Fruit and Vegetable Safety-Microbiological Considerations

Michael P. Doyle

Food Research Institute and Department of Food Science, University of Wisconsin-Madison, Madison, WI 53706

Microorganisms present two important problems to the vegetable and fruit industries. These include: 1) spoilage problems due to microbial degradation of produce that results in economic loss, and 2) human health problems due to the presence and/or growth of microbial pathogens that results in morbidity and mortality. Although both issues are of great concern to the produce industry, only the association of human pathogens with vegetables and fruits will be addressed here.

TYPES OF MICROBIAL PATHOGENS ASSOCIATED WITH PRODUCE-RELATED ILLNESSES

Many of the recognized food-related bacterial pathogens or toxins have been associated with human illnesses caused by eating contaminated vegetables or fruits. Examples include *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium botulinum* neurotoxin, enterotoxigenic *Escherichia coli*, *Listeria monocytogenes*, *Salmonella*, *Shigella*, and *Staphylococcus aureus* enterotoxin (Doyle, 1989). In addition, foodborne viruses such as hepatitis A also have been responsible for produce-related outbreaks. However, compared with other food groups, vegetables and fruits are infrequent vehicles of foodborne illness in the United States. The most recently reported information on outbreaks of foodborne disease provided by the U.S. Centers for Disease Control indicates that, in 1982, 25 of 279 (9%) outbreaks in which a vehicle of transmission was identified were caused by fruits or vegetables (including four outbreaks attributed to poisonous mushrooms) (Macdonald and Griffin, 1986). Most outbreaks were due to botulism that resulted from eating improperly home-canned produce.

INTRIGUING OUTBREAKS FROM EATING VEGETABLES OR FRUITS

Vegetable-related outbreaks

Several outbreaks of gastroenteritis have been caused by eating fresh vegetables. Lettuce was the vehicle in three major outbreaks of shigellosis that occurred in Texas in 1983 and 1986 (Martin et al., 1986; Davis et al., 1988). In one instance, two simultaneous outbreaks of *Shigella sonnei* gastroenteritis occurred at two Texas university campuses 97 km apart (Martin et al., 1986). Tossed salads were determined to be associated with illness at both campuses. Students made salads from a self-serve bar and lettuce was believed to be the vehicle of transmission, since it was the only produce item known to be used in tossed salads in all of the cases. Unlike most outbreaks of shigellosis, these outbreaks were caused by a contaminated food source and not by a food handler.

A more recent lettuce-associated outbreak of *S. sonnei* gastroenteritis occurred in two west Texas counties in 1986 (Davis et al., 1988). Three hundred forty-seven cases of shigellosis were associated with eating foods containing shredded lettuce and tomatoes at specific fast food restaurants. All implicated restaurants received shredded lettuce produced at one lettuce-shredding plant but different lots of tomatoes were received by the restaurants. Investigation of the lettuce-shredding plant suggested that a food handler was the source of contamination and that the method of processing allowed cross-contamination to occur. Laboratory studies revealed that *S. sonnei* can survive on shredded lettuce at 5C for 3 days without a

decrease in number, and for 7 days with only a 1-log₁₀ decrease. *Shigella* grows well in shredded lettuce at 22C, with a generation time of 1.1 hr the organism increased for 1.4×10^3 cfu/g to 1.8×10^6 cfu/g in 12 hr.

Salads containing raw vegetables have been identified as important vehicles of traveler's diarrhea, an illness Americans often experience when visiting developing countries (Menon et al., 1976). A prospective study of 73 physicians and 48 family members attending a medical conference in Mexico City in 1974 revealed that enterotoxigenic *Escherichia coli* was the most common cause of illness. Fifty-nine (49%) of the participants developed traveler's diarrhea, and eating salads containing raw vegetables was associated with enterotoxigenic *E. coli* infection.

Coleslaw was identified as the vehicle of the first conclusively documented foodborne outbreak of listeriosis (Schlech et al., 1983). This outbreak occurred in the Maritime Provinces of Canada in 1981 and involved 34 perinatal cases and seven adult cases. In the perinatal cases, pregnant women generally developed acute febrile illness that was followed by abortion, with high mortality among the fetuses. Meningitis was the principal manifestation of illness in the adult cases. *Listeria monocytogenes* serotype 4b was identified as the causative agent and the epidemic strain was isolated from coleslaw in the refrigerator of a patient and from two unopened packages of coleslaw from the same manufacturer as the patient's sample.

The coleslaw was prepared by a regional manufacturer with cabbages and carrots obtained from several wholesalers and many local farmers. A farmer was identified who raised cabbage and maintained a flock of sheep that had cases of listeriosis. Manure from the sheep was used to fertilize his cabbage fields. After the cabbage was harvested in October, it was held in a large cold-storage shed during the winter and early spring, until it was made into coleslaw. *L. monocytogenes* is a psychrotrophic bacterium that grows at refrigeration temperature. Inoculation studies by Beuchat et al. (1986) revealed that the organism can grow in raw, shredded cabbage during refrigerated storage. These investigators observed growth of *L. monocytogenes* (from 104 cfu/g to 10^8 cfu/g) in cabbage held at 5C for 25 days. Hence, listeriae likely proliferated on the cabbage during cold storage before it was made into coleslaw.

Vegetable sprouts grown in a commercially sold seed sprouting kit were the vehicle of an outbreak of *Bacillus cereus* food poisoning (Portnoy et al., 1976). In 1973, four individuals developed *B. cereus* gastroenteritis within 6 to 15 hr after eating raw soy, mustard, and cress sprouts. The sprouts were grown at 22C for 3 days in a home seed sprouting kit purchased at a health food store. Laboratory studies revealed that *B. cereus* on unsprouted seeds proliferated during germination and reached levels of 8×10^4 to 7.6×10^7 cfu/g.

Recently, four outbreaks of staphylococcal food poisoning were associated with eating imported canned mushrooms from the Peoples' Republic of China (Collins et al., 1989). The outbreaks included: a) 22 cases from eating mushrooms in omelets or hamburgers at a university cafeteria in Starkville, Miss., b) 48 cases from eating mushrooms from a salad bar at a hospital cafeteria in Queens, N.Y., c) 12 cases from eating mushrooms in pizza or parmigiana sauce at a restaurant in McKeesport, Pa., and d) 20 cases from eating mushrooms on pizza at a pizzeria in Philipsburg, Pa. All of the problem mushrooms were packaged in no. 10 cans that were processed in China. Some small producers of Chinese-grown mushrooms were found to hold fresh mushrooms in high salt

solutions for long periods of time. The mushrooms were subsequently canned after a long period of temperature abuse. Investigators speculate that staphylococcal enterotoxin was produced in mushrooms during storage in the salt solution and that the toxin remained active after thermal processing.

Fruit-related outbreaks

Historically, fruits have had an exceptionally good public health record. This may largely be attributed to the natural defense mechanisms that many fruits possess. Mechanisms that prevent fruit-borne transmission of pathogens include the thick skin of many fruits, which is usually removed or thoroughly washed before the fruit is eaten, and antimicrobial substances such as essential oils and/or organic acids that often maintain the pH at <4.6 (Goepfert, 1980). However, there have been occasional food-related illnesses caused by pathogens or bacterial toxins in fruit.

Canned fruits are among the safest of foods. However, a few outbreaks of botulism have occurred from home-canned pimientos, figs, blackberries, peaches, and tomatoes (Goepfert 1980). These are fruits whose pH is above the critical 4.6 to 4.8 for growth of *C. botulinum* or were situations where molds or acid-metabolizing bacteria contaminated the product and used the organic acid of the fruit to raise the pH above the critical level (Mundt and Norman, 1982; Montville, 1982; Odlaug and Pflug, 1979). An example is botulism from home-canned tomatoes. Tomatoes are a high-acid food usually in the pH range of 4.0 to 4.6, which normally would not allow botulinal toxin production (Fields et al., 1977; Mundt et al., 1978). However, since 1899, at least 17 outbreaks have been reported that were due to home-canned tomato products (Odlaug and Pflug, 1979). The major problem has been due to jars or cans with poor seals or low vacuum and minimally heat-processed. Molds contaminating product after heating or bacteria such as *Bacillus licheniformis* that survive in product receiving a minimal heat treatment can metabolize citric acid and increase the pH to allow growth of *C. botulinum* (Montville, 1982; Mundt and Norman, 1982; Odlaug and Pflug, 1979).

Outbreaks of salmonellosis also have been caused by fruit products. An outbreak in England in 1961 was associated with eating marshmallow cookies (Semple et al., 1961). The source of the *Salmonella* was desiccated coconut sprinkled on the cookies. Subsequent analysis of shipments of desiccated coconut from Ceylon revealed that 479 (4.8%) of 9265 samples were contaminated with *Salmonella*. Another interesting outbreak occurred in New

Jersey in 1974, and was caused by *Salmonella*-contaminated chilled, nonsterile apple juice (pH 3.4-3.9) (Morbidity and Mortality, 1975). The source of the salmonellae was thought to be animal manure that was used to fertilize the orchards. *Salmonellae* contaminated the juice because of failure to clean and sterilize the processing equipment. Subsequent studies by Goverd et al. (1979) revealed that salmonellae could grow at 22°C in apple juice at pH 3.7. A solution to this problem is to pasteurize apple juice.

An unusual outbreak of 24 cases of hepatitis A occurred in Aberdeen, Scotland, in 1983 (Reid and Robinson, 1987). Raspberry mousse prepared from frozen raspberries at a local hotel was identified as the source of infection. The raspberries had been gathered from several farms, and three of the producers had been implicated indirectly in a previous outbreak. It was presumed that the contamination occurred either at the time of picking or packing of the raspberries, probably by a virus excreter among the pickers.

SOURCES OF MICROBIAL PATHOGENS CAUSING PRODUCE-ASSOCIATED ILLNESSES

Microbial pathogens contaminate produce through three principal sources. These include soil, feces, and food handlers. Several pathogens, including *B. cereus*, *C. Botulinum*, and *L. monocytogenes*, are soil-borne and are often present on vegetables and fruits that contact soil. Untreated human or animal fecal wastes that are used for fertilizer or irrigation are sources of *C. jejuni*, enterotoxigenic *E. coli*, *L. monocytogenes*, *Salmonella*, *Shigella*, and hepatitis A virus. Food handlers can be fecal carriers of microbial pathogens and may contaminate fruits and vegetables during picking, packing, or food preparation. pathogens such as *C. jejuni*, enterotoxigenic *E. coli*, *Salmonella*, *Shigella*, *S. aureus*, and hepatitis A virus are known to be carried by food handlers and have been causes of vegetable- or fruit-associated illnesses.

PRESENCE OF PATHOGENS ON VEGETABLES

Many surveys have been done to determine the incidence of bacterial pathogens on vegetables. Examples of results of such studies are shown in Table 1. These results represent studies in which pathogens were detected; results of many surveys not reported indicate that no pathogens were found. Hence, although in some instances a substantial percentage of certain vegetables assayed was contaminated with pathogens, most vegetables are pathogen-free.

Table 1. Presence of bacterial pathogens on vegetables.

Vegetable	Country	Pathogen	Prevalence	Reference
Dried asparagus	Taiwan	<i>Salmonella</i>	11/48 (22.9%)	Bockmühl and Wohls (1984)
Arúchoke	Spain	<i>Salmonella</i>	3/25 (12.0%)	Garcia-Villanova Ruiz et al. (1984b)
Cabbage	Spain	<i>Salmonella</i>	7/41 (17.1%)	Garcia-Villanova Ruiz et al. (1987b)
Cabbage	United States	<i>Listeria monocytogenes</i>	1/92 (1.1%)	Heisick et al. (1989)
Celery	Spain	<i>Salmonella</i>	2/26 (7.7%)	Garcia-Villanova Ruiz et al. (1987b)
Chili	Surinam	<i>Salmonella</i>	5/16 (31.3%)	Tamminga et al. (1978)
Cucumbers	United States	<i>Listeria monocytogenes</i>	2/92 (2.2%)	Heisick et al. (1989)
Fennel	Italy	<i>Salmonella</i>	64/89 (71.9%)	Ercolani (1976)
Lettuce	Italy	<i>Salmonella</i>	82/120 (68.3%)	Ercolani (1976)
Lettuce	Netherlands	<i>Salmonella</i>	2/28 (7.1%)	Tamminga et al. (1978)
Lettuce	Spain	<i>Salmonella</i>	5/80 (6.3%)	Garcia-Villanova Ruiz et al. (1987b)
Dried mushrooms	China/Taiwan	<i>Salmonella</i>	11/162 (6.8%)	Bockmühl and Schoeni (1986)
Fresh mushrooms	United States	<i>Campylobacter jejuni</i>	3/200 (1.5%)	Doyle and Schoeni (1986)
Potatoes	United States	<i>Listeria monocytogenes</i>	28/132 (21.2%)	Heisick et al. (1989)
Radishes	United States	<i>Listeria monocytogenes</i>	19/132 (14.4%)	Heisick et al. (1989)
Prepacked salads	England	<i>Listeria monocytogenes</i>	4/60 (13.3%)	Sizmur and Walker (1988)
Vegetables	Spain	<i>Salmonella</i>	46/849 (5.4%)	Garcia-Villanova Ruiz et al. (1987a)
Vegetables ²	United States	<i>Salmonella</i>	4/50 (8.0%)	Rude et al. (1984)

²Celery, carrots, radishes, and spinach.

POTENTIAL FUTURE MICROBIOLOGICAL SAFETY CONCERNS ABOUT VEGETABLES AND FRUITS

Looking ahead, as researchers develop and food producers and processors apply new innovations to growing, processing, and marketing produce, the vegetable and fruit industries should evaluate the microbiological risks these changes in technology introduce. Examples of technological changes that could lead to microbiological safety problems if appropriate measures are not taken to assure safety include: 1) the reduction of naturally occurring preservatives through genetic improvement of crops. 2) innovations in packaging to extend shelf life, and 3) producing refrigerated cooked products that have an extended shelf life.

Reduction of naturally occurring preservatives. Many plants (including their seeds and fruits) contain antimicrobial substances that inhibit growth of spoilage microorganisms and/or human pathogens. For example, cranberries contain benzoic acid, which is a mold inhibitor; wheat endosperm contains purothionins, which inhibit yeasts and some human pathogens; cocoa beans and many fruits contain anthocyanins, which inhibit many types of bacteria; and tomatoes contain α -tomatine, which inhibits fungi and bacteria. In addition, many fruits contain high levels of organic acid(s) that prevent the growth of microbial pathogens. Consideration should be given to changes that may occur in expression of preservatives when plant genes are manipulated for strain improvement. Reduced levels of preservatives may result in more rapid spoilage of produce by microorganisms and/or food safety problems.

An example of a potential food safety problem would be the reduction of the acid levels of tomatoes. Tomatoes normally contain high levels of acid (pH 4.0-4.6) that provide protection against growth and toxin production by *C. botulinum*. Many home canners do not heat tomato products sufficiently to kill spores of *C. botulinum*. Developing low-acid (>pH 4.8) tomatoes, which may result through advances in plant breeding, may lead to a botulinal toxin hazard.

POTENTIAL PROBLEMS WITH INNOVATIONS IN PACKAGING AND STORING FOODS

Because of the high perishability of most vegetables and fruits, the produce industry is very interested in applying new technological developments that would extend the shelf life of products. Many successful methods have been developed to greatly extend product shelf life; however, care must be taken to consider the potential microbiological risks associated with such practices. There are several examples of brilliant technological innovations that prolong product shelf life, but also present serious health risks. For instance, a process was developed in Europe that uses pasteurization to produce vacuum-packed, lightly cooked potatoes that could be stored for 2 to 3 min at room temperature (Lund et al., 1988). The process involves: a) pasteurizing the product at 75 to 85°C for 5 to 15 min. b) vacuum-packaging the product in plastic pouches, c) holding the product at 25 to 35°C for 14 to 24 hr to allow germination of spores, and d) pasteurizing the product at 85 to 90°C for 25 to 35 min. Inoculation studies revealed that *C. botulinum* spores could survive the process and form toxin within 5 to 9 days at 25°C (Lind et al., 1988). Potatoes that are packaged and processed under such conditions must be held refrigerated (<4°C) or treated with a preservative (such as acidified to <pH 4.6) to control *C. botulinum* (Notermans et al., 1985; Dodds, 1989).

Another example of the use of a novel packaging idea was to wrap containers of fresh mushrooms with a polyvinyl chloride (PVC) stretch film. Respiration of the mushrooms consumes the oxygen within the package, thereby creating an anaerobic environment. This greatly extends the shelf life of the mushrooms because oxygen is required for enzymatic browning to occur. However, this anaerobic environment also provides conditions for toxin production by *C. botulinum*. Inoculation studies by Sugiyama and Yang (1975) revealed that botulinal toxin was produced in PVC film-packaged mushrooms held at 20°C for 3 to 4 days, and that the toxic mushrooms appeared to be edible. Subsequent studies revealed that

placing two 3.2-mm holes in the overwrap of the package prevents toxin formation (Sugiyama and Rutledge, 1978; Kautter et al., 1978). Although placing holes in the PVC film reduces the greatly extended shelf life of film-wrapped mushrooms, it is now common practice to put these holes in the package to prevent *C. botulinum* growth.

Controlled atmosphere storage (CAS) of vegetables is another innovation that extends shelf life. Usually, CAS of vegetables uses a lower oxygen concentration and higher carbon dioxide concentration than that normally found in air. CAS decreases the rate of vegetable respiration, thereby retarding ripening and facilitating the retention of higher quality for a longer period of time than would occur under an atmosphere of air. Although the economic benefits of CAS are apparent, the microbiological safety risks are not. *Listeria monocytogenes* is a soilborne bacterial pathogen that can grow at refrigeration temperature (4°C) and frequently contaminates vegetables (Heisick et al., 1989). Additionally, growth of the organism is enhanced in an environment with decreased oxygen concentrations and supplemented with carbon dioxide (Doyle, 1988). Hence, conditions of a CAS environment may enhance the growth of *L. monocytogenes* on vegetables.

Inoculation studies by Bet-rang et al. (1989) were done to evaluate the fate of *L. monocytogenes* on fresh asparagus, broccoli, and cauliflower held under CAS at 4 or 15°C. Studies revealed that CAS at 15°C lengthened the time that vegetables were acceptable for consumption by 2 to 4 days, and at 4°C by 1 week. Although CAS did not influence the growth rate of *L. monocytogenes*, the organism continued to grow on asparagus during extended storage and increased by 1- to 1.5-log₁₀/g during the extended shelf life.

POTENTIAL PROBLEMS WITH REFRIGERATED, COOKED FOODS WITH EXTENDED SHELF LIFE

With today's active lifestyle, time to prepare meals at home has become quite limited. Hence, today's consumer has a market-driving interest in convenient, easy-to-prepare foods. The food industry anticipates meeting this demand by marketing a variety of pre-cooked foods that are packaged for extended shelf life and preserved principally by refrigeration. Such foods carry considerable microbiological risks if they are not properly prepared and controlled. The principal microbiological concerns are *C. botulinum*, *L. monocytogenes*, and *Yersinia enterocolitica*. There are two types of *C. botulinum*, i.e., proteolytic and nonproteolytic, that differ in the heat resistance of their spores as well as in their ability to grow at refrigeration temperature. The proteolytic types are more heat-resistant (spores can survive boiling) and cannot grow at 4°C, whereas the nonproteolytic types are more heat-sensitive (spores usually are killed at 100°C) and can grow at 4°C.

The heat treatment given such foods is usually not sufficient to kill spores of proteolytic *C. botulinum*. Hence, it must be assumed that active spores are present after cooking. The heat treatment given the food typically is sufficient to kill most vegetative bacterial competitors, and this would make the food a medium conducive for botulinal toxin production if the food were temperature-abused (held unrefrigerated). Although nonproteolytic *C. botulinum* spores are more heat-sensitive than the proteolytic types, the heat treatment given many cooked foods is often inadequate to kill these spores.

Nonproteolytic *C. botulinum*, *L. monocytogenes*, and *Y. enterocolitica* are psychrotrophic bacteria, which means that they can grow at refrigeration temperature. Both *L. monocytogenes* and *Y. enterocolitica* are vegetative bacteria that do not require as severe a heat treatment for inactivation as the spore-forming *C. botulinum*. To prevent the psychrotrophic vegetative bacteria from proliferating in cooked foods, such foods should be given sufficient heat treatment to destroy these organisms. It may not be possible to provide enough heat to foods to kill spores of nonproteolytic *C. botulinum* and still have an organoleptically acceptable product. Hence, consideration must be given to means of preventing toxin formation by nonproteolytic *C. botulinum* during refrigerated storage. Restricting the shelf life of the product to a time that would not allow toxin formation is one alternative.

Toxin formation by proteolytic *C. botulinum* can be prevented by proper refrigeration (4C). However, if refrigeration is the sole means of preserving the product, excellent temperature control is essential as is a means of detecting temperature abuse so that abused products can be identified and destroyed. Ideally, the means of identifying temperature abuse should be interpretable by the consumer because the product may not be properly refrigerated in the home. Appropriately designed time-temperature integrators may serve as useful indicators of temperature abuse. Ideally, for safety, at least one other protective factor (beyond refrigeration) should be included to control *C. botulinum*. Examples of such protective factors include an antibotulinal agent such as sodium nitrite, acid to reduce pH below 4.6, or humectants to lower the water activity below 0.93.

Two recent botulism outbreaks caused by a garlic-in-oil product illustrate the importance of proper refrigeration of perishable foods. The outbreaks included 36 cases of type B botulism from eating garlic buttered bread at a restaurant in Vancouver, B.C., in 1985 (St. Louis et al., 1988) and three cases of botulism in 1989 in New York from garlic buttered bread eaten at home. The vehicle in both outbreaks was a chopped garlic-in-soybean oil product. The product was bottled and labeled as "Keep Refrigerated" in very small print. In both outbreaks, the product was held at room temperature. Inoculation studies revealed that botulinal toxin could be produced in 15 days in product with 1 *C. botulinum* spore/g of garlic and held at 35C (Solomon and Kautter, 1988). The appearance and odor of the toxic product was acceptable, and the product would likely have been consumed. The U.S. Food and Drug Administration recently has banned the sale of garlic-in-oil products that require refrigeration for safety. Products acidified to below pH 4.6 are considered safe and marketable.

CONCLUDING REMARKS

Clearly, past history indicates that bacterial pathogens can be associated with vegetables and fruits and cause illness. Although most produce is likely free of pathogens, food processors cannot be complacent and must take appropriate measures to assure food safety. A well-designed and properly implemented hazard analysis critical control point (HACCP) program is the best approach a food processor can take to produce a microbiologically safe product. Many recent advances in food technology have resulted in products with increased quality and/or increased shelf life. However, many of these innovations have potential microbiological risks associated with them. Hence, it is important that the vegetable and fruit industries fully evaluate the microbiological risks associated with new innovations in growing, processing, and marketing produce before adopting such practices for routine use.

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