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Microbiological safety issues in prepared chilled produce

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About the author

Dr. Gillian Francis has a Ph.D in Food Microbiology and used to work as a senior research fellow with the Food Science Research Centre at the University of Limerick. Her research interests have focused on the survival of pathogens (particularly *Listeria monocytogenes* and *Escherichia coli* O157:H7) in high-risk foods, effects of strain variation and stress response systems on pathogen survival, gas-microbe interactions, and development of molecular sub-typing techniques for pathogens.

The Food Science and Health research facility at the University of Limerick is one of the prime European centres for research on the safety and quality of prepared chilled foods. Facilities and expertise are available for pilot scale processing and gas packaging, and for work in microbiology, molecular biology and biochemistry.

Abstract

This article focuses on pathogens of potential importance to prepared chilled produce and on factors affecting their survival and growth. Key findings from investigations undertaken at the University of Limerick are presented.

1. Introduction

The term *produce* may include fruits, leaves, stems, bulbs, roots, tubers or flowers (Burnett and Beuchat 2001). *Prepared chilled* (PC) produce may consist of trimmed, peeled, sliced/shredded and washed fruits and vegetables, which are packaged and stored at refrigerated temperatures. However, PC produce provides substrates and environmental conditions conducive to the survival and growth of microorganisms. Minimal processing treatments such as peeling and slicing disrupt surface tissues, expose cytoplasm and provide a potentially richer source of nutrients for microorganisms than intact produce (Brackett 1994). This, combined with the high water activity and either virtually neutral (vegetables) or low acid (many fruits) tissue pH of produce, facilitates microbial growth in PC fruits and vegetables (Beuchat 1996).

Prepared chilled (PC) produce may consist of trimmed, peeled, sliced/shredded and washed fruits and vegetables, which are packaged and stored at refrigerated temperatures

Although PC fruits and vegetables have a good track record in terms of food safety, a range of human pathogens have been isolated from raw and PC produce (Brackett 1999; Francis *et al.* 1999), while foodborne infections have been linked to the consumption of fruits and vegetables (Tables 1 and 2 - see Appendix, pages 15-16). Increasing consumption of fresh produce in the United States has been paralleled by an increase in produce-linked food poisoning outbreaks (NACMCF 1999). The presence of pathogens in such foods is a consequence of contamination during agricultural production (mainly from contaminated seed, soil, irrigation water, and air), harvesting and manual preparation (via human contact), or machine processing and packaging (contaminated work surfaces/packaging materials/equipment). Cross-contamination by end-users after pack opening can also occur. While considerable progress has been made in our understanding of the microbiological safety of these complex food systems in the past decade, there are still significant gaps in knowledge which require further research.

2. Pathogens

The main pathogens of concern with respect to refrigerated PC vegetables are non-proteolytic *Clostridium botulinum*, *Listeria monocytogenes*, *Yersinia entercolitica* and *Aeromonas hydrophila*; however, there are also important emerging threats from viral and protozoan pathogens. Mesophilic pathogens, such as *Salmonella*, *Shigella* and *Escherichia coli* O157:H7 are unable to grow where temperature control is adequate (i.e. ≤4°C), although they may be able to grow if temperature abuse occurs.

Of these pathogens, *L. monocytogenes* has probably caused most concern. *L. monocytogenes* causes several diseases in man including meningitis, septicaemia, still-births and abortions. The organism is ubiquitous in soils and the agricultural environment generally and, as a consequence, may contaminate fresh produce. *L. monocytogenes* has been isolated from packaged vegetables at rates ranging from 0 to 44%. Of particular concern is the organism's ability to grow at refrigeration temperatures; the minimum temperature for growth is reported to be –0.4°C (Walker and Stringer 1987). It is also facultatively anaerobic and is capable of survival/growth under the low O_2 concentrations within modified atmosphere packages of prepared vegetables. While counts generally remain constant at 4°C (Farber *et al.* 1998), they can increase to high numbers at mild abuse temperatures (8°C), particularly after antimicrobial dipping treatments or within nitrogen flushed packages (Francis and O'Beirne 1997).

3. Factors affecting survival and growth of pathogens on PC produce

Pathogen survival on produce is influenced by a number of interdependent factors, principally storage temperature, raw material type/product combinations (e.g. vegetables combined with cooked ingredients), minimal processing operations (e.g. slicing, washing/disinfection), modified atmosphere packaging and competition from the natural microflora present on produce.

3.1 Storage temperature

Storage temperature is the single most important factor affecting growth of microorganisms on vegetables. Although psychrotrophic organisms, such as *L. monocytogenes*, are capable of growth at low temperatures, maintenance of temperatures of ≤4°C will extend the lag phase and reduce the rate of growth. Work in our laboratory clearly demonstrated the importance of refrigeration, as *L. monocytogenes* populations remained constant or decreased on fresh-cut packaged vegetables stored at 3-4°C. At 8°C, the growth of *L. monocytogenes* was supported on all vegetables, with the exception of coleslaw mix (shredded carrots and cabbage), on which populations decreased. Reducing the temperature from 8 to 4°C also significantly reduced growth of mesophilic *E. coli* O157:H7 on packaged

vegetables. However, viable populations remained at the end of the 4ºC storage period. Thus, even mild temperature abuse during storage permits more rapid growth of pathogens (Berrang *et al.* 1989a; Carlin and Peck 1996; Farber *et al.* 1998; Rodriguez *et al.* 2000). Strict control of temperature is crucial in maintaining microbiological safety and prolonging shelf-life.

3.2 Product type/combinations

Survival and growth of pathogens on produce vary significantly with the type of product (Austin *et al.* 1998; Carlin and Nguyen-the 1994; Jacxsens *et al.* 1999). Dry coleslaw mix was largely unsuitable for *L. monocytogenes* and *E. coli* O157:H7 growth, while significant growth of the pathogens occurred on shredded lettuce (Francis and O'Beirne 2001a, 2001b). Product factors that may affect pathogen survival and/or growth include: pH; presence of competitive microflora and/or naturally occurring antimicrobials; and respiration rate/packaging interactions.

Product pH strongly influences the survival/growth of pathogens. Most vegetables have a pH of ≥5.0, and consequently support growth of most pathogens. Acid tolerance is common in *E. coli* O157:H7 and *Salmonella* serotypes; these organisms can survive and grow in acidic produce (Liao and Sapers 2000; Ukuku and Sapers 2001; Zhuang *et al.* 1995).

PC produce may be combined with cooked ingredients. For example, it has been shown that growth of *L. monocytogenes* on raw endive was probably limited by nutrient availability, but reached higher numbers when cooked sweetcorn was added (Carlin *et al.* 1996; Nguyen-the *et al.* 1996). The addition of cooked products to raw vegetables supplied a source of nutrients and permitted rapid growth of both spoilage and pathogenic populations on such products.

3.3 Minimal processing operations

The unit operations employed during the production of PC produce (handling, peeling, slicing, washing, packaging; see Figure 1 - page 17) cause the destruction of surface cells, affect product respiration rate and pH, and release nutrients and possibly antimicrobial substances from plant cells (Brackett 1994), which will, in turn, affect the behaviour of pathogens.

In general, pathogens will not grow on uninjured surfaces of fresh, intact produce; however, cutting or slicing facilitates contamination by pathogens and subsequent survival and/or growth. Injuries to the wax layer, cuticle and

underlying tissues increase bacterial adhesion and growth (Han *et al.* 2000, 2001; Seo and Frank 1999; Takeuchi and Frank 2001); consequently, minimising damage throughout harvesting and processing reduces the chances of pathogen contamination, penetration and growth (Liao and Cooke 2001).

A variety of antimicrobial wash solutions have been used to reduce populations of microorganisms on fresh produce. Chlorine (50–300 ppm) is the most frequently used disinfectant for fresh fruits and vegetables and is added to water as a solid, liquid or gas (Adams *et al.* 1989; Anon. 1973; Lund 1983). Generally, no more than 2–3 $\log_{10}$ reductions of bacteria have been reported on produce after chlorine treatment (Adams *et al.* 1989; Beuchat 1992, 1999).

A variety of antimicrobial wash solutions have been used to reduce populations of microorganisms on fresh produce

The effects of chlorine in removing pathogens from produce have been studied. The maximum $\log_{10}$ reductions of *L. monocytogenes*, after treatment with chlorine (200 ppm), were 1.7 for lettuce and 1.2 for cabbage (Zhang and Farber 1996). Dipping coleslaw and lettuce in a chlorine solution (100 ppm) reduced initial *L. innocua* and *E. coli* populations, but resulted in their enhanced survival during extended storage at 8ºC (Francis and O'Beirne 2002). Chlorine (100–200 ppm) was only marginally effective at reducing *E. coli* levels on tissue surfaces of lettuce (Beuchat 1999) and apple (Wisniewsky *et al.* 2000; Wright *et al.* 2000). *Salmonella* populations on alfalfa sprouts were reduced by about 2 $\log_{10}$ cfu/g after treatment with 500 ppm chlorine, and were reduced to undetectable levels after treatment with 2000 ppm chlorine (Beuchat and Ryu 1997).

Chlorine, used at concentrations currently permitted by the industry to wash fresh produce, cannot be relied upon to fully eliminate pathogens, while, at the same time, natural competitive organisms may be reduced or removed. *L. monocytogenes* inoculated onto disinfected (10% hydrogen peroxide) endive leaves grew better than on water-rinsed produce (Carlin *et al.* 1996), while dipping lettuce in a chlorine (100 ppm) solution followed by storage at 8ºC significantly enhanced *Listeria* growth compared with undipped samples (Francis and O'Beirne 1997). Disinfection before contamination with the pathogen may increase its growth because populations of competing microflora have been removed (Bennik *et al.* 1996). Therefore, temperature management (i.e. ≤4ºC) after reduction of microbial populations is crucial for microbial safety.

Due to the ineffectiveness of chlorine in removing pathogens from produce and increasing concern over production of chlorinated organic compounds and

their impact on human and environmental safety, a variety of other disinfectants, including acidic electrolysed water (Park *et al.* 2001), peroxyacetic acid (Park and Beuchat 1999), chlorine dioxide (Zhang and Farber 1996), hydrogen peroxide (Sapers and Simmons 1998), organic acids (Karapinar and Gonul 1992), trisodium phosphate (Zhang and Farber 1996) and ozone (Burrows *et al.* 1999) have been evaluated (Beuchat 1999). However, none of the sanitiser treatments tested are likely to be totally effective against all pathogens, and behaviour of pathogens during subsequent storage of produce remains unpredictable (Beuchat and Ryu 1997; Park and Beuchat 1999; Zhang and Farber 1996).

3.4 Modified atmosphere packaging

When a vegetable is subjected to modified atmosphere packaging (MAP), it respires, thereby passively modifying the gas atmosphere inside the package. Ideally, O_2 levels will fall from the 21% found in air to 2–5%, while CO_2 levels will increase to the 3–10% range. These gas levels, combined with refrigeration, slow down both vegetable respiration and microbial growth which serve to delay physiological ageing and extend shelf-life. However, achieving the optimum atmosphere modification can be difficult, and careful attention must be paid to ensure that MAP is applied safely and that product quality is consistently maintained throughout storage.

Application of MAP to refrigerated vegetables presents several areas of concern with regard to microbiological safety. By extending shelf-life and protecting product quality, MAP-prepared produce systems can provide sufficient time for pathogens to grow to significant numbers on otherwise acceptable fresh foods (Berrang *et al.* 1989b). Furthermore, the gas atmospheres and refrigeration temperatures employed may inhibit growth of some aerobic spoilage microorganisms, which may be natural competitors of pathogens; their suppression may therefore facilitate pathogen growth without the product showing obvious signs of spoilage. The risk of food poisoning is greatest in products eaten raw without any further preparation. In addition, although the low levels of O_2 (2–5%) within modified atmosphere

packages of vegetables should inhibit growth of obligate anaerobes such as *C. botulinum*, if modified atmosphere packages are subjected to temperature abuse, they may become anaerobic as a result of increased product respiration. This could enable growth and toxin production by *C. botulinum* to occur. A number of studies have indicated that MAP may select for psychrotrophic facultative anaerobic pathogens.

There are some contradictory reports in the literature concerning the growth of *L. monocytogenes* under MAP. Workers have shown that growth of *L. monocytogenes* on vegetables was not influenced by MAP (97% N_2, 3% O_2), compared to produce packaged in air. In contrast to these findings, other researchers showed that MAP enhanced survival and growth of *L. monocytogenes* on vegetables. Research conducted at the University of Limerick has indicated that vegetable type and product atmosphere influence populations of *L. monocytogenes*, particularly under mild abuse temperatures (8°C). Nitrogen flushing, combined with storage at 8°C, enhanced growth of *L. monocytogenes* on shredded lettuce (Francis and O'Beirne 1997), while increasing CO_2 levels from 0 to 20% had no inhibitory effect on growth of *L. monocytogenes* in a surface model system (Francis and O'Beirne 1998a). Inconsistencies in the literature highlight the need for more research in order to better understand the potential effects of MAP on survival of *L. monocytogenes* and other pathogens.

CO_2 has no inhibitory effect on growth of *E. coli* O157:H7 on shredded lettuce stored at 13 or 22°C; growth potential is increased in an atmosphere of $O_2/CO_2/N_2$: 5/30/65, compared with growth in air. Investigations undertaken within our laboratories have demonstrated the potential of *E. coli* O157:H7 to grow on modified atmosphere packaged vegetables. However, *E. coli* O157:H7 survival was affected more by vegetable type and storage temperature than by gas atmosphere.

The literature contains many reports on the ability of *Salmonella* to grow under modified atmospheres on meat products. In general, increased CO_2 concentrations (50–100%) reduce growth, although these concentrations would cause damage to most vegetables. Information describing the survival characteristics of *Y. enterocolitica* on vegetables stored under different atmospheric conditions remains extremely limited. On meat products, atmospheres containing 40–50% CO_2 have minimal inhibitory effects on *Y. enterocolitica*. Several studies have demonstrated that *A. hydrophila* can grow on vegetables stored at 4–5°C under modified atmospheres, and that growth was not affected by gas atmosphere.

3.5 Competition between pathogens and indigenous microflora

PC produce harbours large populations of native microorganisms including pseudomonads, lactic acid bacteria (LAB) and Enterobacteriaceae (Francis *et al.* 1999; Nguyen-the and Carlin 1994). Background microflora provide indicators of temperature abuse largely by causing detectable spoilage, and can vary significantly for each product and during storage. A concern with refrigerated MAP vegetables is that growth of the natural, competitive microflora of the product may be reduced, potentially favouring pathogen survival or growth. The effects of competition between indigenous microflora and pathogens on MAP produce have not been studied extensively, although a recent study at the University of Limerick examined the effects of gas atmosphere on *L. monocytogenes* and competing microflora (LAB, pseudomonads, Enterobacteriaceae; Francis and O'Beirne 1998a, 1998b). The findings showed that increasing CO_2 levels (20%) increased growth of LAB, which, in turn, inhibited growth of the pathogen. However, growth and inhibitory activities of *Enterobacter* spp. were inversely related to the concentration of CO_2. In 3% O_2 (a level often reached in commercial modified atmosphere packages), growth of an inoculated mixed natural population was reduced, while *L. monocytogenes* proliferated (Francis and O'Beirne 1998a). Cai *et al.* (1997) reported that a large portion of LAB isolates from bean sprouts inhibited growth of *L. monocytogenes*. Strains of LAB were reported to inhibit *A. hydrophila*, *L. monocytogenes*, *Salmonella* serotype Typhimurium, and *Staphylococcus aureus* on vegetable salads (Vescovo *et al.* 1996).

Complex interactions with the indigenous microflora may have significant effects on survival and growth of pathogens. More research needs to be performed to examine the influence of gas atmospheres, background microflora and storage temperatures on the survival and growth of pathogens, including foodborne viruses and protozoan parasites, on produce, in order to ensure that novel mild preservation technology can continue to be applied safely.

Complex interactions with the indigenous microflora may have significant effects on survival and growth of pathogens

4. Minimal processing and stress responses

Pathogenic bacteria can respond or adapt to sub-lethal stresses encountered in PC in ways that increase their resistance to more severe treatments and enable better survival in foods (Abee and Wouters 1999; Buncic and Avery 1998). One of the best studied adaptive tolerance responses is to acid (acid tolerance response, or ATR). Acid adapted *L. monocytogenes*, *Salmonella* and

E. coli O157:H7 survived significantly better in acidic foods such as salad dressings and fruit juices when compared to non-adapted cells (Gahan *et al.* 1996; Leyer and Johnson 1992). Acid adaptation induces acid tolerance to more severe or normally lethal acid, but it can also induce cross-protection against other environmental stresses such as thermal and osmotic stress (Leyer and Johnson 1993; Lou and Yousef 1997). Equally, other stresses can induce acid tolerance. Acid adaptation enhanced survival of *L. monocytogenes* during storage in packages of vegetables which had relatively high in-pack CO_2 levels (25–30% in MAP coleslaw and bean sprouts; Francis and O'Beirne 2001b). *E. coli* O157:H7 survived in an acidic environment better at 4ºC than at 10ºC, which implies that induction of acid tolerance may enhance resistance to low temperatures (Conner and Kotrola 1995).

5. Implications of strain variation among pathogens

The selection of strain(s) of a particular pathogen to be used in survival studies is extremely important, as different strains may behave differently on MAP produce. Work carried out by the author has shown that strains of *L. monocytogenes* differ significantly in their inherent ability to survive and grow on packaged vegetables. In addition, there was significant variation among strains in their inherent stress resistance characteristics; some strains may be more resistant to the stressful conditions encountered in foods and during food processing. The ability of *E. coli* O157:H7 to tolerate heat, for example, was strain dependent (Clavero *et al.* 1998; Duffy *et al.* 1999), and survival of *E. coli* O157:H7 on vegetables depended on bacterial strain and product type (Francis and O'Beirne 2001a). Different strains of pathogens may respond differently to treatments including mild acid, low temperature and gas atmosphere, which may result in variations in the ability of surviving populations to cause human disease (Buncic *et al.* 2001).

6. Improving the safety of PC modified atmosphere packaged produce

The pathogen risks from PC packaged produce cannot be totally eliminated, but they can be minimised by applying best practice at every stage – agricultural production, pre-processing, processing, distribution, and final use. At all stages, strategies to minimise contamination by pathogens, product storage at ≤4ºC and education/training of workers and consumers are

important recurring themes. Clearly, Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) need to be put in place to minimise hazards, and many Codes of Practice have been published by national agencies (e.g. FSAI 2001) and industry sectors.

Starting at harvest, bruising and cutting of produce should be minimised prior to processing (Liao and Cooke 2001). Immediately prior to processing, preliminary decontamination should be carried out by removing outer leaves, soil etc. from produce, using sharp sanitised knives for any cutting that is performed. Peeling, cutting, shredding, etc. should be carried out with equipment designed to cause the minimum of tissue disruption, as severe processing may facilitate more effective contamination and subsequent growth by pathogens (Gleeson *et al.* 2002). GMP should include effective surface and machine sanitisation to eliminate the risk of pathogen contamination from the processing environment, or from machines used in processing (Zhang and Farber 1996; Nguyen-the and Carlin 1994).

Although their benefits have been questioned (Brackett 1999), antimicrobial dipping techniques are probably valuable tools for reducing numbers of potential pathogens (Beuchat and Ryu 1997), although special care should be exercised to avoid contamination after dipping. Post-processing risks introduced by antimicrobial dips (Bennik *et al.* 1996; Francis and O'Beirne 1997; Carlin *et al.* 1996) should be addressed in HACCP protocols; the most important of these are measures to ensure that products are stored at ≤4°C at all times, as well as the use of conservative use-by dates. Where alternatives to the use of chlorine as a sanitiser are being introduced, any differences in their antimicrobial effects should be understood and taken into account.

Packaging materials must be carefully selected to ensure that their gas permeability properties match the respiration rates of the products being packaged. This is necessary in order to achieve package atmospheres within the technically useful range of 2–5% O_2 and 3–10% CO_2 (Cliffe-Byrnes *et al.* 2003). Poor 'package-product compatibility' will result in the creation of unintended atmospheres with uncertain microbiological implications (Bennik

et al. 1998). Furthermore, ensuring that temperatures are kept ≤4ºC throughout the cold chain is essential for microbial safety and requires considerable attention to detail.

7. Conclusion

Demand for fresh-cut fruits and vegetables has led to an increase in the quantity and variety of products available to the consumer. Vigilance with respect to the safety of these products must be maintained, as the potential for outbreaks of foodborne illness to occur is always present, especially if some problem arises which causes a break in the chill chain. Research trends driven by the needs of this sector include: greater understanding of emerging pathogens, particularly viruses and protozoan parasites; greater understanding of processes of produce contamination generally, and of how to prevent them; the development of new effective decontamination technologies; and development and application of active and intelligent packaging. In order to improve surveillance for foodborne illness, there is a need for greater use of molecular techniques for sub-typing of pathogens. This technology can help establish sources and points of contamination, indicate links between geographically isolated outbreaks of food poisoning with a common source (NACMCF 1999), and provide other types of data which will help develop HACCP protocols which can then be validated.

8. Appendix

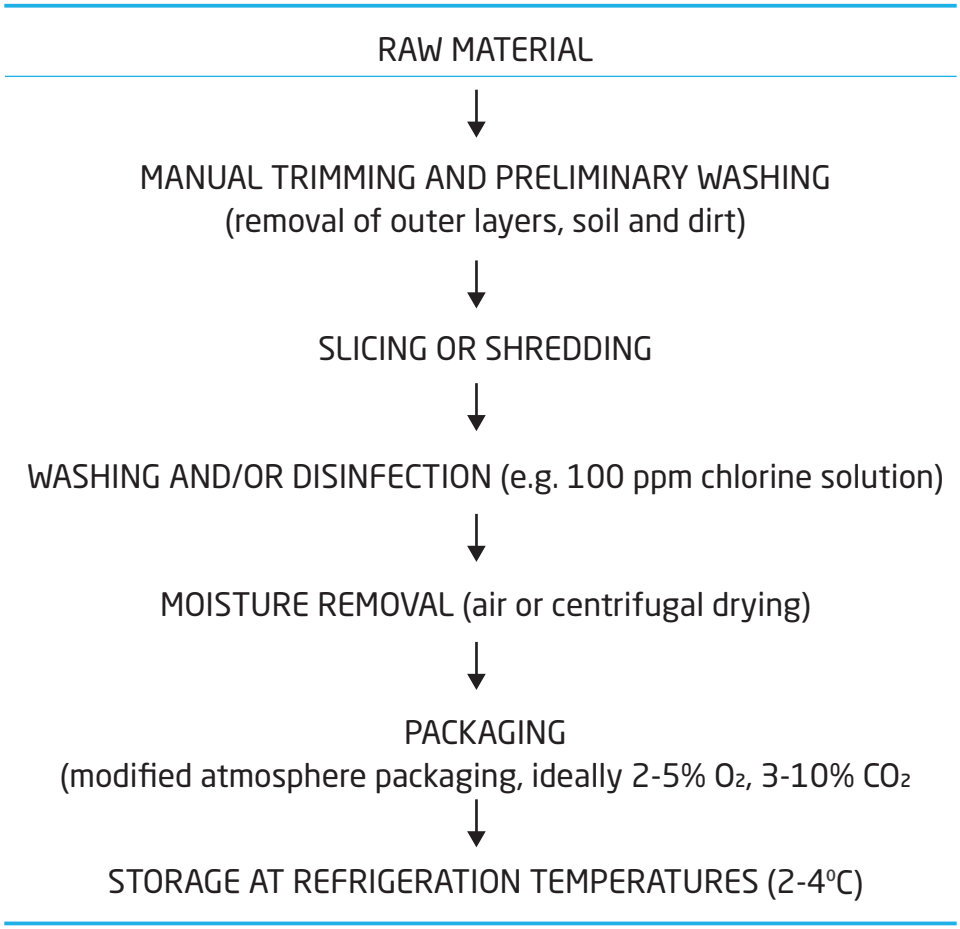
Table 1: Occurrence of pathogens on prepared chilled produce

Vegetable	Number (and %) of positive samples	Country and comments	Reference
Listeria monocytogenes			
Cucumber slices	4/5 (80%)	Malaysia	Arumugaswamy *et al.*, 1994
Bean-sprouts	6/7 (85%)	Malaysia	Arumugaswamy *et al.*, 1994
Coleslaw	2/92 (2.2%)	Canada	Schlech *et al.*, 1983
	2/50 (4%)	Singapore	Doris and Seah, 1995
	3/39 (7.7%)	United Kingdom	MacGowan *et al.*, 1994
Pre-packed mixed salads	3/21 (14.3%)	Northern Ireland	Harvey and Gilmour, 1993
	4/60 (6.7%)	United Kingdom	Sizmur and Walker, 1988
Chopped lettuce	5/39 (13%)	Canada	Odumeru *et al.*, 1997
Cut and packaged lettuce	3/120 (2.5%)	Australia	Szabo *et al.*, 2000
Prepared mixed vegetables	8/42 (19%)	United Kingdom (contamination during processing suspected; <200/g present)	Velani and Roberts, 1991
Fresh cut salad vegetables	11/25 (44%)	The Netherlands ($<10^2$/g present)	Beckers *et al.*, 1989
Chicory salads	(8.8%)	France (<1/g present)	Nguyen-the and Carlin, 1994
Prepared vegetables	1/26 (3.8%)	United Kingdom	MacGowan *et al.*, 1994
Processed vegetables and salads	(13%)	United Kingdom	McLauchlin and Gilbert, 1990
Aeromonas spp.			
Cut lettuce	66/120 (55%)	Australia	Szabo *et al.*, 2000
Salad mix	12/12 (100%)	Italy	Marchetti *et al.*, 1992
Prepared salads	(21.6%)	United Kingdom	Fricker and Tompsett, 1989
Escherichia coli O157:H7			
Salad mix	0/63 (0%)	US	Lin *et al.*, 1996
Clostridium botulinum			
MAP salad mix	2/350 (0.6%)	US	Lilly *et al.*, 1996
MAP cabbage	1/337 (0.3%)	US	Lilly *et al.*, 1996
MAP green pepper	1/201 (0.5%)	US	Lilly *et al.*, 1996
Salmonella spp.			
Salad mix	1/159 (0.6%)	Egypt	Saddik *et al.*, 1985
Endive	2/26 (7.7%)	Netherlands	Tamminga *et al.*, 1978
Yersinia spp.			
Cut and packaged lettuce	71/120 (59%)	Australia	Szabo *et al.*, 2000
Campylobacter jejuni			
Mushrooms	3/200 (1.5%)	US	Doyle and Schoeni, 1986

Table 2: Foodborne infections linked to the consumption of fruits and vegetables

Pathogen	Product suspected	No. of cases	Location	Reference
Bacteria				
L. monocytogenes	Shredded cabbage in coleslaw	41	Canada	Schlech *et al.*, 1983
	Raw tomatoes, lettuce and celery	20	Boston, US	Ho *et al.*, 1986
Cl. botulinum	Shredded cabbage in coleslaw	4	Florida, US	Solomon *et al.*, 1990
	Chopped garlic in oil	37	British Colombia, US	Solomon and Kautter, 1988
Salmonella spp.	Sliced watermelon	39	Michigan, US	Blostein, 1993
	Cantaloupe melon	22	Canada	Deeks *et al.*, 1998
	Cress sprouts	31	UK	Feng, 1997
	Mung sprouts	143	UK	O'Mahony *et al.*, 1990
	Tomatoes	85	Multi-state US	Susman, 1999
	Tomatoes	174	Multi-state US	Tauxe, 1997
E. coli O157:H7	Cantaloupe melon	9	Oregon, US	Del Rosario and Beuchat, 1995
	Radish sprouts	6561	Japan	WHO, 1996
	Alfalfa sprouts	108	US	CDC, 1997
	Lettuce	70	Montana, US	Ackers *et al.*, 1998
	Lettuce	23	Canada	Preston *et al.*, 1997
Shigella sonnei	Watermelon	15	Sweden	Freudlund *et al.*, 1987
	Shredded lettuce	347	Texas	Davis *et al.*, 1988
	Lettuce	140	Texas	Martin *et al.*, 1986
	Lettuce	118	Norway, UK, Sweden, Spain	Kapperud *et al.*, 1995
	Parsley	310	Multi-state US	CDC, 1999
Bacillus cereus	Soy, mustard and cress sprouts	4	Texas	Portnoy *et al.*, 1976
Yersinia enterocolitica	Beansprouts	16	US	Cover and Aber, 1989
Camylobacter jejuni	Salad	330	Canada	Allen, 1985
	Lettuce	14	Oklahoma, US	CDC, 1998a
Viruses				
Hepatitis A virus	Raspberries (frozen)	24	Scotland	Reid and Robinson, 1987
	Strawberries (frozen)	242+14	Multistate US	Hutin *et al.*, 1999
	Lettuce	103	Florida, US	Lowry *et al.*, 1989
	Watercress	129	Tennessee, US	CDC, 1971
	Diced tomatoes	92	Arkansas, US	Lund and Snowdon, 2000
Norwalk virus	Melon	206	UK	Lund and Snowdon, 2000
	Fresh-cut fruit	>217	Hawaii, US	Herwaldt *et al.*, 1994
	Raspberries (frozen)	>500	Finland	Lund and Snowdon, 2000
Parasites				
Cyclospora cayetanensis	Raspberries	1465	20 US states & Canada	Herwaldt and Ackers, 1997
	Blackberries	104	Canada	Herwaldt, 2000
Cryptosporidium parvum	Green onions	54	Washington, US	CDC, 1998b
Giardia	Lettuce and onions	21	New Mexico	CDC, 1989

Figure 1: A flow diagram for the production of prepared chilled vegetables



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