Potential Contamination Sources on Fresh Produce Associated with Food Safety

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ABSTRACT - The health benefits associated with consumption of fresh produce have been clearly demonstrated and encouraged by international nutrition and health authorities. However, since fresh produce is usually minimally processed, increased consumption of fresh fruits and vegetables has also led to a simultaneous escalation of foodborne illness cases. According to the report by the World Health Organization (WHO), 1 in 10 people suffer from foodborne diseases and 420,000 die every year globally. In comparison to other processed foods, fresh produce can be easily contaminated by various routes at different points in the supply chain from farm to fork. This review is focused on the identification and characterization of possible sources of foodborne illnesses from chemical, biological, and physical hazards and the applicable methodologies to detect potential contaminants. Agro-chemicals (pesticides, fungicides and herbicides), natural toxins (mycotoxins and plant toxins), and heavy metals (mercury and cadmium) are the main sources of chemical hazards, which can be detected by several methods including chromatography and nanotechniques based on nanostructured materials such as noble metal nanoparticles (NMPs), quantum dots (QDs) and magnetic nanoparticles or nanotube. However, the diversity of chemical structures complicates the establishment of one standard method to differentiate the variety of chemical compounds. In addition, fresh fruits and vegetables contain high nutrient contents and moisture, which promote the growth of unwanted microorganisms including bacterial pathogens (Salmonella, E. coli O157: H7, Shigella, Listeria monocytogenes, and Bacillus cereus) and non-bacterial pathogens (norovirus and parasites). In order to detect specific pathogens in fresh produce, methods based on molecular biology such as PCR and immunology are commonly used. Finally, physical hazards including contamination by glass, metal, and gravel in food can cause serious injuries to customers. In order to decrease physical hazards, vision systems such as X-ray inspection have been adopted to detect physical contaminants in food, while exceptional handling skills by food production employees are required to prevent additional contamination.

Key words : Fresh produce, Biological hazards, Chemical hazards, Physical hazards, Detection
and imported products during all seasons throughout the year. Secondly, there have been increased efforts by the government to promote healthy foods. As the consumption of fresh produce has increased, the number of foodborne illnesses outbreaks associated with fruits and vegetables has risen. Because fresh produce is mostly consumed raw or after minimal processing, pathogen contamination constitutes a potential health risk. Fresh produce is easily contaminated by chemical, biological, and physical hazards during the transition from farm to fork phase. A recent report by the Center for Science in the Public Interest (CSPI) indicates that one of the highest numbers of outbreaks can be attributed to the fresh produce industry in the US between 2006 and 2016. Fresh produce-related outbreaks constitute not only one of the greatest numbers of total illnesses, but also the largest average number of illnesses per outbreak.

In this review, the chemical, biological, and physical hazards associated with food safety in fresh produce will be described, as well as transmission routes, symptoms of contamination, and detection methods.

**Chemical contaminants on fresh produce**

Chemical contaminations involve the presence of chemicals in a food matrix where their concentration exceeds safe level. Chemical hazards are one of the main causes for foodborne disease outbreaks derived from a variety of sources from harvest to processing. Contamination of fresh produce can occur from soil, sewage, external surfaces, and live animals. Chemical hazards have been considered one of the most serious consumer concerns because of long-term carcinogenic potential from chemical contaminant residues. The chemical hazards on fresh produce are classified as agro-chemical, natural toxins, and heavy metals (Table 1). Symptoms caused by chemical contaminants span from mild gastroenteritis to fatal cases of hepatic, renal, and neurological syndromes. In recent years, with industrial development and subsequent environmental pollution, foods contaminated by chemicals have become more serious issues. In Nigeria, 400 to 500 children have died annually due to lead poisoning caused by ingestion of foods contaminated with lead-containing soil and dust. According to the Foodborne Disease Surveillance System of the US and Puerto Rico, 257 chemical hazard outbreaks were reported between 2009 and 2015 including 1,024 illnesses and 5 deaths.

**Agro-chemical hazards**

**Pesticides**

Agricultural pesticides are commonly utilized in farming operations for fruits and vegetables to increase the viability and quality of fresh produce. However, if pesticides are not degraded, these chemical hazards will penetrate plant tissues and can persist into processed products like juices and jams. Another important aspect of pesticides is their transmission to animals or water sources in different ways. Pesticides in fresh foods may result in a number of different health problems such as kidney damage, congenital disabilities, reproductive problems and cancer. In addition, the accumulations of pesticides in the human body may contribute to metabolic degradation. Moreover, pesticides can produce a variety of transformational products (TPs), which can be much more toxic than the parent compounds.

**Fungicides**

Fungicides are widely used chemical agents that provide protection to crops and seedlings in the field and during the storage of foods. Fungicides are unlikely to cause frequent or severe systemic poisoning since a large number of fungicides have low bioavailability and toxicity in mammals. However, the possibilities of chronic health problems and environmental effects have been identified and recently brought to public concern. Dichloran (nitro derivative), flutriafol (triazole), O-phenylphenol (biphenyl), prochloraz (imidazole) and tolclofos methyl (thiophosphate) are representative chemical structures available commercially for different crops which have already been proven to have carcinogenic effects in animal and human. To minimize potential hazards, the European Union Commission and the US Environmental Protection Agency (US EPA) established maximum residue limits (MRLs) in fruits and vegetables to ensure that fungicides are not present at certain levels that may influence health threats to the public.

<table>
<thead>
<tr>
<th>Chemical hazard</th>
<th>Subcategory of chemical hazard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agro-chemicals</td>
<td>Pesticides, fungicides, and herbicides</td>
</tr>
<tr>
<td>Natural toxins</td>
<td>Mycotoxins ( aflatoxin, ochratoxin A, citrinin, and patulin) and plant toxins (cyanogenic glycosides, alkaloids, trypsin inhibitor, and hydrazine)</td>
</tr>
<tr>
<td>Heavy metals</td>
<td>Mercury and cadmium</td>
</tr>
</tbody>
</table>
Herbicides

In order to control weeds in crops and increase the yield and quality of produce, modern agricultural production systems rely on the use of herbicides\(^24\). In general, most herbicides are soil-applied agro-chemicals and their toxicity to mammals is low. Despite the low toxicity, herbicides which are widely used in agriculture, have found their way into public concern due to the presence of their residues identified in foods\(^25\).

Natural toxins

Mycotoxins

Mycotoxins are secondary metabolites produced by fungi. Despite efforts to control fungal contamination, fungi are ubiquitous in the environment and found in most fresh produce\(^26\). *Penicillium*, *Fusarium* and *Aspergillus* are representative fungi that produce mycotoxin compounds which have a toxigenic impact on food safety. There are more than 1,000 varieties of mycotoxins already reported as causes of several health issues\(^27,28\) from milder symptoms including diarrhea, abdominal pain, or other gastrointestinal problems to more severe complications like cancer\(^27\). Among varieties of mycotoxins, most of the toxins occur as aflatoxins, ochratoxin A, and patulin\(^29\). Mycotoxins can contaminate fresh produce via many different routes from pre-harvest to storage since fungi easily colonize crops and contaminate them during harvest or post-harvest stages\(^29\). Mycotoxins in foods may be partially degraded by physical and chemical methods, as well as irradiation. For example, 54% of patulin can be removed from vegetables or fruits through washing steps. Furthermore, washing rotten or damaged apples results in a 10-fold decrease of the concentration of patulin\(^29\).

Plant toxins

Plant toxins, or phytoxins, are secondary metabolic compounds produced from plants that play a role in defense mechanisms against insects and fungi. Plant toxins induce a range of negative health effects in humans, from inhibiting an uptake of specific nutrients to carcinogenic properties\(^31\). Represented plant toxins include potato glycoalkaloids and toxins produced from herbs, such as pyrrolizidine alkaloids and anisatin in certain varieties of star anise\(^25\). Pyrrolizidine alkaloids are one of the most common phytotoxins having carcinogenic, hepatotoxic, genotoxic and teratogenic properties\(^25\). Despite the serious health impact of phytotoxins, regulations are not well established in comparison with other chemical hazards such as mycotoxins or herbicides\(^25\). Until now, there have been a lack of routine methods for the determination of plant toxins due to over 200,000 varieties of secondary plant metabolites reported\(^25\).

Heavy metals

Certain metals (e.g., iron, zinc, manganese and copper) are required micronutrients to maintain proper health. Despite the health benefits of metals, excessive accumulation in animals may induce serious health problems due to the low biodegradability and concentration through the food chain\(^34\). Industrial processing, pesticides or chemical fertilizers, mining, and automobile exhaust are the main sources of heavy metals in the environment and these compounds are easily transmitted to fresh produce\(^35\). Heavy metals can seriously deplete specific nutrients in the body, which can lower the natural immunological defenses, impair psycho-social facilities, and cause intrauterine growth retardation. Heavy metal consumption is also associated with malnutrition, and reports have claimed that heavy metals increase the rates of gastrointestinal diseases and cancer\(^36\). Among various heavy metals, lead (Pb), cadmium (Cd), and mercury (Hg) are the most probable causes of the heavy metal-related diseases\(^37\).

Detection of chemical hazards on fresh produce

US and European governments attempt to define one standard technique to detect chemical hazards from foods, however establishing one standard method to analyze a

| Table 2. Detection methods for chemical hazards on fresh produce |
|---|---|---|
| Category | Detection method | Reference |
| Pesticides | Mass chromatography (MS), tandem-MS, liquid chromatography-mass chromatography(LC-MS), and liquid chromatography-time of flight mass chromatography (LC-TOF-MS). | 18, 39 |
| Fungicides | Gas chromatography (GC), GC-capillary electrophoresis(CE), LC, and LC-CE | 40-42 |
| Herbicides | GC and GC-CE | 43 |
| Mycotoxins | Thin layer chromatography (TLC), High performance liquid chromatography (HPLC), and GC | 44 |
| Plant toxins | LC - MS, LC- MS/MS, and TOF-MS | 45-47 |
| Heavy metals | Inductively coupled plasma-mass spectrometry (ICP-MS), atomic absorption spectroscopy (AAS) MS potentiometric methods, X-ray fluorescence spectroscopy (XR-FS), noble metal nanoparticles (NMPs), quantum dots (QDs), and magnetic nanoparticles or nanotube | 34, 48-50 |
variety of chemical contaminants is very difficult since all chemical hazards have diverse structures and characteristics\textsuperscript{24,38}. Chemical hazard detection methods vary based on the target compounds (Table 2). Classical chemical contamination detection procedures use solvents to extract target analytes. The solvent extraction method is a reliable test, however it is time-consuming, requires a trained technician and large volumes of solvent which produce a large amount of waste\textsuperscript{18,24}. In order to reduce chemical waste and analysis time, a chromatography method has been developed to identify chemical compounds. In the past few years, nanostructured materials such as NMPs, QDs and magnetic nanoparticles or nanotubes have been invented for simple, highly sensitive and selective assessment compared with conventional protocols\textsuperscript{34}.

**Biological contaminants on fresh produce**

Fresh produce such as whole or fresh-cut fruits and vegetables are important dietary constituents, as they contain high levels of vitamins and minerals\textsuperscript{51}. Due to these health benefits, most fresh vegetables and fruits receive minimal processing and are usually consumed as raw. Pathogens can easily contaminate fresh produce, leading to serious health problems\textsuperscript{27,29}. In spite of considerable protection from microbial contamination by low pH, skins, and waxy coatings on fresh produce, the high levels of nutrients and moisture present in fresh produce can create a suitable environment for pathogens\textsuperscript{27,29}. Rupturing plant tissues through peeling or cutting releases nutrients and encourages growth of unwanted microorganisms\textsuperscript{52}. Microbial contaminations easily occur during the different stages from farm to consumer including production, harvest, processing, storage, transportation and can be introduced from environmental, animal or human sources\textsuperscript{53}. Symptoms of foodborne illness range from mild complications as abdominal pain, diarrhea, fever, headaches, vomiting and muscle aches\textsuperscript{11}, to severe health issues such as autoimmune complications, bloody diarrhea, enterotoxin poisoning, meningitis, septicemia, hemorrhagic colitis, hemolytic uremic syndrome (HUS) and miscarriage in pregnant women\textsuperscript{77}. Foodborne pathogens frequently associated with a consumption of fresh produce include viruses (norovirus), parasites, and bacteria (*Salmonella, Escherichia coli* O157: H7, *Shigella, Listeria monocytogenes, Bacillus, Campylobacter*, and *Clostridium*)\textsuperscript{9}. Fig. 1 indicates the number of bacterial outbreaks, illnesses and deaths attributed to fresh produce from 2006 to 2016 according to the National Outbreak Reporting System (NORS) from the CDC web database\textsuperscript{9}. There are a diverse number of pathogens in existence, however this review focuses on the most common illnesses caused by the bacterial foodborne pathogens *Salmonella, E. coli* O157:H7, *Shigella, Listeria* and *Bacillus cereus* as well as norovirus and parasites. The possible routes and sources of contamination in fresh fruits and vegetables are diverse, and the exact mechanisms for introduction of pathogens into fresh produce are still unclear\textsuperscript{44}. Despite intensive efforts to prove accurate pathways of contamination by biological hazards, the routes to fresh produce contamination in the wild are varied from livestock and other sources such as surface water, soil, and ground water (Fig. 2)\textsuperscript{52}.

![Fig. 1. Incidence of biological foodborne (a) outbreaks, (b) illnesses and (c) death associated with fresh produce reported by the CDC in the US from 2006 to 2016.](image)
Bacterial contaminants on fresh produce

**Salmonella**

*Salmonella* is a Gram-negative, non-spore forming bacterium, and is included in the family Enterobacteriaceae. The *Salmonella* genus is composed of two species; *S. enterica* and *S. bongori*\(^5\). *S. enterica*, which are a main cause of gastroenteritis, is subdivided into hundreds of serovars. *Salmonella* can be easily found in the gastrointestinal tract of animals, from livestock to humans\(^50\). Previously, most people suspected salmonellosis was attributed to consuming contaminated poultry products, however an increasing number of outbreaks are associated with fresh produce in the US that can be traced back to bacterial contamination by *Salmonella*\(^55\). In addition, *S. enterica* has been found to easily colonize seeds, leaves, and fruits including watermelon, sprouts, tomatoes, mangoes and lettuce\(^54\). For example, from 2006 to 2016, 131 incidents of foodborne illness outbreaks of *Salmonella* related to vegetables and fruits were reported (Fig. 1)\(^7\). Approximately 60% of human salmonellosis cases reported by CDC were caused by four serotypes of *Salmonella*: *S. Typhimurium*, *Enteritidis*, *Newport* and *Heidelberg*\(^58\).

**Escherichia coli** O157

*Escherichia coli* (E. coli) is a Gram-negative, facultative anaerobe that commonly inhabits the gastrointestinal tract of mammals and belongs to the family of Enterobacteriaceae\(^59\). Though most strains of *E. coli* are harmless, several pathogenic strains have been identified that cause serious clinical problems in humans. Enterotoxigenic *Escherichia coli* (ETEC) is a group of pathogens that have the ability to colonize the small intestine in humans, where heat-stable (ST) or plasmid-encoded heat-labile (LT) enterotoxins are produced. Collectively, these organisms cause hundreds of millions of cases of diarrheal diseases each year, particularly in developing countries, with 300,000 to 500,000 estimated deaths of children annually\(^60\).

Six primary pathogenic groups of *E. coli* (enterotoxigenic, enteropathogenic, entero-invasive, enterohemorrhagic, entero-aggregative and adherent-invasive *E. coli*) have been documented. Of these six main pathogen groups, *E. coli* O157 is one of the most common causes of foodborne outbreaks. Pathogenic *E. coli* strains that belong to the Enterohemorrhagic group of *E. coli* (EHEC) are known for verocytotoxin-producing or Shiga-toxin-producing strains\(^61\). Outbreaks of *E. coli* O157 have been associated with lettuce, unpasteurized apple cider, cantaloupes, and sprouts\(^62\). According to a CDC report, an *E. coli* O157 outbreak linked to romaine lettuce in June 2018 infected 210 people in 36 different states and resulted in 5 deaths\(^63\).

**Shigella**

*Shigella* is a Gram-negative, facultative aerobic intracellular pathogen\(^64\). Species of *Shigella* are most frequently isolated from patients experiencing diarrhea. Between five to fifteen percent of all diarrheal patients worldwide are *Shigella* related\(^9\). *Shigella* is a member of the Enterobacteriaceae family which closely related to *E. coli*. Though *Shigella* and *E. coli* share very similar DNA sequences, they have remained separate species for clinical reasons\(^66\). The infective dose for *Shigella* is very low: only 10 cells of *S. dysenteriae* and 500 cells of *S. sonnei* can be infectious\(^67\). According to CDC reports (Fig. 1), a total of 7 outbreaks, 495 illnesses, and 1 death related to *Shigella* have occurred from 2006 to 2016. In addition, *Shigella* is easily transmissible through person-to-person contact\(^68\).

**Listeria monocytogenes**

The genus *Listeria* consists of Gram-positive, facultative, non-spore forming bacteria. *Listeria* is represented by seven species: *Listeria monocytogenes*, *innocua*, *welshimeri*, *grayi*, *seeligeri*, *ivanovii* and *marthii*. Among the seven species, *L. monocytogenes* is the primary human pathogen and causes a life-threatening disease known as listeriosis\(^69\). *L. monocytogenes* represents a serious threat to the food industry because it can survive conventional food processing conditions, such as high salinity, acidity, refrigeration temperatures and low water activity. Though *Listeria* is unable to survive pasteurization temperatures\(^59\), most fresh produce are provided without heat treatment, leading to serious listeriosis outbreaks. In 2015, listeriosis outbreaks occurred in multiple states in the US. All of the patients were over 19, and one of them died\(^69\).

**Bacillus cereus**

*Bacillus cereus* is a Gram-positive, spore-forming, motile, aerobic rod that can also grow in anaerobic conditions. *B.
cereus easily grows within a temperature range of 10 to 50°C with an optimum between 28 and 35°C, however members of Bacillus survive in a wide variety of environmental conditions due to their ability to form endospores, which are resistant to dehydration, heat, and other physical stresses\(^7\). B. cereus also has the ability to form biofilms on stainless steel\(^\text{72}\), demonstrating an increased level of resistance against environmental factors that typically prevent bacterial growth. It also has the ability to contaminate nearly any agricultural product due to its abundance in soil and the ability to form spores and biofilms\(^7\). B. cereus is readily found on a variety of food products, including vegetables, fruits and grains\(^7\).

**Non-bacterial contaminants on fresh produce**

**Norovirus**

Foodborne viruses are present in high numbers in human feces. The two types of viruses most frequently implicated in foodborne outbreaks are noroviruses (NoVs) and hepatitis A viruses (HAVs)\(^7\). Human NoVs are one of the primary sources of viral gastroenteritis around the world, and are the main cause of foodborne illness in Europe\(^2\) and the US\(^\text{70}\). Fresh produce has been identified as a common vehicle for the transmission of foodborne viruses\(^7\). According to a comprehensive survey of outbreaks identified with fresh produce sources in the US from 2006 to 2016 (Fig. 1), NoVs are the second largest cause of outbreaks (32.8%). Aside from murine strains, NoVs cannot be cultivated in vitro, which prevents classification into distinct serotypes and screening from plating\(^7\).

**Parasites**

Various fruits and vegetables have been identified as vehicles for the transmission of parasites. Parasites associated with vegetable- or fruit-borne outbreaks include helminths such as *Fasciola hepatica*\(^7\), *Ascaris lumbricoides* and *Ascaris suum*\(^7\). Over 1.5 billion people worldwide have been diagnosed with parasitic infection by at least one species of soil transmitted helminth (STH)\(^7\). In many developing countries, the use of inappropriate treated wastewater to irrigate vegetables has contributed to contamination with pathogenic parasites. Poor hygienic practices during production by food handlers also contributes to the number of cases of parasitic infection\(^7\). The lack of a globally acceptable methods for the detection and quantification of STH eggs in environmental samples poses a challenge for comparative assessments of egg concentrations in different sample matrices\(^7\).

**Symptoms of biological hazards on fresh produce**

Biological contaminations of fresh produce induce a variety of symptoms to consumers from mild diarrhea to life-threatening health issues depending on the type of pathogens (Table 3). For instance, the symptoms of salmonellosis, including abdominal cramps, diarrhea, fever, headache, nausea, and vomiting\(^8\), usually develop 8 to 72 h after consumption of contaminated food, and may last from four to seven days. Arthritis-like symptoms may follow three to four weeks after onset of acute symptoms\(^8\). The typical symptoms of shigellosis are also similar with salmonellosis which include bloody diarrhea, abdominal pain, fever, and malaise\(^7\). When it comes to *E. coli* O157, the most common etiological problem is hemolytic uremic syndrome (HUS). The virulence level of *E. coli* O157 strains ranges from asymptomatic colonization within the body to potentially lethal HUS disease. Diarrhea-associated HUS is often attributable to Shiga toxin (Stx) produced by pathogenic *E. coli*\(^8\). Stx-producing *E. coli* (STEC) was named because of the similarity of the toxin generated by the stxl gene to

<table>
<thead>
<tr>
<th>Table 3. Symptoms of biological hazards on fresh produce</th>
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<tbody>
<tr>
<td>Category</td>
</tr>
<tr>
<td>Common symptoms</td>
</tr>
<tr>
<td>Arthritis-like symptoms</td>
</tr>
<tr>
<td>Pathogen specific symptoms</td>
</tr>
<tr>
<td>Anorexia, vertigo and fecal incontinence</td>
</tr>
<tr>
<td>Mild symptoms like eosinophilia to life-threatening health issue depending on the kinds of parasites</td>
</tr>
<tr>
<td>Parasites(^7,9)</td>
</tr>
</tbody>
</table>
the Stx produced by *Shigella dysenteriae*. STEC have an *eae* gene that translates for intimin, which adheres to the outer membrane protein and enables the bacterium to enter the intestinal wall of a host. After the invasion, Stxs which come from STEC can cause life-threatening health complications, especially to infants and the elderly. Additionally, *L. monocytogenes* leads to two types of listeriosis: non-invasive gastrointestinal listeriosis and invasive listeriosis. Both invasive and non-invasive listeriosis are dangerous for elderly and immunocompromised adults, as *Listeria* can manifest as septicemia or meningoencephalitis. Invasive listeriosis is especially dangerous to pregnant women, since perinatal listeriosis is a serious threat to the unborn child and can lead to miscarriage. Additionally, *B. cereus* causes two types of food poisoning that result from different types of toxins, emetic- and entero-toxins, which lead to vomiting and diarrhea, respectively. Emetic toxin syndrome is defined by nausea, vomiting, and abdominal cramping, which occur 1 to 5 h after ingestion of the contaminated food. The illnesses are self-limiting, and recovery usually occurs within 6 to 24 h. Hospitalization is occasionally required due to excessive vomiting, and fatality is rare. The onset of the diarrheal syndrome generally ranges from 8 to 16 h after exposure, and the symptoms resolve themselves in 12 to 14

### Table 4. Detection methods for biological hazards on fresh produce

<table>
<thead>
<tr>
<th>Methods</th>
<th>Assay</th>
<th>Properties</th>
<th>Pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Media/microscopy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culturing method</td>
<td>Culturing on selective media</td>
<td>The only culturable pathogen can analyze in selective media, which takes more than 24 h to 48 h.</td>
<td><em>Salmonella, E. coli O157, Shigella, Listeria monocytogenes, and Bacillus cereus</em></td>
</tr>
<tr>
<td>Microscopic method</td>
<td>Microscopical identification</td>
<td>Separation and concentrations of parasites and quantification through a microscope, but less reliable.</td>
<td>Parasites</td>
</tr>
<tr>
<td><strong>Nucleic acid-based</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymerase chain reaction (PCR)</td>
<td>Multiplex PCR</td>
<td>Identification more than one species target at a time through amplified specific genes.</td>
<td>All pathogens</td>
</tr>
<tr>
<td>Genetic subtyping</td>
<td>Reverse transcription PCR (RT-PCR)</td>
<td>Reverse transcript from RNA to DNA with reverse transcriptase to read sequence from RNA</td>
<td><em>E. coli O157, and NoVs</em></td>
</tr>
<tr>
<td>Microarray</td>
<td></td>
<td>Thousands of specific DNA sequences to be detected on a small glass or silica slide at the same time.</td>
<td><em>Salmonella, Shigella, and E. coli O157</em></td>
</tr>
<tr>
<td><strong>Immunological-based</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enzyme immunology</td>
<td>Enzyme-linked immunoabsorbent assay (ELISA)</td>
<td>Using natural binding affinity of antibodies to antigens. Antibody combined with an enzyme which can react with a substrate to make fluorescence.</td>
<td><em>Salmonella, E. coli O157, Listeria monocytogenes, and NoVs</em></td>
</tr>
<tr>
<td>Non-enzyme immunology</td>
<td>Immunochromatographic</td>
<td>Simple paper-based devices intended to detect the target analytes in a liquid sample (matrix) without the need for specialized and costly equipment</td>
<td><em>Salmonella, E. coli O157, and NoVs</em></td>
</tr>
<tr>
<td><strong>Biochemical analysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metabolic compounds analyze</td>
<td>Chromatography</td>
<td>Analyze the metabolite compounds through chromatography to identify the pathogen</td>
<td><em>Salmonella and Listeria monocytogenes</em></td>
</tr>
<tr>
<td><strong>Biosensor</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biosensor</td>
<td>Biosensor</td>
<td>Recognition signal is generated when specific analytes (immunology or nucleic acid-based parameters) bind to the biological recognition element</td>
<td><em>Salmonella, E. coli O157, and Shigella</em></td>
</tr>
</tbody>
</table>
h). Enterotoxins are proteins causing cytotoxicity marked by fluid accumulation in the ligated ileal loop, dermonecrosis, and lethality in mice. Lastly, the most common acute symptoms of NoVs infection are diarrhea and nausea, followed by vomiting, abdominal pain, fever and fecal incontinence. Various non-specific symptoms are also reported, such as anorexia, thirst and lethargy, headache and vertigo, listed in order of decreasing prevalence. Acute symptoms typically subside after three to four days of illness, whereas non-specific symptoms can persist for up to 19 days.

Detection methods for biological hazards on fresh produce

In order to prevent foodborne illnesses, detection and identification of the specific foodborne pathogen is important and numerous methods have been previously published (Table 4). Conventionally, bacterial pathogens such as Salmonella, E. coli, Shigella, Listeria monocytogenes and B. cereus can be identified with culture-dependent methods. Detection of a specific microbial species from a mixed culture using selective media can be accurate however, it usually requires pre-enrichment steps and culturing methods may take more than 1 to 2 days to obtain results. Furthermore, NoVs are impossible to culture on the media. In recent years, molecular microbiological methods such as PCR, rep-PCR and microarray have also been developed. In theory, DNA from a single bacterial cell can be amplified through PCR within 2 h, which is rapid compared to previously described methods. Immuno-based assays have also been created to detect pathogens through specificity of the antigen-antibody reaction, though immunological methods are less sensitive compared to the nucleic acid amplification and cross reactivity with other closely-related species is also a concern.

With regards to parasites, microscopic methods are traditionally used to identify and quantify eggs of parasites, however newer techniques have been developed. The advent of genomic sequencing and the wealth of data generated by it have markedly increased the feasibility of developing polymerase chain reaction (PCR)-based methods as diagnostic tools for parasites. Another option for detection is analysis of metabolite compounds emitted by pathogen-contaminated food with GC, GC-MS or GT-TOF-MS. Despite the range of detection methods, current protocols are neither fast nor reliable enough to be used in emergency situations.

Physical hazards on fresh produce

Physical hazards result from the introduction of unwanted foreign materials into food which cause physical damage to consumers. Physical hazards may involve a wide range of objects, as are listed in Table 5. The primary sources of physical hazards may include the manufacturing environment, raw materials and ingredients, plant equipment, contractors, and employees. In order to detect any contaminants in-line, automated vision systems, X-ray technology, filters and sieves are required. Employee training programs and Good Manufacturing Practices (GMPs) are also included in a physical hazard control program. More effective control programs to prevent physical hazard contamination can be achieved by support from vendors and suppliers, as the magnitude of the potential threat will dictate the appropriate control strategies. A vision system or X-ray inspection may be necessary for the control of glass contamination, while a properly calibrated metal detector may be effective against both ferrous and nonferrous metal contaminants. Human inspection may be required for the detection and removal of dangerous pits and stems.

### Table 5. Potential physical hazards in the food industry

<table>
<thead>
<tr>
<th>Material</th>
<th>Injury potential</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass</td>
<td>Cuts and bleeding</td>
<td>Bottles, jars, and covers</td>
</tr>
<tr>
<td>Wood</td>
<td>Cuts, infection, and choking</td>
<td>Field sources, boxes, and building materials</td>
</tr>
<tr>
<td>Stones, gravel</td>
<td>Choking and breaking teeth</td>
<td>Fields and building materials</td>
</tr>
<tr>
<td>Insulation</td>
<td>Choking, long-term if asbestos</td>
<td>Building materials</td>
</tr>
<tr>
<td>Plastic</td>
<td>Choking, cuts, and infection</td>
<td>Packaging, pallets, and equipment</td>
</tr>
<tr>
<td>Personal effects</td>
<td>Choking, cuts, and break teeth</td>
<td>Employees and customers</td>
</tr>
</tbody>
</table>

Summary

Fresh produce is easily contaminated from farm to table by chemical, biological and physical hazards. Among three different type of contaminants, agrochemical, natural toxins and heavy metal contaminants are representative of chemical hazards, which can be detected using solvents, chromatography and nano-techniques. The wide range of chemical structures makes it difficult to establish a single and standardized method to detect target compounds.

Furthermore, fruits and vegetables are a common source of foodborne pathogens, including bacterial pathogens (E. coli O157, Shigella, Salmonella, Listeria monocytogenes and Bacillus cereus) and non-bacterial pathogens (norovirus and parasites). In order to detect food pathogens and prevent foodborne illnesses, conventional culture dependent methods...
have been utilized. However, culturing methods require significant time and effort, and some pathogens, such as noroviruses, are unculturable. To detect foodborne pathogens derived from fresh produce more efficiently, immunological and nucleic acid-based methods should be applied. Finally, physical hazards including glass, plastics and stone contamination of fresh produce result in serious injuries such as choking, broken teeth and bleeding. To avoid serious injuries from physical hazards, vision systems or X-ray inspections are recommended in-line for use by well-trained employees.

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References

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Potential Contamination Sources on Fresh Produce Associated with Food Safety


