

REVIEW ARTICLE

A risk assessment approach for fresh fruits

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Abstract

Aims: To describe the approach used in conducting a fit-for-purpose risk assessment of microbiological human pathogens associated with fresh fruit and the risk management recommendations made.

Methods and Results: A qualitative risk assessment for microbiological hazards in fresh fruit was carried out based on the Codex Alimentarius (Codex) framework, modified to consider multiple hazards and all fresh (whole) fruits. The assessment determines 14 significant bacterial, viral, protozoal and nematodal hazards associated with fresh produce, assesses the probable level of exposure from fresh fruit, concludes on the risk from each hazard, and considers and recommends risk management actions. A review of potential risk management options allowed the comparison of effectiveness with the potential exposure to each hazard.

Conclusions: Washing to a recommended protocol is an appropriate risk management action for the vast majority of consumption events, particularly when good agricultural and hygienic practices are followed and with the addition of refrigerated storage for low acid fruit. Additional safeguards are recommended for aggregate fruits with respect to the risk from protozoa.

Significance and Impact of the Study: The potentially complex process of assessing the risks of multiple hazards in multiple but similar commodities can be simplified in a qualitative assessment approach that employs the Codex methodology.

Introduction

Consumption of fresh fruits and vegetables continues to increase in many countries owing to consumer preferences for fresher, more nutritious foods that also happen to meet the needs of busier lifestyles. The availability of fresh produce has increased such that these products are obtainable at all times during the year, and this relies heavily on the import of goods from regions all over the globe. Globalization of the food supply introduces hazards from these regions into other areas and disseminates pathogens over wide geographical areas. In addition to their increasing popularity in consumption patterns, fresh fruits and vegetables have also become increasingly important vehicles in foodborne disease statistics. In the United States, these products were responsible for only 1% of foodborne disease cases in the 1970s, and in the 1990s, this has increased to 12% (Sivapalasingam 2004).

Between 1990 and 2003, the US Centre for Science in the Public Interest showed that fresh fruit and vegetables caused 554 foodborne disease outbreaks in the United States, outnumbering the number linked to poultry for the same period.

There have been some notable outbreaks of illness in recent years that demonstrate the increasingly important role of fresh fruits and vegetables in foodborne disease. These include: a multistate outbreak of enterohaemorrhagic *Escherichia coli* O157 linked to bagged fresh spinach in the United States in 2006 (Anon 2006), affecting more than 183 persons, of which 52% were hospitalized and 16% developed haemolytic uraemic syndrome, and at least one person died; outbreaks of norovirus-associated gastrointestinal disease linked to consumption of frozen raspberries in Denmark, imported from Poland and China in 2005 and 2007 (Anon 2005, 2007), affecting more than 1000 people in total; an outbreak of *E. coli*

O157-associated disease in Sweden in 2005, caused by iceberg lettuce, affecting 120 people (Anon 2005); an outbreak of salmonellosis in Finland, linked to lettuce imported from Spain in 2005 (Anon 2005). These products tend to pose a greater risk than other types of food products because they are consumed raw or are minimally processed.

It is possible that some of these apparent increases may be attributed to improved epidemiology/surveillance and detection, but it is also likely that there are genuine increases in disease. There is evidence that changes in production, processing and distribution practices have led to some of these outbreaks (Beuchat 2002), but it is also the case that pathogens, such as *E. coli* O157, are now more prevalent in the environment than they used to be. There are a number of potential sources of the human pathogens that can contaminate fruits prior to processing.

Soil (on its own) is not generally regarded as an important source of human pathogens on produce (De Roever 1998). Exceptions to this include a number of bacteria that can be isolated from soils free from faecal contamination, namely *Clostridium botulinum*, *Clostridium perfringens* and *Bacillus cereus*, and to a lesser extent, *Listeria monocytogenes* (Lund 1992; Nguyen-the and Carlin 1994; De Roever 1998). Other *Bacillus* spp. would also be expected to be present in soil (Brackett 1994). The majority of diseases associated with fresh fruits and vegetables are primarily those transmitted by the faecal-oral route (De Roever 1998), and therefore, are a result of contamination at some point in the process.

Contamination of vegetables (and hence likely fruit also) frequently occurs through agricultural practices, such as irrigation with polluted water or fertilization with manure or sewage sludge (Nguyen-the and Carlin 1994). Irrigation water can become polluted either through the direct introduction of sewage, or through nonpoint pollution sources, such as ground water running off (De Roever 1998). Manure or sewage sludge can contaminate produce by being improperly composted, produce being harvested too soon after application, or even as a result of the protection and survival of organisms, sometimes for several months or longer (Nguyen-the and Carlin 1994; De Roever 1998).

Wild and domestic animals, including mammals, birds, reptiles and insects, are also sources of pathogenic bacteria in agricultural environments, by direct contamination of the crop and contamination of irrigation water (De Roever 1998). Birds can be particularly important owing to their ability to transmit bacteria over substantial distances.

Farm workers have an important impact on the microbiological safety of the produce they handle. The lack of good hygienic practice can lead to cross-contamination; this appears to be particularly important in the transmis-

sion of enteric viruses, such as hepatitis A (De Roever 1998) and noroviruses. Furthermore, cross-contamination of harvesting equipment may also serve as a vehicle for contamination, as can processing equipment and further human handling beyond the harvest stage (e.g. food service, factory workers) (De Roever 1998; Brackett 1999).

Understanding the contribution of these factors to the risks from consuming fresh produce is complex. Codex has outlined an approach to risk assessment that is now commonly used in the assessment of risks posed by microbiological pathogens. These risk assessments are usually focussed on a single hazard/commodity combination or, for risk ranking, a single hazard and multiple commodities. The nature of fresh fruit and vegetable production and consumption leads to a wider range of potential hazards of concern being identified. Risk assessments for fresh fruits and vegetables would therefore ideally cover multiple hazards/multiple commodities; however, owing to the complexity of such a task and possibly the visibility of the disease burden, there is a lack of risk assessments for these commodities published in the public domain to date.

This paper outlines a qualitative risk assessment approach for the hazards and risks associated with fresh fruit, based on the Codex framework. While this assessment focusses on fresh fruit, it could easily be adapted to consider fresh vegetables, as much of the data come from the broader fresh produce domain. It was conducted for a specific scenario, to inform on risk management practices for products incorporating fresh fruit. The assessment determines the significant hazards associated with fresh produce, assesses the probable level of exposure from fresh fruit, concludes on the risk from individual hazards, and considers and recommends risk management actions.

Materials and methods

Risk assessment scope and approach

The scope of the assessment was limited to pathogenic micro-organisms in fresh fruit. 'Fresh fruit' was defined as 'perishable fruit that has not been frozen or manufactured into articles of food of a different kind or character'. Elements of a USDA definition in the regulations (7 CFR Part 46; United States Department of Agriculture) of the Perishable Agricultural Commodities Act were used in this definition. The output of the risk assessment was a list of organisms that constitute a significant risk (considering both likelihood and consequence) in the consumption of fresh fruits, and selection of appropriate risk management measures.

The Codex elements of risk assessment (hazard identification, hazard characterization, exposure assessment and

risk characterization) were applied qualitatively to identify and assess each potential hazard in turn (Codex Alimentarius Committee 1999). Quantitative data were used but not combined using mathematical or simulation models to come to an estimate of risk. Instead, any data (or the lack thereof) were weighed subjectively in the estimation of risk from each hazard.

Commodity grouping

The fruits considered in the assessment are listed in Table 1. To simplify the risk assessment, a number of commodity characteristics were examined *via* published literature for a suitable means to group fruits on a factor affecting the presence, growth and survival of micro-organisms. The factors considered were growth habitat (proximity to ground), pH, acid type, water activity, nutrients, surface characteristics (e.g. presence of peel), competing microflora, antimicrobial substances, abuse potential (susceptibility to damage) and ripening ability (climacteric or not).

Hazard identification

An initial list of potential hazards was developed based on literature evidence of isolates or outbreaks involving fresh fruit or vegetables, where hazards had been linked (definitively or purportedly) to human illness. Vegetable data were included owing to the paucity of information on fresh fruit alone, making the assumption that contamination routes and outcomes are similar for both groups.

Hazard profiles

The remaining elements of the Codex framework (hazard characterization, exposure assessment and risk characterization) were applied in what was termed 'Hazard Profiles'. These profiles considered: the characteristics of the organism, including growth, inactivation and survival parameters; characteristics of the disease, including available evidence of the dose–response relationship; epidemio-

logy, including pathogen reservoirs, presence on produce and information on disease outbreaks; and risk estimation, a summation of the significance of the hazard in fresh fruit considering the potential for the organism to be present, survive and grow in fruit, the possibility that disease may be caused without growth, epidemiological data on outbreaks caused by produce, and the individual or public health consequences of the disease.

Consumption data of individual or all fruits were not considered. Where the hazard was determined to be significant in fresh fruit, exposure of consumers at levels sufficient to cause illness was assumed to occur.

Risk management

The published literature was reviewed for evidence on the effectiveness of the following risk management options: good agricultural, manufacturing and hygienic practices (GAP, GMP and GHP); maintaining integrity; storage temperature; washing; disinfectants; modified atmosphere packaging (MAP); heat; and alternative technologies. Control measures to manage the risk of significant hazards on fresh fruit are recommended. References used for all stages of the risk assessment cover published literature prior to mid-2004.

Results

Commodity grouping

The following factors affecting microbial survival or growth were examined to conclude on a mechanism of grouping fruits for simplification.

pH

Along with storage temperature, pH is cited as the principal determinant of growth of bacterial micro-organisms on fresh fruit (De Roever 1998). Many acidic fruits do not support the growth of human pathogens and even inactivate them. However, some fruits (e.g. melon) have a significantly higher pH and can support microbial growth. The fact that outbreaks of human disease have more commonly been associated with higher pH fruits suggests that there is a relationship between pathogen presence at consumption and pH of the fruit.

The effectiveness of pH on the inhibition of micro-organisms is affected by the type of organic acid in the food, with some acid types being more effective than others (Corlett and Brown 1980). However, fruits may contain one or many different types of organic acids (Wiley 1994), and information on minimum pH for growth, given any of the number of organic acid combinations, could not be found.

Table 1 Fruits considered

Citrus	Pip	Stone	Soft	Tropical/other
Orange	Grape	Peach	Raspberry	Mango
Grapefruit	Apple	Plum	Strawberry	Pineapple
Lemon	Pear	Cherry	Blackcurrent	Kiwifruit
Lime	Melon	Apricot	Cranberry	Passionfruit
			Redcurrent	Banana
			Gooseberry	Papaya
				Guava

Surface characteristics

Although researchers note that the thickness and nature of the epithelium and protective cuticle are important with respect to microbial growth, there have been no attempts to categorize individual fruits according to these characteristics (Jay 1996; Brackett 1997; Beuchat 2002). The inedible peel of some fruits could be reasoned to act as a barrier to contamination of the edible portion; however, the potential for cross-contamination could remove this advantage. Differentiation on this characteristic was not considered useful.

Abuse potential

Damage to the fruit integument by bruising, penetration or cutting leads to faster spoilage (Jay 1996; Zagory 1999). Although the effect of damage on pathogens has not been quantified, *E. coli* and *L. monocytogenes* have been shown to grow well on bruised or wounded apples (Janisiewicz *et al.* 1999; Conway *et al.* 2000; Dingman 2000), and *Salmonella* on cut surfaces of tomatoes (Lund 1992).

Despite the fact that fruits would have differing susceptibilities to abuse, it is possible to ensure that all fruit is conveyed to the point of consumption without significant damage. Therefore, although recommendations on the prevention of loss of integrity may be made, it is not practical to distinguish fruits on the basis of abuse potential for the purposes of assessing pathogen growth and/or survival.

Ripening ability

Climacteric fruits, those that can ripen after removal from the plant, are subsequently more susceptible to microbial infection and spoilage as time from harvest increases (Brackett 1997). This is attributed to a drop in acid content and a subsequent rise in pH (Lund and Snowdon 2000). Examples of climacteric fruits include banana, pear, peach, apple, plum and kiwifruit. Examples of nonclimacteric fruits include citrus, grape, pineapple, strawberry and cherry.

Climacteric fruits may therefore provide a better environment for growth of organisms that are pathogenic to humans. However, there is a lack of information on any (or no) relationship between these fruits and pathogens levels.

Other factors were considered but ruled out as either not being useful or there being a lack of data around their effect. These factors were: growth habitat (ground or tree); organic acid type; water activity; nutrients; competing microflora; and naturally occurring antimicrobial substances.

Considering the growth limits for microbial pathogens (International Commission on Microbiological Specifications for Foods 1996), we assumed that growth is unlikely

Table 2 pH values of fruits

Fruit	Approximate pH range
1. Citrus fruits	
Orange	2.6–4.4 ^c
Grapefruit	2.9–3.4 ^a
Lemon	2.2–2.6 ^a
Lime	1.6–3.2 ^c
2. Pip fruits	
Grape	3.0–4.5 ^a
Apple	2.9–4.5 ^c
Pear	3.4–4.7 ^a
Melon, cantaloupe	6.2–6.5 ^b
Melon, honeydew	6.3–6.7 ^b
Watermelon	5.8–6.0 ^b
3. Stone fruit	
Peach	3.1–4.2 ^b
Plum	2.8–4.6 ^b
Cherry	3.2–4.7 ^b
Apricot	3.3–4.4 ^a
4. Soft fruits	
Raspberry	2.9–3.5 ^a
Strawberry	3.0–3.6 ^a
Blackcurrant	2.6–3.1 ^c
Cranberry	2.5–2.7 ^b
Redcurrant	2.6–2.9 ^d
Blackberry	3.0–4.2 ^a
Gooseberry	2.8–3.1 ^d
5. Tropical/other	
Mango	3.8–4.7 ^a
Pineapple	3.2–4.0 ^b
Kiwifruit	3.1–4.0 ^b
Passionfruit	2.6–3.3 ^b
Banana	4.5–5.2 ^b
Papaya	4.5–6.0 ^b
Guava	4.3–4.7 ^d

Source: (a) ICMSF, Vol. 6, p. 252 (International Commission on Microbiological Specifications for Foods 1998); (b) Lund and Snowdon 2000; (c) ICMSF, Vol. II, p. 645 (International Commission on Microbiological Specifications for Foods 1980); (d) Other (unsubstantiated – WWW, abstracts).

to occur on fruits of pH < 4, irrespective of the types of acid in the fruits concerned. Splitting fruits into those that are below (or equal to) a pH of 4.0, where growth is unlikely to occur, and those above pH 4.0, where growth is more likely to occur, was chosen as the grouping with the most potential. The split was made using the highest pH values cited for a particular fruit (Table 2). The selected fruits that fall into the respective groups are listed in Table 3.

Hazard identification

Hazards identified as potentially contaminating fruit that have also been linked to human illness (but not necessarily as a result of produce contamination) are listed in

Table 3 Fruit groups

Group I – High acid fruit (pH ≤ 4.0)	Group II – Low acid fruit (pH > 4.0)
Grapefruit	Melon, cantaloupe
Lemon	Melon, honeydew
Lime	Watermelon
Pineapple	Banana
Kiwifruit	Papaya
Passionfruit	Guava
Raspberry	Orange
Strawberry	Grape
Blackcurrant	Apple
Cranberry	Pear
Redcurrant	Peach
Gooseberry	Plum
	Cherry
	Apricot
	Mango
	Blackberry

Table 4 Hazard identification

Bacteria	Viruses
<i>Listeria monocytogenes</i>	Hepatitis A virus
<i>Salmonella</i> spp.	Noroviruses
<i>Clostridium botulinum</i>	Protozoa
<i>Clostridium perfringens</i>	<i>Cryptosporidium</i> spp.
<i>Bacillus cereus</i>	<i>Cyclospora cayetanensis</i>
Other <i>Bacillus</i> spp. (<i>Bacillus subtilis</i> , <i>Bacillus licheniformis</i> , <i>Bacillus pumilus</i>)	<i>Giardia intestinalis</i>
<i>Streptococcus</i> spp.	<i>Entamoeba histolytica</i>
<i>Aeromonas</i> spp.	
<i>Campylobacter</i> spp.	Nematodes
<i>Shigella</i> spp.	<i>Ascaris</i> spp.
<i>Yersinia enterocolitica</i> and <i>Yersinia pseudotuberculosis</i>	
<i>Escherichia coli</i>	
<i>Staphylococcus aureus</i>	
<i>Vibrio cholerae</i>	
<i>Pseudomonas aeruginosa</i>	
Other or nonspecific Enterobacteriaceae	

Table 4. Where a number of species have been implicated with similar characteristics, they are grouped together under the genus name. It was assumed that any of the pathogens could be initially present on any fruit.

Fungal species producing mycotoxins were not included in the review. While these are important organisms in terms of public health, exposure to mycotoxins from produce is considered to be less significant than from low water activity foods (e.g. grains, nuts) intended for longer term storage (European Commission–Scientific Committee on Food 2002).

Hazard profiles

The nature of the contamination data available and the factors used to come to a decision on the significance of each of the pathogens listed in Table 4 are summarized in Table 5. The references cited for each pathogen inform whether or not the pathogen has been isolated (I) from either fruits or vegetables, or fruits and vegetables have been linked to an outbreak (O) of disease owing to that micro-organism. Where the references cite quantitative information, this is included in the table, although concentrations reported per amounts larger than 1 g have been adjusted to a per gram basis.

Giving the incidence, concentration and outbreak data on vegetables, the same weighting as those available for fresh fruit (i.e. assuming similar mechanisms, contamination prevalence and concentration, and survival), the remainder of Table 5 was populated on a subjective evaluation of the available evidence, with conclusions being applied specifically to fresh fruit only.

Whether or not growth was noted as required to cause illness was based on the comparison between the levels of the organism recorded in produce and (qualitative or quantitative) levels cited as responsible for causing infection, or where toxin is the usual cause of illness. Where quantitative data on the levels in produce were not available, this assessment was based solely on the available dose–response data.

Whether growth conditions were considered to be favourable or not depended on the nature of the organism (e.g. anaerobic, aerobic etc.), the usual matrix associated with food poisoning (e.g. *B. cereus* and cooked food), and the worst case pH of fruit (Table 2) being in the cited growth range. For nonbacterial organisms, growth in fruit will not occur, and it was assumed that any low level on fruit would be sufficient to cause illness.

Fruit was determined to be a significant public health burden with respect to any pathogen where either multiple or large outbreaks (e.g. salmonellosis), or a single/few outbreak(s) with severe individual consequences (e.g. shigellosis, ascariasis) have been attributed conclusively to fresh produce.

The decision on whether a particular pathogen was determined to be a significant hazard in fresh fruit depended on the fulfillment of one of the following criteria:

- 1 Fruit being concluded as posing a significant public health burden.
- 2 A significant burden of disease attributed to other commodities, yet isolated from produce and able to cause illness with a low dose (e.g. campylobacteriosis) or where conditions in fruit (usually higher pH or damaged) could allow growth to harmful levels (e.g. listeriosis).

Table 5 Hazard profile results

Pathogen	Fruit data		Vegetable data		Conc. (CFU g ⁻¹)	Growth required for illness	Favourable growth conditions	Significant public health burden	Significant hazard	Refs.
	I	O	I	O						
<i>Listeria monocytogenes</i>	√ (juice)	x	√	√	<1–<100	√	√	x	√	Farber et al. 1989; Arumugaswamy et al. 1994; Petran et al. 1988; Breer and Baumgartner 1992; Macgowan et al. 1994; Percudani and Gola 1995; Gola et al. 1990; Wong et al. 1990; Desimon et al. 1992; Harvey and Gilmour 1993; Sizmur and Walker 1988; Schlech et al. 1983; Ho et al. 1986; Laine and Michard 1988; Beckers and Veld 1989; Odumeru et al. 1997; Thunberg et al. 2002; Heisick et al. 1989; Monge and Arias 1996; Sado et al. 1998
<i>Salmonella</i> spp.	√	√	√	√	NE	x	√	√	√	Jerngkinchan and Saitanu 1993; Mahon et al. 1997; Madden 1992; Blostein 1993; Saddik et al. 1985; Ruiz et al. 1987; O'Mahony et al. 1990; van Duynhoven et al. 2002; Weissinger et al. 2000; Mohle-Boetani et al. 1999; Tamminga et al. 1978; Parish 1997, 1998; Tauxe et al. 1997; Hedberg et al. 1999; Rasinaul et al. 1988; Rude et al. 1984
<i>Clostridium botulinum</i>	x	x	√	√	0.41	√	x	x	x	Lilly et al. 1996; Notermans et al. 1989; Solomon and Kautter 1986; Hauschild et al. 1975; Insalata et al. 1969; Solomon et al. 1990; St Louis et al. 1988; Otofuiji et al. 1987; Horwitz et al. 1975
<i>Clostridium perfringens</i>	x	√ ¹	x	√ ¹	NE	√	x	x	x	Bean and Griffin 1990
<i>Bacillus cereus</i>	x	x	√	√	>10 ²	?	x	x	x	Harmon et al. 1987; Portnoy et al. 1976; Kaneko et al. 1999; Becker and Holzappel 1997; Splittstoesser et al. 1983.
Other <i>Bacillus</i> spp.	x	x	x	√	>10 ²	?	x	x	x	Kramer and Gilbert 1989
<i>Streptococcus</i> spp.	√ (juice)	√ ¹	√	√ ¹	10 ³ –10 ⁴	?	?	x	x	Bean and Griffin 1990; Tamminga et al. 1978; Ercolani 1976; Soriano et al. 2000; Khalaf et al. 1988
<i>Aeromonas</i> spp.	x	x	√	x	10 ² –10 ⁶	?	?	x	x	Saad et al. 1995; Nishikawa and Kishi 1988; Callister and Agger 1987; Mattick and Donovan 1998

Table 5 Continued

Pathogen	Fruit data		Vegetable data			Conc. (CFU g ⁻¹)	Growth required for illness	Favourable growth conditions	Significant public health burden	Significant hazard	Refs.
	I	O	I	O	O						
<i>Campylobacter</i> spp.	x	√ ¹	√	√ ¹	√	NE	x	x	x	√	Park and Sanders 1992; Bean and Griffin 1990; Doyle and Schoeni 1986
<i>Shigella</i> spp.	x	√	√	√	√	NE	x	√	√	√	Dunn et al. 1995; Kapperud et al. 1995; Martin et al. 1986; Saddik et al. 1985; Davis et al. 1988; Tauxe et al. 1997; Fredlund et al. 1987
<i>Yersinia enterocolitica</i> , <i>Yersinia pseudotuberculosis</i>	x	x	√	√	√	NE	?	√	√	√	Brocklehurst et al. 1987; Cateau et al. 1985; Darbas et al. 1985a,b; Gola et al. 1990; Johannessen et al. 2002; Salamah 1993; Tassinari et al. 1994; Cavazzini et al. 1982, 1989; Nuorti et al. 2004
<i>Escherichia coli</i> (EHEC, EPEC, ETEC)	√	√	√	√	√	NE	x	√	√	√	Besser et al. 1993; Zepeda et al. 1995; Odumeru et al. 1997; Parish 1997; Tauxe et al. 1997; Beuchat 1996; Cavazzini et al. 1989; Bell and Kyriakides 1998; Franco et al. 1987; Merson et al. 1976; Singh et al. 1995; Benoit et al. 1994; Nairi et al. 2003; Okafo et al. 2003; Velaudapillai et al. 1969; Khalaf et al. 1988
<i>Staphylococcus aureus</i>	√	x	√	x	x	10 ² -10 ³	√	x	x	x	Prokopowich and Blank 1991; Saddik et al. 1985; Abdelnoor et al. 1983; Houang et al. 1991; Khalaf et al. 1988
<i>Vibrio cholerae</i>	x	x	x	√	√	NE	x	√	√	√	Shuval et al. 1985
<i>Pseudomonas aeruginosa</i>	√	x	√	x	?	10 ³ -10 ⁶	?	√	x	x	Becker and Holzapfel 1997; Gras et al. 1994; Khalaf et al. 1988
Other or nonspecific Enterobacteriaceae	√	x	√	x	?	10 ¹ -10 ⁶	?	√	x	x	Oconnor and Mitchell 1991; Becker and Holzapfel 1997; Torriani and Massa 1994; Simonsarkadi et al. 1994; Jockel and Otto 1990; Cavazzini et al. 1989
Hepatitis A virus	x	√	√	√	√	NE	x	x	√	√	Ramsay and Upton 1989; Reid and Robinson 1987; Rosenblum et al. 1990; Hutin et al. 1999; Monge and Arias 1996; Niu et al. 1992; Tauxe et al. 1997; Pebody et al. 1998; Dentinger et al. 2001

Table 5 Continued

Pathogen	Fruit data		Vegetable data		Conc. (CFU g ⁻¹)	Growth required for illness	Favourable growth conditions	Significant public health burden	Significant hazard	Refs.
	I	O	I	O						
Noroviruses	x	√	x	√	NE	x	x	√	√	Lieb <i>et al.</i> 1985; Iversen <i>et al.</i> 1987; Griffin <i>et al.</i> 1982; Warner <i>et al.</i> 1991; Herwaldt <i>et al.</i> 1994; Gaulin <i>et al.</i> 1999; Ponka <i>et al.</i> 1999
<i>Cryptosporidium</i> spp.	x	√ (juice)	√	x	0-03 (cysts)	x	x	x	√	Millard <i>et al.</i> 1994; Ortega <i>et al.</i> 1997; Robertson and Gjerde 2001; Monge and Arias 1996
<i>Cyclospora cayetanensis</i>	x	√	√	x	NE	x	x	√	√	Herwaldt <i>et al.</i> 1997; Ortega <i>et al.</i> 1997; Sherchand and Cross 2001
<i>Giardia intestinalis</i>	√	√	√	√	0-01-0-08 (cysts)	x	x	x	√	Mintz <i>et al.</i> 1993; Robertson and Gjerde 2001, 2001; Monge and Arias 1996; Porter <i>et al.</i> 1990
<i>Entamoeba histolytica</i>	x	x	√	x	NE	x	x	x	√	Monge and Arias 1996
<i>Ascaris</i> spp.	x	x	√	√	NE	x	x	x	√	Rude <i>et al.</i> 1984; Raisanen <i>et al.</i> 1985

I, incidence; O, outbreak; √, yes; x, no; NE, not estimated.

*One report attributed to either fruit or vegetable (Bean and Griffin 1990).c

Table 6 Significant hazards and growth boundaries

Hazard	pH boundaries	Temp boundaries (°C)
<i>Listeria monocytogenes</i>	4-39-9-4	-0-4-45
<i>Salmonella</i> spp.	3-8-9-5	5-2-46-2
<i>Campylobacter</i> spp.	4-9-9-0	32-45
<i>Shigella</i> spp.	4-9-9-34	6-47
<i>Yersinia enterocolitica</i> and <i>Yersinia pseudotuberculosis</i>	4-2-9-6	-1-3-42*
<i>Escherichia coli</i> (EHEC, EPEC, ETEC)	4-4-9-0	7-46
<i>Vibrio cholerae</i>	5-0-9-6	10-43
Noroviruses	-	-
Hepatitis A virus	-	-
<i>Cryptosporidium</i> spp.	-	-
<i>Giardia intestinalis</i>	-	-
<i>Cyclospora cayetanensis</i>	-	-
<i>Entamoeba histolytica</i>	-	-
<i>Ascaris</i> spp.	-	-

*Temperature range cited for *Y. enterocolitica*.

The final list of significant hazards is displayed in Table 6, with the pH and temperature growth boundaries (International Commission on Microbiological Specifications for Foods 1996).

Risk management options

GAP/GMP/GHP

GAP, GMP and GHP are management practices aimed at controlling and reducing microbial food safety hazards. The following broad areas affecting the microbiological status of fresh produce would receive consideration in these programmes (US Department of Health and Human Nutrition and FDA 1998):

- Water;
- Animal manure and municipal biosolids;
- Worker health and hygiene;
- Sanitation;
- Specific process steps.

There is a lack of published data on the extent that these programmes are able to control specific pathogens in fresh fruit. In one study that compared the numbers of natural bacterial flora on raw vegetables prepared under GMP and non-GMP conditions, the results were variable (Koek *et al.* 1983). Pathogen groups were not isolated in sufficient numbers to assess the two methods. Another study did find a significant difference in numbers of coliforms (plus APC aerobic plate count, yeast and moulds) in a variety of foods between ready-to-eat food stores classified according to GMP behaviours observed (de Sousa *et al.* 2002).

There is no direct evidence that GAP/GMP/GHP programmes can be relied upon to provide a definitive risk management solution for pathogens on fresh fruit.

However, these programmes can reduce faecal contamination, and by association, could reduce faecal pathogens. Basic good hygienic practices (GAP/GMP/GHP as appropriate) should therefore be employed as a minimum during the growth and preparation of fresh fruit, in particular with respect to human and equipment contact with the edible product.

Maintaining integrity

As noted earlier, damaged or bruised fruit would support the persistence and growth of both spoilage and pathogenic bacteria. It is suggested that the mechanism by which damage allows the persistence and growth of pathogens is by the creation of a micro-environment (Janisiewicz *et al.* 1999; Conway *et al.* 2000). The pH of acidic fruit has been noted as rising to within growth limits upon bruising (Dingman 2000), and the same is likely to occur with other forms of damage. The sugar content of exposed fruit is also a potential source of nutrients for microbes (Brackett 1997).

Some authors suggest the preservation of integrity and the temperature of storage are more useful ways to manage spoilage than the removal of micro-organisms (Zagory 1999). Fruits have a thicker epidermis than vegetables, the protection from which is eliminated either by accidental damage or deliberate cutting of the fruit (Brackett 1997).

Maintaining integrity as long as possible prior to the consumption of raw fruit is therefore a sensible risk management option for all fruit/(bacterial) pathogen combinations. In this respect, damage is defined as bruising and cuts to fruit epidermis either deliberate (in processing) or accidental (e.g. in transport). The loss of integrity caused by the growth of spoilage micro-organisms (e.g. fungi) should also be prevented.

Storage temperature

Temperature is a major determinant of bacterial growth. Although pH and a_w are also significant, optimal growth temperatures allow micro-organisms to overcome a wider range of pH and a_w conditions. However, unlike the pH and a_w of fruit, the storage temperature, once a fruit is harvested, is under the control of the harvester/processor. A low storage temperature is a sensible risk management option, particularly in fruit/(bacterial) pathogen combinations where growth can occur or the conditions become favourable for growth.

Washing

Washing fruit with water alone can reduce micro-organism levels, in some instances, as effectively as using disinfectants. The effect will depend on the fruit type, organisms and the method of washing.

Rinsing apples was shown to reduce (inoculated) *Salmonella* and *E. coli* (but not *L. monocytogenes*) by 0.5 log₁₀ (Beuchat *et al.* 1998). Soaking them with a water spray for 1–10 min prior to rubbing and rinsing reduced *Salmonella* by 3 logs and *E. coli* by 0.5 log₁₀ (with 10 min soaking only), but left other organisms relatively unaffected.

A 5 min wash (with gentle stirring and a 5 s rinse) was shown to reduce high levels of *E. coli* O157:H7 inoculated onto apples by 2 logs, and was just as effective as a 10 min wash, although not as effective as using commercial produce disinfectants (3 logs in 5 min) (Wisniewsky *et al.* 2000).

Immersion in deionized water for 8 min achieved about a 2 log reduction of a mixture of *E. coli* strains on oranges, which was comparable with the reduction achieved by a 200 ppm chlorine solution (Pao and Davis 1999). A reduced efficacy was noted at the stem scar area.

Brushwashing oranges resulted in a 60–70% (0.4–0.5 log) reduction in microbial surface population in one study reported in an FDA review of decontamination (Anon 2003).

Extended washing of cut lettuce in water (20 min) by continuous circulation reduced microbial counts (APC) as effectively (1–2 log) as washing with a 100 ppm hypochlorite solution (for 5–30 min) (Adams *et al.* 1989). Other studies on the normal bacterial population present on vegetable products have shown reductions in the order of 0.5–1 log (Garg *et al.* 1990; Becker and Holzapfel 1997). Although there is limited information on viruses, the reduction of a surrogate virus on produce was reported as typically less than 1 log (CCFRA newsletter, November 2002).

The effectiveness of washing on protozoal organisms appears to be inadequate at higher levels of contamination, while contamination at lower levels produced inconsistent findings (Lee and Lee 2001). The large number of crevices in raspberries and their fragile nature may prevent effective washing with respect to protozoa in this particular fruit type (Herwaldt *et al.* 1997). In another study using *Toxoplasma gondii* as a surrogate for *Cyclospora*, it was demonstrated that raspberries retained more oocysts than a smooth berry type (blueberries) (Kniel *et al.* 2002). This was attributed to the fine hairlike projections covering raspberries.

The World Health Organization (WHO), in a review of surface decontamination of fruit and vegetables, has suggested that vigorous washing can be as effective as treatment with water containing 200 ppm chlorine, which generally reduces microbial populations by 10–100-fold (Beuchat and World Health Organization 2002). 'Vigorous' is not defined. The WHO review also recommends using water at a higher temperature than the fruits or

vegetables being washed (to prevent ingress of water and micro-organisms), and double washing for heavily contaminated produce.

Mechanical drying (e.g. with paper towelling) after washing can further reduce the levels of some organisms. One study has demonstrated a reduction of an additional 0.4 log of *Salmonella* inoculated onto apples (Parnell and Harris 2003). Drying in warm air may also reduce contamination levels, the amount of reduction dependent on the organism (Beuchat *et al.* 2001).

Therefore, washing is a possible risk management option, where it is known that levels of initial contamination are low, and the conditions postwashing do not allow opportunity for bacterial growth. Further study on the effectiveness of washing is required, particularly on organisms other than bacteria and viruses. It should be noted that removal of spoilage bacteria may reduce the competition for pathogenic bacteria, should they remain or be reintroduced, and subsequently allow them to grow faster (Zagory 1999).

Disinfectants

In a US FDA review, it is reported that liquid chlorine and hypochlorite are the most commonly used methods for disinfecting produce – generally at concentrations of 50–200 ppm for 1–2 min (Anon 2003). The report also notes that a differing effect can be seen depending on the micro-organism and the produce type. Many chlorination studies on vegetables demonstrate a reduction of 1–2 logs, with an increase in contact time or concentration not giving a commensurate rise in lethality.

Chlorine is also noted to have an effect on viruses. Using feline calicivirus as a surrogate for noroviruses, a 1000 ppm (fresh) solution was found to give a 4 log inactivation after 10 min (Doultree *et al.* 1999). However, this effect was demonstrated *in vitro*, and a much higher concentration was required if the solution was not freshly reconstituted.

It can be concluded that the disinfection of pathogens on fruit by chlorine is likely to be variable, and will be dependent on the target organism, the surface characteristics of the fruit and the level of organic material, among other factors.

A number of other compounds are cited as having potential use for disinfecting produce, including hydrogen peroxide, acid and alkali compounds, bromine, iodine, peracetic (peroxyacetic) acid, ozone and plant derivatives. There are limited data on the effectiveness of many of these compounds, and those data often report variable results (Anon 2003).

Disinfectants are a possible risk management option; however, given the variable efficacy, any compound utilized would require validation of its effectiveness against

the pathogens likely to occur, for a specific product and process.

Modified atmosphere packaging (MAP)

Modification of the air space is used in the packaging of produce, primarily to maintain quality. Typically, this involves reducing the concentration of O₂ and increasing the concentration of CO₂ in the airspace (Berrang *et al.* 1989). This has the effect of reducing the rate of respiration, retarding the ripening process and increasing the shelf life of the produce.

Changing the atmosphere in packaging can also have an effect on bacterial survival or growth. However, these effects appear to be variable depending on the organism, produce type and the concentrations of O₂ and CO₂. For example, on endive, *L. monocytogenes* has been shown to survive and grow, while *Bacillus cereus* was reduced (Gorris 1994). Conflicting results regarding the effect on *Campylobacter* have also been noted (Farber 1991). Other studies have been cited as showing that MAP has no effect on *E. coli* O157, mesophilic bacteria and *L. monocytogenes* in vegetables (Zagory 1999).

There is a concern that MAP may actually increase the risk from pathogens in that the normal process of spoilage is delayed, allowing more time for the growth of pathogens (Francis *et al.* 1999). Under conditions of temperature abuse, the atmosphere could also favour the growth of anaerobic organisms. MAP appears to have little effect on virus survival (CCFRA Newsletter, Nov 2002), and based on the biology of oocysts, unlikely to have any effect on protozoa. In conclusion therefore, MAP does not appear to offer a reliable risk management option for managing pathogens in fresh fruit.

Heat

Heat is commonly used to inactivate pathogens in processed food. However, the use of heat on fresh fruit would, in many cases, change the sensory properties of the fruit. Where heat might be used with minimal sensory change would be on fruits with an integument that would be removed before consumption. The reduction of 5 log of *E. coli* on oranges, e.g. was achieved using water immersions of 70°C for 2 min or 80°C for 1 min (Pao and Davis 1999). The microbial load of the final juiced orange was also reduced, without loss of sensory quality. On raspberries, heating for 1 h at 80°C has been shown to remove the infectivity of a coccidial substitute for *Cyclospora cayetanensis* (Lee and Lee 2001).

Heat is therefore a possible risk management option for fresh fruit in the following situations:

– where changes in sensory qualities are not important to the product;

- where an outer integument will protect the edible portion of the fruit;
- where facilities are available to treat with steam.

Alternative technologies

There are a large number of more novel technologies that are being investigated for their efficacy in reducing microbial contamination (Anon 2003). These include irradiation, high pressure, biocontrol, pulsed electric field, pulsed light, oscillating magnetic fields, ultrasound and UV treatment. For most of these technologies, there is sparse evidence of their effectiveness for fresh fruit.

Irradiation is one technology where there is more evidence available, although consumer resistance, and in some countries, legislative barriers prevent its widespread use. Irradiation has been used to eliminate *E. coli* O157 from apple juice and *Toxoplasma gondii* and *Cyclospora cayentanensis* from raspberries (Anon 2003). However, many fruits will not tolerate the medium- and high-level doses required to inactivate some of the more resistant organisms. In addition, irradiation is more suited to the inactivation of Gram-negative bacteria (Brackett 1992).

High pressure is also building in popularity, although there are limited data on pathogen inactivation on fruit and possible organoleptic issues with soft fruits (Hoover 1997). CO₂ in combination with lower pressures has been suggested as an improvement for this latter issue.

Further information on effectiveness is required before most of these technologies can be recommended as risk management options for fresh fruit. Irradiation is a suitable control measure where it has been validated for specific pathogens, although its use depends on local legislation and consumer attitudes.

Risk management recommendations

Based on the risk management option analysis, generic recommendations for all fruits were made, regardless of the significant hazards that could be present (Fig. 1).

Recommendations specific for each significant hazard were then made, taking into account the likely extent of contamination, the potential for growth in fruit and the need for growth to cause illness. Survival was assumed at the least for all hazards.

The hazard-specific risk management recommendations (working not shown) focussed on measures to remove contamination prior to consumption and the prevention of growth to significant levels during storage. The survey data, combined with the generic recommendation of good hygienic practices led to the recommendation of a control measure targeting only low levels of contamination for all hazards. Prevention of significant growth during storage can be achieved by storage at a temperature lower than the minimum cited for growth (Table 6), and as some organisms grow at refrigeration temperatures, also by limiting the time of storage. For these organisms (*L. monocytogenes* and *Yersinia* spp.), the USDA Pathogen Modeling Program (PMP6.1) was used with a near worst case pH = 6.5 (Table 2) to predict the time taken to reach 100 CFU g⁻¹ (*L. monocytogenes*) or for a 2 log increase (*Yersinia* spp.) at well-controlled refrigeration temperatures (4 or 5°C). A starting level of 10 CFU g⁻¹ for *L. monocytogenes* was assumed and a 2 log reduction from the decontamination step factored in. With less evidence on starting levels for *Yersinia* spp. in fruit or an upper action level, a maximum 2 log increase, where a decontamination step would be performed, was considered acceptable.

The final recommendations for storage temperature and time were the most conservative considering all the individual hazard recommendations (Fig. 2).

Recommendations were split into high acid fruit, where no growth will occur, and low acid fruit, where the pH might be at levels within the pH growth boundaries (Table 6). The recommended temperature and time to control the growth of psychrotrophic organisms at a moderate pH was 4°C for 4 days. For fruits that would be damaged at this temperature, a shorter period of storage (2 days) at the higher temperature of 15°C was recommended. Poststorage, a recommendation is made for the removal of low levels of organisms by washing. For some fruits (e.g. apples, pears) there is an absence of epidemiological evidence that storage for longer periods compromises safety (or quality). Holding periods for these fruits could be extended in line with historical safe practice.

Additional recommendations for aggregate fruits, such as blackberries and raspberries, were made owing to the

- Fruit should be free from bruising, punctures and visible spoilage.
- After any decontamination steps (including washing) recontamination should be prevented.
- Basic good hygienic practices (GAP/GMP/GHP as appropriate) should be employed in the growing and harvesting of fresh fruit and preparation of fresh fruit products.

Figure 1 Generic recommendations – all fruit.

- Specific recommendations for high acid fruit ($\text{pH} \leq 4.0$ always recorded):
- Fruit should be washed immediately prior to preparation. If washing takes place earlier than immediately before use, fruit should be dried prior to storage.
- Specific recommendations for low acid fruit ($\text{pH} > 4.0$ sometimes or always recorded):
- Fruit should be washed immediately prior to preparation. If washing takes place earlier than immediately before use, fruit should be dried prior to storage.
 - Store fruit at a temperature $\leq 4^\circ\text{C}$ for a maximum of 4 days before use.¹
- or
- Where 4°C storage (or below) cannot be attained due to potential fruit injury, store at the lowest temperature possible but not higher than 15°C for a maximum of 2 days.¹
 - Storage should be followed by washing and peeling (if applicable) immediately prior to consumption or further processing (to a state where growth of bacterial pathogens is prevented).

Figure 2 Recommendations for high and low acid fruits. ¹Where there is history of safe use at longer storage times this period may be extended.

- Recommendations relevant to the pH of the fruit and (one of the following)
 - Irradiate with a dose of at least 1kGy.
- or
- heat at 80°C for 1 h.
- or
- Keep frozen at -18°C for 24 h.

Figure 3 Recommendations for aggregate fruits.

apparent difficulty to remove protozoa by washing and the low dose required to cause illness (Fig. 3). Fresh characteristics would however be lost with heating or freezing. Based on the data considered on the effectiveness of washing, a best practice protocol was developed (Fig. 4).

It is important that any processing equipment, water, or air that comes in contact with the fruit does not add significant microbial contamination, particularly after washing. Good hygienic practices have a role in preventing such contamination. Recommendations for cut fruits

were not made as a result of this assessment; these would be the result of a separate risk assessment.

Discussion

In this study, we have described application of the Codex principles for risk assessment to a wide range of fruits and considered a comprehensive list of biological hazards that may be associated with this produce (Codex Alimentarius Committee 1999). The approach taken was qualitative rather than quantitative and was conducted to identify higher risk products and effective risk management measures. There are few examples of this type of application available, although some recent publications have used quantitative risk assessment approaches to relate contamination rates to adverse public health consequences for fresh produce. In a report from Stine *et al.* (Stine 2005), the authors describe a quantitative approach to calculate the concentration of particular infectious pathogens in irrigation water necessary to achieve a 1 in 10 000 annual risk of infection, which is the accepted level of risk used for

- Washing method
- Use potable water that is at a higher temperature than the fruit being washed (e.g. $2\text{--}3^\circ\text{C}$ higher).
 - Soak fruit for 5–10 min, preferably with agitation.
 - Rinse fruit with potable water.
 - Dry fruit after washing, either mechanically or with warm air.
 - Double wash fruit with heavy surface soiling/contamination.

Figure 4 Recommended washing method.

drinking water by the US Environmental Protection Agency. Such studies however remain rare and do not cover the wide range of produce considered here nor do they consider the factors investigated here. Other risk assessments commonly focus on single hazards, such as *E. coli* O157:H7 in apples (Duffy 2002), but food producers must assess the risks posed by a number of potential hazards. Attempting to analyse the risk of each pathogen/commodity combination separately and quantitatively, following the risk assessment approach of the Codex Alimentarius Commission, was not practical owing to the large number of pathogens considered, the wide range of fruits and the prohibitive data requirements.

We grouped fruits based on intrinsic factors that are relevant to the survival and growth of biological hazards, developed hazard profiles based on Codex-defined risk assessment elements and used a number of factors to estimate whether hazards were significant or not. Dealing with an extensive list of hazards diverts limited resources and creates unnecessary difficulties for risk management, and it is important that food producers and manufacturers focus on those most relevant to their produce and products. The significant hazards identified in this study may come from a variety of sources and by profiling each hazard, we have been able to identify the most relevant risk management options that should control them. Of fundamental importance in all the situations considered here, despite the lack of hard data, is the prevention of contamination at source and the application of effective good agricultural practice (US Department of Health and Human Nutrition and FDA 1998), good manufacturing practice and good hygiene (US Department of Health and Human Nutrition and FDA 2007). With these in place, we conclude that the quality of the raw material (harvested produce) will meet a minimum standard that coupled with good handling to prevent damage and prevention of subsequent (cross-)contamination, should ensure that fresh produce is free from harmful levels of microbiological hazards.

We have shown that for particular types of fruits, there are further risk management options that can be used to reduce risk even further. There may be relatively little impact of disinfectants or decontaminating solutions and washing with potable water can provide similar (limited) reductions in numbers. These limited effects emphasize the importance of good quality and prevention of pathogen contamination at source. Storage of fresh produce at low temperatures was also shown to impact on risk, although again, this may have limited impact on some low-infectious dose pathogens that can survive low/freezing temperatures. The highest risk products are low-acid fruits (able to support growth of some infectious pathogens) and those that are difficult to clean because of their

surface properties and structure, such as raspberries, blackberries and mulberries. For the low-acid fruits, these should have a limited shelf life at chill and should be washed/peeled prior to consumption or further processing. Some processing technologies (e.g. irradiation) can be used to destroy contaminating micro-organisms and offer the rare options of delivering a defined log reduction without affecting the sensory and organoleptic properties of fresh produce, but such technologies are not always readily accepted by consumers.

Quantitative risk assessment for fresh produce is still hampered by a lack of data and information, particularly in relation to the sources of contamination during growing. Outbreak information sometimes points to environmental sources of pathogens, e.g. from irrigation water contaminated by run-off from livestock (Ackers 1998), wastewater discharge or faecal contamination from farm and wild animals (Sagoo 2003), but there is often little hard evidence to identify the exact source or sources. In a recent investigation of irrigation water quality in the United Kingdom, Tyrrel *et al.* (Tyrrel 2006) emphasized the need for risk assessment of sources, credible monitoring practices and benchmarking of monitoring results against a reference point or standard. It is not unusual to see an emphasis on microbiological testing as method for control in harvested produce, but such trust can be misplaced because of the low likelihood that contamination will be detected, particularly for low-level contamination with low infectious-dose pathogens. For these reasons, it is important that the emphasis is placed on practices and measures for control and that testing is used to verify that these are operating as expected. Food producers should use HACCP (Hazard Analysis and Critical Control Point) and GAP/GMP to assure safety of fresh produce, and applying a risk assessment approach prior to this can provide manufacturers with some insight into effective management options. These will vary depending on the local conditions and supply chains used and the validation of identified interventions, which can be 'cumulative' to deliver a defined performance objective, and is critical to assure the safety of fresh produce. Additional measures may be required for quality aspects, e.g. the control of spoilage.

The approach outlined in this paper necessarily contains many simplifications, which may lead to risk management recommendations not appropriate for a specific situation. Therefore, it is important to take account of other data and evidence relevant for the situation being assessed.

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