology provides a framework for combining a number of milder preservation techniques to achieve an enhanced level of product safety and stability.

b. Mechanism

Microorganisms react homeostatically to stress factors. When their environment is disturbed by a stress factor, they usually react in ways that maintain some key element of their physiology constant. Microorganisms undergo many important homeostatic reactions (Table 3). Preservative factors functioning as hurdles can disturb one or more of the homeostasis mechanisms, thereby preventing microorganisms from multiplying and causing them to remain inactive or even die. Therefore, food preservation is achieved by disturbing the homeostasis of microorganisms. The best way to do this is to deliberately disturb several homeostasis mechanisms simultaneously thus a combination of multiple hurdles (hurdle technology) could increase the effectiveness of food preservation.

The success of hurdle technology depends on ensuring metabolic exhaustion. Most stress reactions of microorganisms are active processes, and this often involves the expenditure of energy, e.g. to transport protons across the cell membrane, to maintain high cytoplasmic concentrations of 'osmoregulatory' or 'compatible' solutes. Restriction of the availability of energy is then a sensible target to pursue. This probably forms the basis of many the successful, empirically derived, mild combination preservation procedures exemplified by hurdle technology. As an example, if a food can be preserved by lowering the pH, then it is sensible also to include a weak acid preservative which will amplify the effect of the protons or to allow a milder, higher pH to be employed. It is sensible if proton export is made more difficult by the additional requirement that cells be forced to regulate osmotic strength. Then, if the food can be enclosed in oxygen-free vacuum or modified atmosphere packaging, facultative anaerobes will be further energy-restricted at a time when the various stress and homeostatic reactions are demanding more energy if growth is to proceed (Gould, 1995).

However, environmental stresses can provide varying results because some bacteria may become more resistant or even more virulent under stresses through stress reactions such as synthesis of protective stress shock proteins (Leistner, 2000).

TABLE 3. Homeostatic responses to stress by microorganisms (Adapted from Ohlsson and Hengtsson, 2002)

Stress factor	Homeostatic response
Low levels of nutrients	Nutrient scavenging; oligotrophy; 'stationary-phase response'; generation of 'viable non-culturable' forms
Lowered pH	Extrusion of protons across the cell membrane; maintenance of cytoplasmic pH; maintenance of transmembrane pH gradient
Lowered water activity	Osmoregulation; accumulation of 'compatible solutes'; avoidance of water loss; maintenance of membrane turgor
Lowered temperature for growth	'Cold shock' response; changes in membrane lipids to maintain satisfactory fluidity
Raised temperature for growth	'Heat shock' response; membrane lipid changes
Raised levels of oxygen	Enzyme protection (catalase, peroxidase, superoxide dismutase) from H_2O_2 and oxygen-derived free radicals
Presence of biocides	Phenotypic adaptation; reduction in cell wall/membrane permeability
Ionizing radiation	Repair of single-strand breaks in DNA
High hydrostatic pressure	Uncertain; possibly low spore water content
High voltage electric discharge	Low electrical conductivity of the spore protoplast
Competition from other microorganisms	Formation of interacting communities; aggregates of cells showing some degree of symbiosis; biofilms

It has been reported that synthesis of protective stress shock proteins is induced by several stresses including heat, pH, a_w, ethanol, oxidative compounds, and starvation. And, although each stress has a different spectrum of antimicrobial action, those stress reactions might have a non-specific effect, since due to a particular stress, microorganisms become also more tolerant to other stresses i.e. 'cross-tolerance' (Cheng et al., 2002). For instance, acid-shock or acid-adapted cells became tolerant to a range of other environmental stresses in several pathogenic bacteria including *E. coli* O157:H7, *S. typhimurium*, and *L. monocytogenes* (Leyer et al, 1995; Leyer and Jonson, 1993; Farber and Brown, 1990). Conversely, the heat shock response that follows mild heating can result in cells becoming more acid tolerance (Rowbury, 1995). Therefore, the various stress responses of microorganisms might hamper food preservation and could turn out to be problematic for the application of hurdle technology when hurdles are used consequitively. However, the use of different stresses at the same time (combination treatment) may also prevent the synthesis of those protective proteins because simultaneous exposure to different stresses will require energy-consuming synthesis of several or at least much more protective stress shock proteins which in turn may cause the microorganisms to become metabolically exhausted (Leistner, 2000). This antimicrobial action of combining hurdles is known as 'multitarget preservation' introduced by Leistner (1995). The concept of multitarget preservation increases the effectiveness of food preservation by using a combination of different hurdles which have different spectra of antimicrobial actions. It has been suspected for some time that combining different hurdles for good preservation might not have just an additive effect (explained below) on microbial stability, but they could act synergistically (Leistner, 1978). A synergistic effect (explained below) could be achieved if the hurdles in a food hit, at the same time, different targets (e.g. cell membrane, DNA, enzyme systems, pH, a_w, Eh) within the microbial cells and thus disturb the homeostasis of the microorganisms present in several respects. Thus repair of homeostasis as well as the activation of stress shock proteins becomes more difficult (Leistner, 1995). Therefore, simultaneously employing different hurdles in the preservation of a particular food should lead to optimal microbial stability. In addition, no one preservative factor is active against all the spoilage microorganisms present in foods. An attempt is therefore made to compensate for this deficiency by combining various preservative factors having different spectra of action (Lück and Jager, 1997). Since from this multitargeted approach, hurdle technology could more effective than single targeting, it allows the use of individual hurdles of lower intensity for improving product quality as well as for food preservation.

c. Limitation

As described above, hurdles used in food preservation could provide varying results depending on bacterial stress reactions such as the synthesis of protective proteins. These stress reactions or cross-tolerance may not exist when combined hurdles are used. However, although hurdles are applied simultaneously in combined form, there are three possible results whereby the action may be changed by combining two or more preservative factors: (i) addition or additive effect, (ii) synergism or synergistic effect, (iii) antagonism or antagonistic effect. The term additive effect denotes that the effects of the individual substances are simply added together. Synergistic effect is the expression used when the inhibitory action of the combination is reached at a concentration lower than that of the constituent substances separately. An antagonistic effect is the opposite of this latter, i.e. one where the mixture concentration required is higher than that of the individual constituents. Among these results, first two are desirable results and the main reason the hurdle technology is employed for food preservation rather than one hurdle. Generally, it is accepted that the combination of hurdles has a higher inhibitory effect than any single hurdle. However, recently, some studies showed that combination treatments were less effective at reducing levels of microorganism than were single treatments (Casey and Condon, 2002; Cole et al., 1990; Jordan et al., 1999). These effects of combining hurdles were antagonistic. In some cases, application of the hurdle concept for food preservation may inhibit outgrowth but induce prolonged survival of microorganisms in foods (Uyttendaele et al., 2001). The various responses of microorganisms under mild stress conditions of hurdle technology might hamper food preservation and could turn out to be problematic for the application of hurdle technology. However, no general statements can be made about the actions of any particular preservative method on other factors (Lück and Jager, 1997). Additionally, in many studies, any synergistic effects observed in laboratory tests were so weak as to have no significance for practical food preservation. It was mainly for commercial reasons that most combination products formerly marketed in large numbers were preferred to straight preservations.

Although some combinations of hurdles showed less or not significant effectiveness for killing microorganisms in foods, many promising hurdles have been identified so far. However, the application of the idea in the food industry has been largely restricted to the meat sector. Recent studies, however, emphasize a much wider potential application, e.g., in bakery products, fish, and dairy products, More specifically, the concept was introduced into mild processing of fruits and vegetables. However, there is only limited information available about the effect of combined hurdles in these types of foods. The vast majority of preserved foods that are consumed in different countries rely on combinations of preservative factors for their stability and microbiological safety. Therefore, it will be important to understanding the interactions of different hurdles in various foods to find types and intensity of that hurdles are needed for the desired microbial safety and stability of a particular food.

4. Pickling fruits and vegetables

Pickling is an age-old method for preserving vegetables and fruits. The manufacture of pickles, relishes, and condiments has become one of the most important food industries. Although the preservation of vegetables and fruits in pickled form began as a household art, at present most of the world's supply of pickles is produced in commercial plants. Any vegetables or fruit may be pickled, although the quality of some pickled foods is poor (Thomas and Holly Berry, 1999). The cucumber, one of the most important raw material used for pickling, is packed in many forms, e.g., in plain or spiced sweet vinegar in jars, kegs, or cans or fermented in spiced brine as dill pickles; packed in mustard; or in chopped form in various relishes (Brink, 1958). Green tomatoes, peppers, cauliflower, and onions are common ingredients of mixed pickles, chowchow, etc. Moreover, sauerkraut and olives are also important pickled products. Recently, more types of fruits and vegetables have been used to produce pickled products, including asparagus, beets, peaches, figs, pears, and so on.

Ingredients used for producing pickled products include salt, vinegar (acetic acid), spices, sugar, and water. Pickled fruits and vegetables are made by immersing raw materials in brine containing vinegar (acetic acid) and salt, and then heat treated. Spices and sugar are used as additional ingredients to improve flavor. Therefore, salt, acetic acid, and heat are considered as major factors for increasing the microbial safety of pickled products. However, sometimes other preservatives (such as benzoate and sorbate) are also added to enhance microbial safety. However, the U.S. Food and Drug Administration regulations do not allow the use of preservatives as the primary barriers to the growth of microbial pathogens in acidified foods (Breidt et al., 2004). For these food products, the Code of Federal Regulations (21 CFR Part 114) states only that acid or acid ingredients which will be acetic acid (vinegar) must be added so that the pH is maintained at or below 4.6; a heat treatment must be included in the process, if necessary, to prevent the growth of microbial pathogens. These regulations were designed to control the growth and toxin production by *Clostridium botulinum*. The regulations do not take into account the amount or type of organic acid present in acidified foods (Breidt et al., 2004). For thermal processing, the pickle industry usually uses a procedure established in the early 1940s that recommended an internal pasteurizing temperature of 74°C for 15 min followed by prompt cooling. Most companies, however, have developed their own processes for specific products.

Heat processing or pasteurization in pickling is performed to increase microbial food safety. However, some vegetables used in pickling are very heat sensitive and quality indicators such as color and texture are usually degraded to a large extent during thermal treatment (Lau et al., 2000). Therefore, some pickled vegetables such as fresh-pack pickles are produced without heat processing (Miller and Wehner, 1989). Moreover, consumers insist upon the freshness of food products. The demand for high-grade, mild (low salt and low acid), refrigerated pickles has increased over the past 10 years, and the pickling industry has responded with a range of products. Overnight dills or refrigerated dills might be an example of pickled products produced without heating. The refrigerated dill is essentially a non-heated, well-acidified, low-salt content, refrigerated green cucumber, containing one or more preservatives with spices and flavoring (Miller and Wehner. 1989). Typically, overnight dills are made from the freshest cucumbers which are washed and packed by hand into containers. The cucumbers are then covered with a brine consisting of water, acetic acid (vinegar), and salt. The equilibration pH desired is 4.2 to 4.3 with a titratable acidity of 0.3 to 0.5% as acetic acid. Always dill, and usually garlic, along with other flavoring agents, plus sodium benzoate as a preservative are added. The containers are closed, cased, and moved to a refrigerated warehouse. Overnight dills are kept refrigerated in marketing channels until purchased by the consumer (Miller and Wehner. 1989).

5. Preservative factors in pickling

a. Acetic acid

Acetic acid (CH_3COOH; pKa = 4.75; MW 60.05) is the primary component of vinegar. Its sodium, potassium and calcium salts are some of the oldest known food antimicrobials. Acetic acid is used in food preservation in two forms, namely as 5 to 10% vinegar and as 25 to 80% aqueous solutions of synthetic acetic acid. The 5 to 10% vinegar is obtained either by diluting synthetic acetic acid or blending acetic acid derived from fermentation and synthetic acetic acid, or by fermentation alone. Depending on the nature of the starting substance, a distinction is drawn between wine, fruit, beer, malt, spirit and other vinegars.

The action of acetic acid is based essentially on lowering the pH value of the product to be preserved. Compared with other preservative acids, however, the concentrations of acetic acid required for this purpose are very large. Only above a concentration of some 0.5% does acetic acid display an antimicrobial action in penetrating the cell wall and denaturing the protein of the cell plasma (Reynolds, 1975). If the food destined for preservation is adjusted to a pH of about 3 by the addition of acid, the antimicrobial effect of the acetic acid is 10 to 100 times as powerful as that of other acids, such as hydrochloric acid (Reynolds, 1975). This difference is due to the fact that undissociated, somewhat lipophilic acetic acid penetrates more readily to the interior of the cell. Although acetic acid has an antimicrobial effect extending beyond its dissociation constant which is comparable to propionic acid and sorbic acid, acetic acid remains in undissociated form even in a higher pH range, although, unlike sorbic acid and propionic acid, its effect is not great. Between pH 6 and pH 5 the action of acetic acid only doubles (Woolford, 1975), whereas the undissociated portion increases about sevenfold over this range. Therefore, there is no positive correlation between the undissocated acid portion and antimicrobial efficacy.

Acetic acid is generally more effective against yeasts and bacteria than against molds (Ingram et al., 1956). Other studies show that acetic acid tends to be more effective against film-forming yeasts and molds than against bacteria. However, overall, its action is weak compared to other preservatives. At pH 5, the growth of common yeasts is retarded by additions of as little as 1% acetic acid. Growth is inhibited entirely in the presence of 3.5 to 4% acetic acid (Yamamoto et al., 1984b). Salt improves the action of acetic acid, mainly by lowering the water activity (Yamamoto et al, 1984a). Acetic acid increases the heat-sensitivity of bacteria but not that of yeasts or molds (Lück and Jager, 1997). The effect of acetic acid against lactic acid bacteria is only slight (Yamamoto et al, 1984a). Since acetic acid does not generally have a strong preserving action, it is often combined with physical methods of preservation, such as pasteurization, or with salt and/or the more powerful preservatives sorbic acid or benzoic acid.

Besides its preservative action, acetic acid is very important as a flavoring; indeed, in many foods its primary function is a flavoring and its preservative function is of secondary importance. The action of acetic acid on protein may also have an influence on flavor. At low concentrations, acetic acid causes partial protein hydrolysis, especially in animal tissues, which may lead to the production of agreeably flavored cleavage products. This effect is important chiefly in the production of fish marinades. Whereas common salt tends to make fish meat firmer, vinegar has a tenderizing effect.

b. Salt

Salt (NaCl; MW 58.44) is one of the most important adjuncts in food preservation for centuries. It is employed on large scale, especially for meat, fish and vegetables. Salt has retained its importance in food preservation to the present day, although it is now used less as a preservative in its own right than in combination with other preservatives and preservation methods. Salt is obtained from rock salt deposits and seawater. The rock salt obtained by mining is not sufficiently pure for use in food. To produce salt for culinary purposes, rock salt is dissolved underground in water and, after appropriate purification, is dried by evaporation in large pans. To obtain sea salt, sea water is allowed to evaporate in shallow tanks in hot countries by solar heat, thus causing the individual salts contained in seawater to crystallize out in succession (Kaufmann, 1960).

Salt lowers the water activity of a system and thus renders conditions less favorable to microbial life. Its mode of action is therefore comparable with that of drying; hence the term “chemical drying” to describe the used of salt. However, since the water activity value of saturated salt solution is only about 0.75 and a number of microorganism varieties are able to grow even below this limit, it is impossible to protect a food stuff reliably from all microbial attack by using salt alone, unless the flavor becomes completely unacceptable (Kushner 1971). The foods to be preserved can be immersed in solutions containing greater or lesser amounts of salt (brines). Alternatively, dry salt can be added to food. The resulting osmotic removal of water from the food reduces the water activity to a level according to the quantity of salt added. Table 4 shows this relationship. According to their salt tolerance, microorganisms are defined as slightly halophilic (salt-tolerant), moderately halophilic or extremely halophilic. Halophilic bacteria grow best in the presence of some 1-5% salt. Moderately halophilic microorganisms tolerate 5-20% salt and extremely halophilic strains up to 30%. The effect of salt on lowering water activity does not in itself adequately explain its antimicrobial action. Certain clostridia strains, for example, grow in the presence of salt only if the water activity is 0.96 or more, but in the presence of glycerin these strains can continue to grow even if the water activity is as low as 0.93 (Baird-Parker and Freame, 1967). In addition, bacteria are more inclined to accumulate certain amino acids when the water activity is lowered, thus salt inhibits their growth. Lastly, salt reduces the oxygen solubility in water. The direct effect of the Cl^- ion is reduced oxygen tension, and interference with the action of enzymes (Desrosier, 1959). Hence, the quantity of oxygen available to aerobic microorganisms in products containing high concentrations of salt is only a fraction of that in substances with a low salt content (Lück and Jager, 1997). From concentrations of as little as 2% upwards, salt intensifies the action of preservatives in the narrow sense of the term (Lück and Jager, 1997). The minimum inhibitory concentration of sorbic acid for yeasts and molds in the presence of 4 to 6% salt is between one-half and one-third, and in the presence of 8% salt about one-quarter of the concentration of sorbic acid used on its own (Lück and Jager, 1997). This effect is especially marked in the acid pH range in relation to yeasts (Smittle, 1977) and clostridia (Baird-Parker and Freame, 1967). Also, salt can have a stimulatory effect on the growth of *L. monocytogenes* (Cole et al. 1990), and *Salmonella* spp. (Radford and Board, 1995; Larson et al., 1993) at low pH. The combination of salt with physical methods of preservation, especially refrigeration and drying, is also of considerable practical importance (Sofos, 1983; Barbuti et al., 1989; Papageorgiou and Marth, 1989). Salt increases the heat resistance of molds (Doyle and Marth, 1975) and bacteria (Bean and Roberts, 1975) as a result of osmotic effects. It is documented as having the reverse effect on clostridia (Hutton et al., 1991). As salt acts chiefly by reducing the water activity, its spectrum of action is governed by the demands imposed on the water activity by the various microorganisms (Ingram and Kitchell, 1967).

TABLE 4. Water activity (a_w) of salt solution (adapted from Robinson and Stokes, 1959)

a_w value	Content of solution in g NaCl/100g H_2O
0.995	0.88
0.99	1.75
0.98	3.57
0.96	7.01
0.95	8.82
0.94	10.34
0.92	13.50
0.90	16.54
0.88	19.40
0.86	22.21
0.85	23.55
0.84	24.19
0.82	27.29
0.80	30.10
0.78	32.55
0.76	35.06
0.75	36.06

The limiting values of water activity for some important microorganisms occurring in foods are shown in Table 5. Of the microorganisms that tolerate relatively high salt concentrations, mention should be made of *Torulopsis* and *Torula* yeasts, oospora, various staphylococci and lactic acid bacteria. The direct enzyme-inhibiting action of salt is of little practical consequence in explaining its antimicrobial action. Indeed, there are some enzymes whose activity is actually increased by low concentrations of salt. The pickling is a phenomenon closely related to the salting of foods. In pickling, the addition of salt initiates a microflora selection process which favors bacteria that form lactic acid.

For vegetable products, salt is used alone as a preservative in what are known on the European mainland as “salt vegetables”. These are intermediate products intended for further industrial processing, the main vegetables preserved in this way being asparagus, beans, cabbage, carrots, turnips, pearl onions, mushrooms and olives. These are placed in salt solution of 15-25%, according to the vegetables concerned. Owing to the high salt concentration, virtually no lactic acid fermentation takes place but the occurrence of film yeasts is not impossible. Vegetables pickled in weak solutions of salt and subjected to lactic acid fermentation, e.g. sauerkraut, pickled gherkins and olives, are not included under the term “salt vegetables”. The main preservative in this instance is not salt but the organic acids added or formed by the fermentation.

Besides its preservative action, salt has a considerable number of other effects, the majority of which are not undesirable. The property of salt as a flavor enhancer should be mentioned first, as in many foods this rather than a preservative action, is its main function. The concentrations of salt required for flavor enhancement are generally much lower than those needed for preservation purposes; so in principle foods preserved with salt alone are rarely suitable for direct consumption. Either they are used as raw materials for further industrial processing or else they need to be desalted by immersion in water. Salt has a variety of influences on proteins. At high concentrations of salt as a preservative, this is not surprising. These influences include a swelling effect on meat, which affects its water-binding capacity, and the properties of salt in making fish palatable.

On the whole, foods preserved with salt have a greater tendency to oxidize, especially for the fat constituent to turn rancid. The cause of this is salt itself, although traces of metals such as iron and copper in salt may also tend to promote oxidation. The action of salt in encouraging oxidation is of practical importance chiefly in meat and fish products. Finally, mention should be made of the fact that salt removes water-soluble ingredients such as minerals, vitamins and proteins from the food by the osmotic extraction of water. Consequently the biological nutrient value of foods preserved with salt is generally lower than that of the corresponding fresh products.

TABLE 5. Minimum levels of water activity for various important microorganisms occurring in foods (adapted from Leistner et al., 1981)

a_w	Bacteria	Yeasts	Mold
0.98	*Clostridium* (1), *Pseudomonas*[a]	-	-
0.97	*Clostridium* (2), *Pseudomonas*[a]	-	-
0.96	*Flavobacterium, Klebsiella*, *Lactobacillus*[a], *Proteus*[a], *Pseudomonas*[a], *Shigella*	-	-
0.95	*Alicaligenes*, *Bacillus*, *Citrobacter*, *Clostridium* (3), *Enterobacter*, *Escherichia*, *Propinibacterium*, *Proteus*, *Pseudomonas*, *Salmonella*, *Serratia*, *Vibrio*	-	-
0.94	*Bacillus*[a], *Clostridium* (4), *Lactobacillis*, *Microbacterium*, *Pediococcus*, *Streptococcus*[a], *Vibrio*	-	*Stahybotrys*
0.93	*Bacillus* (5), *Micrococcus*[a], *Lactobacillus*[a], *Strptococcus*	-	*Botrytis*, *Mucor*, *Rhizopus*
0.92	-	*Pichia*, *Rhodotorula*, *Saccaromyces*	-
0.91	*Corynebacterium*, *Streptococcus*		-
0.90	*Bacillus* (6), *Lactobacillus*[a], *Micrococcus*, *Pediococcus*, *Staphylococcus* (7), *Vibrio*[a]	*Hansenula*, Saccharomyces	-
0.88	-	*Candida*, *Debaryomyces*, *Hanseniaspora*, *Torupolis*,	*Cladosporium*
0.87	-	*Debaryomyces*	-
0.86	*Micrococcus*[a], *Staphylococcus* (8), *Vibrio* (9)	-	-
0.84	-	-	*Alternaria*, *Aspergillus*[a],*Paecilomyces*
0.83	*Staphylococcus*[a]	*Debaryomyces*[a]	*Penicillium*[a]
0.81	-	*Saccharomyces*[a]	*Penicillium*[a]
0.79	-	-	*Penicillium*[a]
0.78	-	-	*Aspergillus*[a], *Emericella*
0.75	*Halobacterium*, *Halococcus*	-	*Aspergillus*[a], *Wallemia*
0.70	-	-	*Aspergillus*[a], *Chrysosporium*
0.62	-	*Saccharomyces*[a]	*Eurotium*[a]
0.61	-	-	*Monascus* (*Xeromyces*)

[a] Various strains:
(1) *Clostridium botulinum* type C;
(2) *Clostridium botulinum* type E and various strains of *C. perfringens*;
(3) *Clostridium botulinum* type A and B as well *as C. perfringens*;
(4) various strains of *C. botulinum* type B;
(5) various strains of *Bacillus stearothermophilus*;
(6) *Bacillus subtilis* under certain conditions;
(7) *Staphylococcus aureus* in anaerobic growth conditions;
(8) *Staphylococcus aureus* in aerobic growth conditions;
(9) various strains of *Vibrio costicolus*

c. Heating

Thermal methods are extensively used for the preservation and preparation of foods. Thermal treatment leads to desirable changes such as protein coagulation, starch swelling, textural softening and formation of aroma components. However, undesirable changes also occur such as loss of vitamins and minerals, formation of thermal reaction components of biopolymers and, in minimal processed foods, loss of fresh appearance, flavor and texture. Thermal treatment is highly effective at killing microorganisms and heat is frequently applied in combination with other preservation methods. Generally, in the presence of preservatives, the temperature/time values required to kill microorganisms are lower than in the absence of preservatives. In other words, microorganisms are killed more swiftly in the presence of most preservatives than at the same temperature in their absence. For the combination of heat with preservatives, the relationships between these two factors have been confirmed in laboratory tests with many strains of bacteria and most of the usual preservatives, e.g. with yeasts and benzoic acid or salicylic acid (Benuchat, 1981a, 1982, 1983), yeasts and pimaricin (York, 1966), yeast and sorbic acid and benzoic acid (Beuchat 1981b), molds and sorbic acid (Beuchat, 1981b) as well as *Salmonella* and sorbic acid (Tuncan and Martin, 1985). The addition of sorbic acid or benzoic acid, however, restores the sensitivity of yeasts even if the water activity is reduced (Beuchat, 1981c, 1981d). However, some additional factors could decrease the effectiveness of thermal treatment. Reducing the water activity by adding salt or sucrose for example, increases the resistance of yeast cells to the effects of heat (Doyle and Marth, 1975; Beuchat 1981c).

In pickling of fruits and vegetables, thermal treatment is sometimes employed to increase the microbial stability or safety of food products since vinegar (acetic acid) alone or even combined with salt may be insufficient to prove reliable long-term preservation. However, some vegetables used in pickling are very heat sensitive and quality indicators such as color and texture are usually degraded to a large extent during thermal treatment (Lau et al., 2000). Therefore, thermal processing may not be desirable for maintaining product quality.

6. *E. coli* O157:H7

Since enterohemorrhagic *E. coli* O157:H7 was first identified as a foodborne pathogen in 1982, this microorganism has become one of most important foodborne pathogens because it has been found in a wide variety of foods and can cause potentially life-threatening illness (Benjamin and Datta, 1995). *E. coli* O157:H7 causes hemorrhagic colitis that is occasionally complicated by hemolytic uremic syndrome which preponderantly afflicts small children, the elderly, and immunocompromised people (Griffin and Tauxe, 1991; Padhye and Doyle, 1992). It is implicated in over 73,500 cases of illness and 60 deaths each year in the United States (Mead et al., 1999). Although most outbreaks with *E. coli* O157:H7 have been associated with undercooked ground beef and raw milk (Doyle, 1991), a variety of acidic foods traditionally considered being of low risk have subsequently been implicated in outbreaks, including unpasteurized apple juice, salami, yoghurt, and mayonnaise (Besser et al., 1993; CDC, 1996; CDC, 1997; Zhao and Doyle, 1994). The temperature range for *E. coli* O157:H7 growth is 2.5 to 45°C, although it grows poorly at 44 to 45°C, and growth on food is rarely if ever seen below 8 to 10°C. Typically, growth occurs with in the pH range of 4.4 to 9.0, but *E. coli* O157:H7 can survive pH values as low as 1.5 in simulated gastric fluid (Roering et al., 1999).

A considerable amount of research has been directed at attempting to understand the factors that contribute to the ability of this organism to become a successful food pathogen. Its low infective dose, possibly as few as 10 organisms ingested with food (Gorden and Small, 1993; Keene et al., 1994; Willshaw et al., 1994), certainly contributes to this. However its ability to resist acid is also important. Survival of *E. coli* O157:H7 in low pH (≤ 4.5) foods such as mayonnaise (Raghubeer et al., 1995; Weagant et al., 1994), apple cider (Besser et al., 1993; Zhao et al., 1993), and fermented dairy products (Arocha et al., 1992) has been reported. The survival of *E. coli* O157:H7 in fresh unpasteurized cider has been shown to well exceed the typical 1 to 2 week refrigerated shelf life (Miller and Kaspar, 1994; Robinson et al., 1977; Zhao et al., 1995). The ability of *E. coli* O157:H7 to survive well in low pH synthetic gastric fluid has also been reported (Arnold and Kaspar, 1995; Roering et al., 1999; Uljas and Ingham, 1998), suggesting that survival while passing through the human stomach may be an important determinant of infectivity.

For *E. coli* strains, both the acid tolerance response (ATR) and the acid resistance response (AR) have been reported. An important feature of AR is that it can function within the confines of a minimal medium, whereas AR requires components of complex medium for induction and/or function (Lin et al., 1995). Furthermore, AR enables survival at pH 2, whereas the maximum limit of ATR is pH 3. However, the differentiation of these two terms is still confusing and they might be used differently in some reports. ATR has been shown to be growth phase-dependent. Stationary phase cultures are considerably more acid-tolerant than mild-exponential phase cultures (Arnold and Kasper, 1995), although this resistance is lost rapidly on subsequent growth (Jordan et al, 1999). A more stable acid tolerance can be induced in mild-exponential phase cultures by exposing the cells to mild acid prior to exposure to low pH. This inducible adaptive tolerance response (ATR) requires protein synthesis and confers a considerable degree of acid tolerance to the cells (Jordan et al., 1999). Induction of ATR increases the survival of *E. coli* O157:H7 in acidic foods. Various studies have demonstrated that acid adaptation of *E. coli* O157:H7 enhanced survival in acidic foods including fermented dairy products, fermented meats such as shredded hard salami (Leyer et al., 1995), and in acidic fruit juices, particularly apple cider (Leyer et al., 1995; Miller and Kaspar, 1994). In contrast, acid adaptation was reported to decrease resistance of *E. coli* O157:H7 to 2% acetic acid spray in washing of carcasses (Berry and Cutter, 2000). Exposure to acidic environments also significantly increased the heat tolerance of various strains of *E. coli* O157:H7 (Ryu and Beuchat, 1998, Buchanan and Edelson, 1999a). D-values of acid-adapted cells were significantly higher than were those of acid-shocked or non-adapted cells. Increased heat-resistance was also observed in milk and chicken broth, but not with apple juice, indicating that the intrinsic parameters of a food affect the resistance properties of the adapted cells (Ryu and Beuchat, 1998). However, survival of *E. coli* O157:H7 in dried beef powder was not significantly enhanced by acid adaptation, suggesting that this stress response did not afford cross protection against dehydration or osmotic stresses (Ryu et al., 1999).

However, the survival of *E. coli* without adaptation in extreme acid pH could be explained by the AR response of *E. coli.* Four different AR mechanisms have been identified in *E. coli* and they include (i) the oxidative or glucose-repressed system, (ii) the glutamate-dependent AR system, (iii) the arginine-dependent AR system, and (iv) the lysine-dependent AR system. Stress responses and cross-protection have been studied extensively in nonpathogenic *E. coli* (Storz and Hengge-Aronis, 2000; Matin et al., 1989; Rowbury, 1995). These studies using traditional laboratory strains showed that acid "habituation", nutrient starvation, and growth into stationary phase yielded populations of cells that were more resistant to various stresses than were control populations.

6. Conclusions

The microbial stability and safety as well as the sensory and nutritional quality of most preserved foods are based on a combination of several empirically applied preservative hurdles, and more recently on knowing employed hurdle technology. The physiological responses of microorganisms during food preservation such as homeostasis, metabolic exhaustion, and stress reaction are the basis for the application of hurdle technology. The disturbance of the homeostasis of microorganisms is the main mechanism of food preservation. And the use of combined hurdles could increase the disturbance of homeostasis and cause the metabolic exhaustion of microorganisms. Since different hurdles have different spectra of antimicrobial action, the combined hurdles could attack microorganisms in different ways and may increase synergistically the effectiveness of preservation ('multitarget preservation'). Although recently, there have been many studies that investigated the effect of combining hurdles in laboratory media and foods, the interactions of many hurdles are still not clear. Also, studies of hurdle technology have been restricted to specific foods such as meat products. A better understanding of the occurrence and interactions of different preservative factors (hurdles) in foods can form a powerful and logical basis for improvements in food preservation. If all the hurdles operating in a particular food are known, the microbial stability and safety of that food, and its quality, might be optimized by changing the intensity or the character of these hurdles (Leistner, 1999a).

Fruits and vegetables can become contaminated with pathogenic microorganisms from different sources. Pickling or fermentation is one traditional method used to preserve fruits and vegetables. Acidified pickled food products may not be safe against some pathogens which have high resistance to acidic pH. Recently, outbreaks of foodborne pathogens such as *E. coli* O157:H7 and *Salmonella* spp. in acidified foods (pH < 4.5) were reported. *E. coli* O157:H7 could be a pathogen of greatest concern in pickled products because of its low infectious dose and high acid tolerance. In pickled products, acid, salt, and heating are major factors that contribute to food preservation and these factors are applied in combination. Current regulations and industrial practices regarding acidified pickled foods are possibly out of date because of the scarcity of information on pathogen control in these foods. Therefore, finding the combined effects of three major factors is necessary to formulating the correct application of preservation factors that can increase the microbial safety and total quality of pickled products.

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