Microbial update fresh produce

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The consumption of fresh produce increases year on year. There are many reasons for this but key ones are current advice on healthy eating, where the 'five portions per day' message is widely known, and the expansion of the market place, where fresh produce is available throughout the year (not just in the local growing season), where more exotic fruits and vegetables are in stores and of course produce is available in an 'easy to use' or 'ready to eat' format.

It has been estimated that over 400 million packs of prepared salad are sold in the UK every year, and this number increases every year.

There is no doubt that the consumption of increasing amounts of fresh fruits and vegetables is beneficial to the health of the consumer. Microbiologically, however, there are some real challenges to the production of fresh produce.

Growth of fresh produce

Produce is generally grown in soil and outdoors. Both scenarios will allow a very free access to a whole range of micro-organisms to the crop. Soils will contain a very high microbiological microflora and will be in direct contact with parts of the plant for its whole life cycle, whilst the outside environment is almost uncontrollable from a microbiological viewpoint. The impact of soil flora and the external environment, on the microbiology of a plant will vary depending

on a variety of conditions. The major ones include:

- The closeness of the plant to the soil. Lower areas of a plant may be expected to have a greater chance of contamination with soil flora than higher parts.
- Rainfall. Whilst rain is often required to provide water for plant growth, it will also cause soil splash onto lower parts of the plant.
- Irrigation. In driers areas or at particular parts of the growing cycle, it may be necessary to irrigate the plants. This provides a number of potential microbiological challenges. Firstly, the water used will not usually be from a mains drinking supply. It may come from farm reservoirs, or be extracted from streams or rivers, so the microbiological quality of the water may impact on the microbiology of the plant. Secondly, the way the water is provided to the plant may affect its microbiology. If it is sprayed on from the top, it has great contact with all of the upper external surfaces, and will deposit any organisms contained in the water on these areas, additionally the 'splash issue' noted with rain, will also occur. If the water is drip irrigated onto the soil directly under the plants, then it will not contact upper plant surfaces, and splash is very unlikely.
- Plant/soil nutrition. It is common to place fertilisers on or in soils to give a better plant growth. There has been a move away from the use of artificial fertilisers to the use of natural manures. In their 'fresh state' manures contain all of the faecal organisms from the animal from which they came. The transformation of faeces into manure should include a period under suitable conditions that allow sufficient temperatures to develop, to eliminate potential pathogens. Additionally, after adding manures to soils, it is unusual to grow produce immediately, a period of time is left to allow a natural soil bacterial flora to develop.
- The need to obtain common crops on a year round basis and the consumer demand for 'exotic' fruits and vegetables, has resulted in a worldwide sourcing of produce items. This means that some items easily available from stores, will have been grown in areas where the natural microbiology is very different to that at the point of consumption, and where standards of hygiene may be different.
- Animals and people. Field growth gives every opportunity for animals (birds, rodents, wild mammals, even domestic pets such as dogs) to come into contact with the produce. This can result in animal faecal material being deposited onto growing plants. Additionally, much fresh produce is hand picked, so that the hygiene of the pickers is an important issue to consider. Many growers now install field toilets complete with good hand wash facilities to improve hygiene in this area.
- Some crops are now grown in hydroponic conditions. This considerably reduces contamination issues relating to the environment, but such material will still require picking, thus picker hygiene is still important.

- Once picked, produce will have to be transported to pack houses/processing areas where the material will be portioned and packed ready for the consumer. Vehicles and containers used for this need to be kept clean and should not be used to transport other items (dirty items, agricultural equipment, animals etc).

All of these potential points of microbiological contamination may be of reduced importance if the plant or crop is to be cooked before consumption. The cooking

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process will usually be effective at eliminating any potentially pathogenic organisms, making the food safe to eat.

However, much fresh produce (salad crops, fruits etc) are eaten in a raw state, and here the presence of contaminating micro-organisms, particularly pathogens, could cause problems for the consumer.

Microbiology

As fresh produce is a natural material, it will always have its own natural microflora. This means that it would not be unusual to find total viable counts on ready to eat fresh produce of over 10^6/g. This is perfectly acceptable.

The types of organisms will generally include fluorescent pseudomonads, Enterobacter spp., Klebsiella spp. Serratia spp., Flavobacterium spp., Xanthomonas spp., Chromobacterium spp., Alcaligenes spp. and Leuconostoc spp., and yeast and mould species including Cryptococcus, Candida, Rhodotorula, Sporobolomyces, Cladosporium, Aureobasidium, Aspergillus, Penicillium, Phoma, Botrytis, Fusarium, Epicoccum and Geotrichum.

It should be noted that it is common to find Enterobacteriaceae on fresh produce. They form part of the normal flora of plants and their presence should not be looked upon in the same negative way as a high Enterobacteriaceae count on, for example, a ready to eat meat product.

The natural flora of fresh produce are an essential part of the plant, indeed some researchers have hypothesised that the presence of high levels of generally harmless organisms could help to inhibit the development of possible spoilage organisms or even human pathogens on such material.

Role of pathogens

Of course, no discussion on fresh produce microbiology would be complete without some discussion of the role of pathogens in these materials. As detailed previously, the way produce has to be grown makes much of it open to potential contamination from the soil or organisms originating directly from animals, including a range of potential human pathogens.

There has been considerable research concerning the potential for plants to take up pathogens directly from the soil into the roots. There is concern that if this could occur, the 'internalised' pathogens would be very difficult to treat or remove in any way.

There is little conclusive data to confirm that internalisation from the root uptake occurs, however it is certainly possible that micro-organisms could become trapped in damaged plant tissue or plant structures like stomata, and thus be very difficult to remove.

Good reviews of the range and types of

pathogens that can be found on fresh produce are available, but can include bacterial pathogens: Listeria monocytogenes, Clostridium botulinum, Shigella, E. coli O157:H7, Salmonella, Staphylococcus aureus, Bacillus cereus, Vibrio cholerae, campylobacter; viral pathogens: Hepatitis A and Norovirus; and Protozoan pathogens: Cryptosporidium parvum, Giardia and Cyclospora cayetanesis.

In addition to contamination of the growing plant, seeds are known, on occasion, to be contaminated with human pathogens. This can be an issue if the seed is to be eaten directly, or made into a ready to eat product (there have been salmonella contamination issues and outbreaks of salmonellosis, linked to sesame seeds and sesame products such as tahini and houmous, and to germinated alfalfa seeds consumed as sprouted seeds).

There have been several well documented outbreaks of illness throughout the world, linked to consumption of raw fruits and vegetables.

These include salmonella from lettuce, tomatoes, bean sprouts and melons; E. coli O157:H7 from spinach, radish sprouts and fresh unpasteurised apple juice (apple cider in the USA); listeria from lettuce, shigella from lettuce, and Clostridium botulinum from vegetable juice.

The range of pathogens causing illness is large. On many occasions the conditions in or on the food would appear to be antagonistic to the growth of the organism, however survival and consumption of the pathogen is sufficient to cause illness in many cases. Some types of fresh produce will offer conditions that allow growth, and this can be a particular problem.

The conditions used to sprout seeds require moisture and some warmth, ideal for microbial growth. In fruit juices the pH is usually low enough to stop growth of most pathogens (the organisms may survive), however in many vegetable juices the pH will be higher and be in a range in which microbial growth can occur. Such products require care in setting shelf life and processing/storage conditions.

In the production of ready to eat, raw fresh produce there is no step that will eliminate pathogens. Any form of process that could do this will usually change the organoleptic properties of the product to such an extent that it would become unacceptable to the consumer.

The only step open to producers of raw fresh produce to reduce microbial loading is washing. Washing will remove dirt and debris from the surfaces of produce, and has some effect at removing surface microflora. Instead of relying on washing solely with water, most producers of ready to eat fresh produce add forms of sanitiser to the water used for washing.

This helps to keep the microbial loading low, and helps to decrease the risk of pathogen presence in these products.

Over recent years there has been much discussion on the use of chlorine in vegetable washing. This has led to considerable research both into chlorine washing itself and its effects and into alternative ways to help remove microflora from fresh fruits and vegetables.

Produce washing

Until recently, chlorinated water was the most common form of wash agent used to reduce the microbial loading of fresh produce. The most common form of chlorine used is sodium hypochlorite, which reacts in water to form different types of chemical component such as sodium hydroxide (NaOH) and hypochlorous acid (HOCl).

It is the hypochlorous acid (free chlorine) that is the active biocide.

This free chlorine can bind with other compounds such as organic matter; this product is then termed total chlorine. The amount of hypochlorous acid formed is pH-dependent and more hypochlorous acid remains in solution at pH 5 than at pH 7.

If too much acid is present, then this can corrode the washing tanks and it is therefore best practise to control chlorine solutions at pH 7.0, where 78% of the hypochlorous acid remains in solution and this achieves a good decontamination effect.

The natural tendency of chlorine solution is to be alkaline and it is best practice to ensure that washing systems are maintained

at pH 7 by the addition of citric acid. It must be noted that monitoring total chlorine gives no information on the disinfecting power of a solution.

In order to monitor this, free chlorine must be monitored. It is thought that most of the biocidal activity occurs within the first few minutes of washing and increasing concentration above a few hundred ppm does not increase the biocidal effect.

Washing fresh produce in chlorinated water can reduce microbial levels by 10 to 100-fold, as long as the washing is carefully controlled.

Washing studies at Campden BRI have shown that a 10-fold (1-log) reduction in salmonella, E. coli and listeria levels can be achieved by washing inoculated lettuce in water containing 100ppm free chlorine for five minutes.

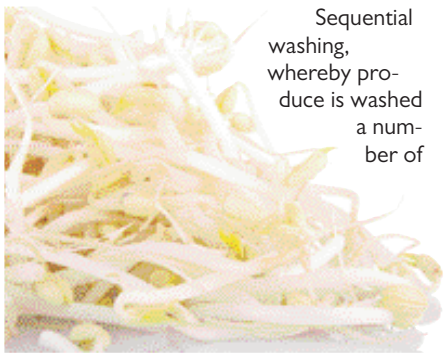
Over the last decade there has been an increasing concern of the potential for formation of harmful by-products such as organochlorine, when fresh produce is washed in chlorine containing wash waters. Whilst there is no real consensus on the presence or effects of these chemicals in washed produce, it has initiated research into alternative sanitisers that could be used.

Alternatives to chlorine

Current popular alternatives to chlorine washing include chlorine dioxide and organic acids. Chlorine dioxide is considered a useful alternative to hypochlorite as it is not affected by pH and does not react with organic matter to the same degree as hypochlorite.

Chlorine dioxide can be unstable and requires on-site generation, but stabilised (liquid forms) are now commercially available. There is also a trend to move towards the use of organic acids for the washing of fresh produce. Some commercially available wash agents containing mixes of organic acids are available and some of these can be used to wash organic produce.

There are many other techniques that are being investigated as potential alternatives to chlorine; these include: UV, ultrasound, ozone, irradiation, electrolysed water, peracetic acid, hot water, bio control agents and natural compounds like essential oils. With many of these it is too early to say whether widespread commercial use will ever occur.



Sequential washing, whereby produce is washed a number of times with either the same wash agent or different agents, is also being studied and may have some added benefits to microbial reduction in some cases.

With any washing system, it is of the greatest importance to ensure that a proper validation of the washing system is done before it is used commercially, and that the systems used commercially are monitored and controlled. In most cases, with ready to eat fresh produce, the washing step is the only point in the production process where the level of micro-organisms can be controlled, as such it is of the greatest importance.

Sprouted seeds

No current article on fresh produce would be complete without some mention of the microbiological risks associated with sprouted seeds. Seeds themselves will usually be considered a microbiologically stable material.

They are dry and whilst they may have a microbiological flora, it will be unable to grow due to the low water activity.

Undoubtedly, however, seeds will contain a rich microbiological flora which will originate from the growing environment and may include organisms from soil, fertilisers, irrigation water, wild animals, pickers and the transportation/distribution system.

There is a risk that some of these organisms could be human pathogens. If we move from the seed itself to the 'sprouting' process, this will usually require seed irrigation and an elevated temperature, ideal conditions to allow the resident flora on the seed to grow.

Research has indicated that any pathogens present on seeds could increase in numbers by 100 to 1000 fold during the first 24 hours of germination.

Outbreaks of food poisoning have been associated with sprouted seeds for decades. In 1973 soy, mustard and cress was associated with a small outbreak of Bacillus cereus poisoning. Since then outbreaks of yersiniosis, salmonellosis and verocytotoxin producing E. coli (VTEC) poisoning have been associated with sprouted seeds. Perhaps the largest occurred in Japan in 1996 where estimates suggest between 6000 and 9000 people were affected with E. coli O157 food poisoning.

The latest reported association between sprouted seeds and a food poisoning outbreak began in Germany in May 2011, and is linked to an unusual serogroup of VTEC, O104.H4. It has affected around 4000 people with a severe food poisoning with approximately 25% of those infected having a kidney related symptom known as haemolytic uraemic syndrome (HUS).

Since the German outbreak, there have been further reports of this same E. coli serogroup causing a smaller outbreak in France, also potentially linked to sprouted seeds.

These recent issues have led to various agencies, such as the UK Food Standards Agency, recommending that consumers should not eat raw sprouted seeds but to cook them before eating.

Conclusions

There is no doubt that increasing the consumption of fresh fruits and vegetables is good for a healthy diet. We should all understand that as a raw product that is generally grown in an outside environment, fruits and vegetables will have a large and varied microflora on their outer surfaces. Some of these organisms may, on occasion, be human pathogens.

Understanding good agricultural practices should help reduce the instances when pathogens can contaminate fresh produce, and this combined with good washing practices and chilled storage during production, retail sale and domestic storage, will result in the production of a healthy nutritious and safe product. The recent outbreaks of E. coli food poisoning linked to sprouted seeds clearly show us the large problems that can occur from the production and consumption of pathogen contaminated produce, and the care needed to obtain seeds, germinate the seed and grow the plant, then harvest, transport, pack and distribute those items in a controlled way that minimises the potential for pathogen contamination and growth. ■

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