14 Safety of Minimally Processed, Acidified, and Fermented Vegetable Products

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CONTENTS

14.1   INTRODUCTION

Food fermentation technology likely originated sometime between 8,000 to 12,000 years ago as plants and animals were being domesticated in the Middle East, Africa, and Asia [1–3]. The development of primitive pottery technology likely led to early fermentation experiments, either planned or unplanned. Cheese, bread, and alcoholic beverages may have resulted from the fermentation of milk, grains, fruits, and vegetables stored in ceramic jars or pots. If these "spoiled" or fermented products were found to have desirable sensory properties, they may have been developed as the first processed or fermented foods [2]. An important characteristic of fermentation was the increase in the storage lifetime during which foods could be safely eaten. The microbial nature of food fermentation or foodborne illnesses was not understood, however, until the advent of the science of microbiology in the late 19th century. The fermentation of vegetables by lactic acid bacteria (LAB) is now well understood as an effective means of preserving and ensuring the safety of foods [4,5]. LAB are being considered for use in nonfermented vegetable products as a means of ensuring safety and preventing spoilage [6–8]. Fermented and acidified vegetable products, such as sauerkraut, kimchi, olives, and cucumber pickles, not only have desirable sensory qualities, but also have an excellent safety record with no known reported cases of foodborne illness.

14.2   VEGETABLE MICROFLORA

The microflora on fresh fruits, grains, and vegetables can range from as low as 10^2 to 10^9 colony forming units (CFU) per gram [9,10]. On pickling cucumbers, for example, the aerobic microflora is typically between 10^4 to 10^6 CFU/ml for fresh fruit, with LAB less than 10^1 CFU/g [11]. In the absence of processing, degradative aerobic spoilage of plant material by mesophylic microorganisms occurs, with *Pseudomonas* spp., *Enterobacter* spp., and *Erwinia* spp. initiating the process [10]. A variety of pathogens, including *Salmonella* spp., *Shigella* spp., *Aeromonas hydrophylia*, *Yersinia enterocolitica*, *Staphylococcus aureus*, *Campylobacter*, *Listeria monocytogenes*, *Escherichia coli*, and others, may be present on fresh vegetable products [12–15]. Pathogens on fruits and vegetables may also include enteric, hepatitis, or polio viruses [16]. A variety of sources may contribute to the occurrence of pathogenic bacteria on fruit and vegetable crops, including exposure of plants to untreated manure or contaminated water, the presence of insects or birds, personal hygiene practices of farm workers, postharvest washing or hydrocooling water, and conditions of storage during distribution [12,14]. A study comparing the use of organic fertilizer (composted manure) and inorganic fertilizer from farms in Minnesota showed significantly higher coliform counts on the organically grown vegetables [17]. However, in this and related studies [18,19], pathogens, including *E. coli* O157:H7, were not detected.

Removal of pathogenic and spoilage bacteria from fruits and vegetables has proved difficult. Surface adherence of bacteria (Figure 14.1) may serve
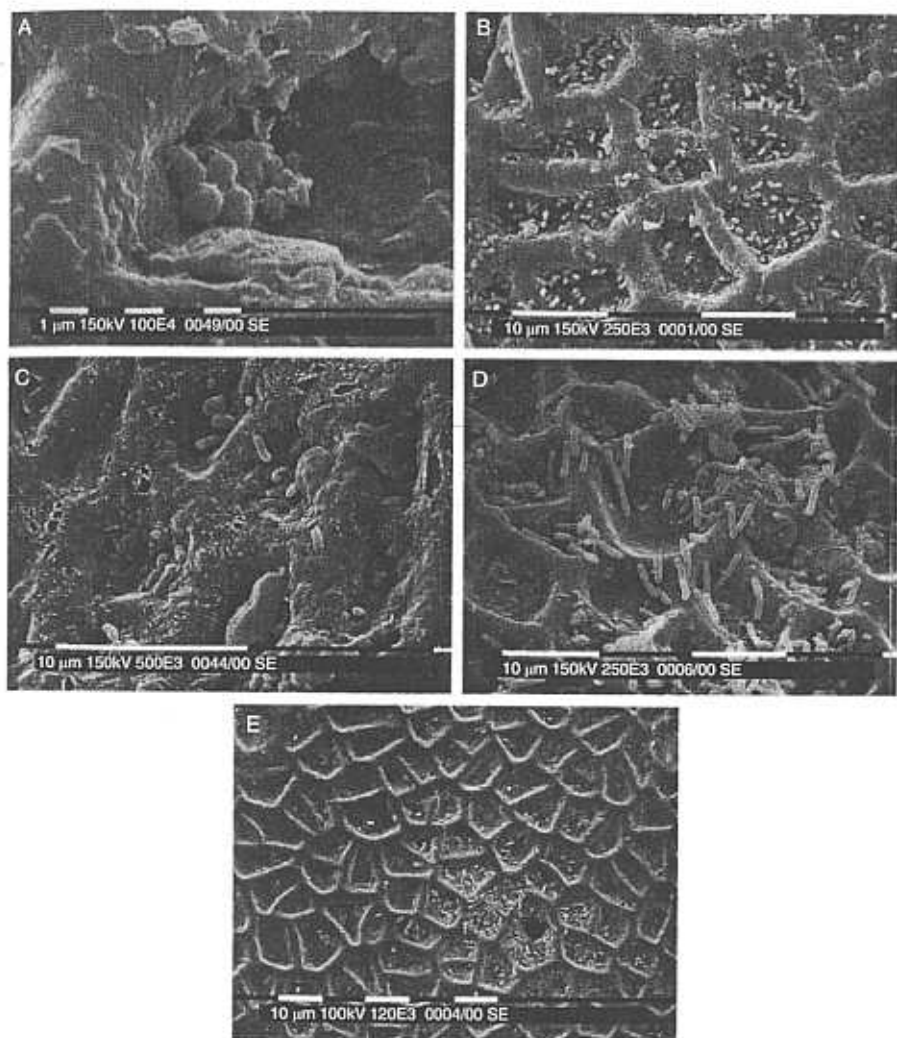
FIGURE 14.1 Attachment of pathogenic bacteria to cucumber fruit. Adhesion of bacteria to the surfaces of pickling cucumbers (Calypso variety) with wax: (A) *Staphylococcus aureus*; (B) *Lactobacillus plantarum*; (C) *Listeria monocytogenes*; (D) *Salmonella typhimurium*; (E) *Enterobacter aerogenes* ATCC 13048. Bar 5, 10 mm. (From Reina, L.D., Fleming, H.P., and Breidt, F., *J. Food Prot.*, 65, 1881–1887, 2002.)

to enhance survival of bacteria during washing or sanitizing treatments. Bacterial cell surface charge and hydrophobicity measurements have been found to correlate with the attachment of cells to surfaces of cantaloupes and cucumbers [20,21]. Dewaxing cucumber fruit led to increased adhesion of *L. monocytogenes* and decreased adhesion for other bacteria with higher relative surface hydrophobicity, including salmonella, lactobacilli, and

staphylococci [20]. Biofilms of bacteria may be more resistant to sanitizing agents and organic acid treatments than free or planktonic cells [22–24]. It is likely that the vast majority of microorganisms in food processing environments occur in multispecies or multistrain biofilms on food or equipment surfaces [25,26].

14.2.1 WASHING PROCEDURES

Washing procedures with water or chemical sanitizers typically result in only a 1 to 2 $\log_{10}$ decrease in bacterial cell numbers [24]. Hydrocooling procedures used for some fruits immediately after harvest may even serve to increase internalization of bacteria due to the vacuum created as internal gases in fruits and vegetables contract with the reduction in temperature [27,28]. Bacteria may be protected in inaccessible locations on fruits and vegetables, such as the cores and calyx of apples [29]. Attachment to wounded regions or entry into the interior of fruits and vegetables through wounded regions or stomata, pores, or channels may occur [20,30–32].

The packaging and storage conditions for minimally processed vegetable products, including the use of modified atmosphere packaging, may significantly alter microbial ecology. The extended shelf life of some minimally processed vegetable products may result in an undesirable "safety index," a concept developed to define the risks associated with modified atmosphere packaged foods [33]. This safety index is defined as the ratio of spoilage to pathogenic bacteria in foods, measured as the relative cell concentrations of these organisms. It has been argued, however, that the primary effect of modified atmosphere packaging in extending the sensory quality of vegetable products may be to decrease the metabolic activity of the vegetable material [34]. In a model system, it was found that growth rates for *L. monocytogenes*, *A. hydrophilia*, and *Bacillus cereus* may be reduced by modified atmosphere conditions, but final cell density was not affected [35]. One major source of concern is that *Clostridium botulinum* spores have been isolated from a variety of vegetables, and this organism may, under the right conditions of temperature, pH, and atmosphere, grow and produce toxin in minimally processed vegetable products if the O_2 concentrations drop to 1% or lower [10].

14.2.2 BIOCONTROL IN MINIMALLY PROCESSED VEGETABLE PRODUCTS

The survival and growth of bacteria on vegetable products can depend on the competitive microflora present and the environmental conditions and processing treatments [15,36]. The use of competitive microflora to enhance the safety of minimally processed foods, including vegetable products, has been proposed by a number of authors [5,37–39]. LAB have been nominated for this role, partly because of their GRAS (generally regarded as safe) status and their common usage in food fermentations. Application of this approach for minimally processed fruit and vegetable products has led to mixed results. Vescovo

and co-workers isolated LAB from salad vegetables and, subsequently, re-inoculated the vegetables with both the biocontrol cultures and selected food pathogens, including aeromonas, salmonella, staphylococcus, and listeria species [6,40]. The added LAB cultures were found to reduce or prevent the growth of microbial pathogens. Conversely, a *Lactobacillus delbruckii lactis* strain, known to inhibit *E. coli* on chicken skin due to the production of hydrogen peroxide, did not alter the survival of *E. coli* O157:H7 on fresh-cut vegetables, possibly due to the presence of catalase on the plant surfaces [8].

Competition from aerobic microflora isolated from fresh vegetables, other than LAB, including yeasts, *Bacillus* spp. and *Pseudomonas* spp., can influence the survival and growth of microbial food pathogens. *Pseudomonas* spp. have been shown to enhance [41], inhibit [42–44], or have no effect [45] on the growth of *L. monocytogenes* in fruits and vegetables. A variety of pseudomonas and aeromonas isolates from fresh vegetables were found to confer inhibitory activity against *E. coli*, salmonella, listeria, and staphylococcus strains using an agar diffusion assay [46]. Competition studies have shown iron sequestration by siderophores may influence the competition between pseudomonads and *L. monocytogenes* [42,47], although some *Listeria* spp. may be able to use exogenous siderophores as an iron source [48]. Buchanan and Bagi [49] demonstrated that the effects of salt and temperature can control the outcome of competitive growth of a *L. monocytogenes* Scott A and a *Pseudomonas fluorescens* culture that was screened for the inability to produce siderophores or bacteriocins. In a study by Del Campo *et al.* [45], competition for nutrients between a Scott A strain of *L. monocytogenes* and saprophytic bacteria from green endive was investigated. Enterobacteriaceae and pseudomonas were grown in competition with *L. monocytogenes* in minimal media and media supplemented with yeast extract. In this case, enterobacteriaceae but not pseudomonads species were effective in reducing the growth of the *L. monocytogenes* culture. Because culture filtrates from enterobacteriaceae were found to have no inhibitory effects in broth supplemented with yeast extract, the data indicated that competition for nutrients (not end product inhibition) was responsible for the inhibitory effect [45].

These studies illustrate the complexity of microbial interactions in and on fruit and vegetable products. Varying environmental conditions may include changes in the availability of nutrients, salt concentration, temperature, atmosphere, pH, and others. While further research is clearly needed, the use of protective cultures should only be considered as a supplement to good manufacturing practice, not as a substitute for the proper handling and packaging of vegetable products [5]. The use of biocontrol cultures may, therefore, be considered to enhance existing hurdle technology to prevent the growth of pathogens in foods. The hurdle concept [50] advocates the use of multiple preservative factors to prevent the growth of pathogens. In fresh fruit and vegetable products, the main factors affecting the growth of the indigenous bacterial populations are sanitation, modified atmosphere packaging, and refrigeration, as well as the competitive interactions of bacteria.

Bacteria cultures selected for use in biocontrol applications should ideally be isolated from the products for which they are intended to be used [39]. Development of successful biocontrol strategies for fresh fruit and vegetable products may include the following steps: (1) isolation of potential biocontrol LAB from the product for which they are intended to be used; (2) reduction of the total microflora in and on the vegetable product by one of a variety of procedures, including heat, washing using chemical sanitizers, irradiation, or others; (3) addition of the biocontrol culture to achieve an appropriate initial population, as determined experimentally; (4) storage of the product under refrigeration temperatures [39]. The shelf life of the product would then be dictated by the growth of the biocontrol culture, but, to be successful, the growth rates of a biocontrol culture presumably should be faster than that of the target pathogens. While rapid growth and production of inhibitory metabolites may be desirable from a safety standpoint, this may be a liability as far as the quality of the product is concerned. Breidt and Fleming [7] investigated the kinetics of acid production and inhibition of *L. monocytogenes* by *L. lactis* using a mathematical modeling approach [7]. It was observed that the growth and death of the *L. monocytogenes* culture could only be accurately predicted by the model if pH was assumed to be the limiting variable, rather than acid concentration, with cessation of growth around pH 4.6. Further studies to characterize the kinetics of bacterial competition are needed to aid in the development of biocontrol strategies.

14.3 FERMENTED VEGETABLES

Under the anaerobic conditions found with brined vegetables, rapid fermentation by LAB and yeasts occurs, resulting in the destruction of most other microflora, usually within a few days of the onset of fermentation [51]. In the U.S., cucumber pickles and sauerkraut represent the majority of fermented vegetable products. For pickles, fermentation was the primary means of preservation until the 1940s, when direct acidification and pasteurization of cucumber pickles was introduced (reviewed by Fleming *et al.* [51]). Currently, fermented cucumbers represent roughly 30% of commercial production of pickles, mostly for institutional markets (hamburger dill slices), with the majority of the retail market being nonfermented acidified pickles which are pasteurized to destroy vegetative microflora.

Vegetable fermentations typically begin with heterofermentative LAB, such as *Leuconostoc mesenteroides* and end with the most acid-resistant homofermentative LAB, usually *Lactobacillus plantarum* [1,52,53]. *Lactobacillus plantarum* is able to tolerate a lower internal pH than other LAB, and this feature may allow it to predominate in the terminal stages of most vegetable fermentations [54]. During the fermentation of cucumbers and cabbage, hexose sugars, including glucose and fructose, are typically converted to lactic acid by homofermentative LAB via the Embden–Myerhof–Parnas pathway, while the heterofermentative LAB will produce a combination of

lactic acid and acetic acid or ethanol, along with CO_2 via the phospho-ketolase pathway [55]. When fructose is present, LAB can use this sugar as an electron acceptor, producing mannitol, which subsequently can be converted anaerobically to lactic acid with an appropriate electron acceptor [56]. In cucumber fermentation where malate is present, *L. plantarum* and other LAB have been found to carry out a decarboxylation of malate to produce lactic acid and CO_2 [57]. This one-step reaction occurs via malolactic enzyme, and is analogous to the amino acid decarboxylation reactions described below [119]. During the reaction, a proton is taken up from the surrounding medium, which helps to buffer cellular pH and causes the pH in the surrounding medium to rise.

14.3.1 FERMENTATION CHEMISTRY

In the U.S., commercial cucumber fermentations are typically carried out with 5 to 6% NaCl, while cabbage fermentations are carried out with 2 to 3% NaCl [51]. During the growth of LAB in vegetable fermentations, a variety of antimicrobial metabolic end products are produced, including org-anic acids, peroxides, amines, thiols, bacteriocins, and other enzymes and compounds [1,4,5,58–61]. These inhibitory compounds begin to accumulate in the initial stages of fermentation. A combination of several factors, including organic acids from the fermentation (up to 2 to 3% organic acids may be produced), complete fermentation of available sugar, terminal pH values around 3 to 3.5, and salt, can serve to destroy most vegetative bac-terial cells, including human pathogens. Desirable textural and nutritional properties of the fermented vegetables may be maintained during storage in the fermentation brine for extended periods of time (a year or more) without refrigeration.

14.4 ACIDIFIED VEGETABLES

For nonfermented, acidified vegetable products, acetic acid is commonly used as an acidulant. At a concentration of 3.6% or greater, acetic acid-acidified foods can be preserved without the addition of other antimicrobial agents or use of heat treatments [62,63]. For pickled pepper products, acidification with 2% acetic acid to pH values around 3.2 was found to prevent microbial growth for 6 months or more [64]. In general, preservation by organic acids alone results in products that can only be consumed in small amounts, as condi-ments, or as ingredients in other foods. Many acidified vegetable products contain between 0.5 and 2% acetic acid and are pasteurized to prevent spoilage, as well as to ensure safety. For nonfermented pickled vegetables, the combination of heat treatments, acid, and sugar concentration (for sweet pickles) serves to prevent microbial growth. Fresh-pack cucumber pickle products typically contain between 0.5 and 1% acetic acid. A recommended pasteurization procedure consists of heating to an internal temperature to 74°C for 15 minutes [65].

Both acidified and fermented vegetable products have enjoyed an excellent safety record with few or no reported cases of foodborne disease resulting from consumption of these products. Recently, however, there have been reports of disease outbreaks in juice products with pH values below 4.0, in the same range as many fermented and acidified vegetable products. *Escherichia coli* O157:H7 and salmonella serotypes have caused serious illness and death from the consumption of apple cider and orange juice [66,67]. These disease outbreaks have raised questions about the safety of acidified and fermented vegetable products. While pathogenic microorganisms have not been found to grow in these products due to the low pH (typically below 4.0), these microorganisms may adapt to acid conditions and survive for extended periods [68]. Acid types and concentrations vary considerably for acidified foods. Factors affecting acid inhibition of microbial pathogens include the pH of the product, as well as specific effects of the acid or acid anion on cellular enzymes or membranes, and the ability of bacteria to transport protons and organic acids out of the cell interior [69–72].

14.4.1 DEFINITIONS AND REGULATIONS FOR ACID AND ACIDIFIED FOODS

Acid foods are defined in the U.S. Code of Federal Regulations (21 CFR part 114) as foods that have a natural pH value at or below 4.6. These foods include fermented vegetables; vegetable fermentation is considered a "field process" and typically results in a product with a final pH below 4.6. A pH value of 4.6 is used in the definition of acid foods because this is a limiting pH at or below which *C. botulinum* spore outgrowth and neurotoxin toxin production is prevented [73]. Foods with pH values above 4.6 are defined as low-acid foods, and, when packaged in hermetically sealed containers, must be made commercially sterile as defined in 21 CFR part 113. Acidified foods are defined in 21 CFR part 114 as foods to which acid or acid food ingredients have been added that have a water activity (a_w) greater than 0.85 and have a finished equilibrium pH value at or below 4.6. The regulation requires producers of acidified foods to verify that the final equilibrium pH is maintained at or below 4.6 to ensure safety. This regulation governing acidified foods in the U.S. was promulgated by the U.S. Food and Drug Administration (FDA) in 1979. At that time, vegetative pathogenic microorganisms were not considered to be a significant risk for acidified or fermented food products. Included in the regulation, however, is the requirement for a heat process "to the extent that is sufficient" to destroy vegetative cells of microorganisms of public health significance or those of nonhealth significance capable of reproducing in the product. The regulations governing acidified foods are, therefore, based primarily on the pH needed to prevent botulism, and do not include any specification about the type or concentration of acid needed to meet the pH requirement.

In a study of beef carcass wash water, a treatment with 0.2% (33.3 mM) acetic acid and a pH of approximately 3.7 showed that an *E. coli* O157:H7