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Safety

Fungi and bacteria will infect fresh fruit and vegetables especially during prolonged storage. In some cases, the infection may be entirely superficial but would still make the crop either entirely unmarketable or at least reduce its commercial value. In other cases, they can cause it to rot completely. Some of these organisms can produce toxic metabolic products. Those produced by fungi are called mycotoxins; those produced by bacteria are called by the name of the organism that produces them.

Church and Parsons (1995) have dealt with legislation related to safety of fruit and vegetables in a detailed review. There is considerable legislation related to food sold for human consumption. In the United Kingdom for example, the Food Safety Act 1990 states that it is an offence to sell or supply food for human consumption if it does not meet food safety requirements. In the late 1990s, the British Government set up a Food Standards Agency, one of whose concerns is food safety.

The use of modified atmosphere packaging has health and safety implications. One factor that should be taken into account is that the gases in the atmosphere could possibly have a stimulating effect on microorganisms. Farber (1991) stated that while modified atmosphere packaged foods have become increasingly more common in North America, research on the microbiological safety of these foods was still lacking. The growth of aerobic microorganisms is generally optimum at about 21% O_2 and falls off sharply with reduced O_2 levels, whereas generally for anaerobic microorganisms, optimum growth is at 0% O_2 and falls as the O_2 level increases (Day 1996). With many modified atmospheres containing increased levels of CO_2, the aerobic spoilage organisms, which usually warn consumers of spoilage, are inhibited, whereas the growth of pathogens may be allowed or even stimulated which raises safety issues (Farber 1991). Hotchkiss and Banco (1992) stated that extending shelf life of refrigerated foods might increase microbial risks in modified atmosphere packaged produce in at least three ways:

- increasing the time in which food remains edible increases the time in which even slow growing pathogens can develop or produce toxin;
- retarding the development of competing spoilage organisms;
- packaging of respiring produce could alter the atmosphere so that pathogen growth is stimulated.

In the United States, the Food and Drug Administration's Bacteriological Analytical Manual sets out culture-based methods for detecting the presence of dangerous bacteria in food. Shearer *et al.* (2001) tested a polymerase-chain-reaction-based detection system for its sensitivity in detecting

Fruit and Vegetables: Harvesting, Handling and Storage, Volume 1, Third Edition. A. K. Thompson.

Salmonella enteritidis, *Escherichia coli* O157 : H7, *Listeria monocytogenes* and other *Listeria* species. The technique was tested on fresh alfalfa sprouts, green peppers, parsley, white cabbage, radishes, onions, carrots, mushrooms, leaf lettuce, tomatoes, strawberries, cantaloupe, mango, apples and oranges. Generally, the method was more sensitive than culture-based methods, but neither method was successful with low levels on any of the crops. However, this method allowed detection of *S. enteritidis*, *E. coli* O157 : H7 and *L. monocytogenes* at least 2 days earlier than the conventional culture methods.

Micotoxins

Many microorganisms produce secondary metabolic products that are toxic. Over 150 species of fungi have been shown to be capable of producing mycotoxins and new ones are constantly being found.

Aflotoxin

The most common of these mycotoxins is aflatoxin, which is produced by *Aspergillus flavus* and *A. parasitic* and has been shown to infect dozens of food products including groundnut kernels, coconut, wheat, rice, flour, dry beans and some leafy foods. Aflotoxins are highly toxic and carcinogenic. The presence of the fatty acid hydroperoxides, which can form in plant material either preharvest under stress or postharvest under improper storage conditions, correlates with high levels of aflatoxin production (Goodrich-Tanrikulu *et al.* 1995). Micotoxins can also be found in fresh fruit although it is much less common than in dried products. Singh and Sumbali (2000) found *A. flavus* infection in Indian jujube fruits (*Ziziphus mauritiana*) associated with decay and that approximately 86% of the *A. flavus* isolates were toxic. Exposure of cultures of *A. flavus* to methyl jasmonate vapour was shown to inhibit toxic production. The amount of aflatoxin produced depended on the timing of the exposure (Goodrich-Tanrikulu *et al.* 1995).

Patulin

The Guardian newspaper of 11 February 1993 reported that there was considerable public concern in the United Kingdom when apple juice in a supermarket was found to be contaminated with levels of the mycotoxin patulin (4-hydroxy-4H-furo[3,2-c]pyran-2(6H)-one). This toxin has carcinogenic properties and has a maximum permitted level of 50 μg L^{-1} in fruit juices. Its presence results from apples or pears that have been contaminated with *Penicillim patulum*, *P. expansum*, *P. urticae*, *Aspergillus clavatus* or *Byssochlamys nivea* and stored for too long before being processed. The toxin was found to occur in apple juice at levels 500–2500 μl L^{-1} if it was made from rotting apples (Steiner *et al.* 1999).

In a market survey in South Africa by Leggott *et al.* (2001), 8 of the 31 fruit juice samples had patulin with concentrations ranging between 5 and 45 μl L^{-1} with a mean of 10 μl L^{-1}. Of six whole fruit products, two samples were contaminated with 10 μl L^{-1} patulin, and of 10 infant fruit juices; six samples had patulin concentrations ranging between 5 and 20 μl L^{-1}, but infant fruit purees showed no detectable patulin contamination. In the Cote d'Ivoire, 8 out of 44 samples of fruit juice tested by Ake *et al.* (2001) were found to contain patulin, but a level of over 50 μl L^{-1} was found in only one traditionally manufactured sample. In 11 Australian apple and pear juices tested by Steiner *et al.* (1999), the patulin content was below the limit of 50 μl L^{-1}. In the apple juices, the patulin content was more than 20 μl L^{-1}, whereas in the pear juices, patulin could not be detected. Lavrik *et al.* (2000) showed that Sorbilen, which is an ethylene absorber, decreased storage decay and accumulation of patulin.

Edible mushrooms

As well as the fungi that infect fruit and vegetables, it is well known that a number of other fungi produce various types of poisons. The toxic mushrooms, or toadstools, may be as large as some of the edible mushrooms and some of the most poisonous species closely resemble edible species. Toxicity obviously depends on species, but can also be affected by the environment and growing conditions. Poisonous mushrooms are mostly members of the class Basidiomycetes, although some are Ascomycetes, such as the poisonous false morel (*Gyromitra esculenta*). The jack-o-lantern (*Clitocybe illudens*) is an orange yellow fungus of woods and stumps and glows in

the dark but superficially resembles the delicious edible chanterelle (*Cantharellus cibarius*). *Lactarius* has milky or bluish juice and this genus contains the edible *L. deliciosus* as well as several poisonous species. Most deaths attributed to mushroom poisoning result from eating members of the genus *Amanita*, especially the destroying angel *A. virosa* or the death cap *A. phalloides*. Sterry (1995) gives more details of toxic species, which includes many photographs and drawings. Other species that are poisonous, but may look like edible mushrooms, include:

Inocybe patouillardii
Inocybe fastigiata
Clitocybe dealbata
Cortinarius orellanus
Entoloma sinuatum
Hypholoma fasciculare

In the 5 years they studied, 1989, 1992, 1993, 1994 and 1995, there were 20–49 human cases of fungal poisoning seen annually at a hospital in Parma in Italy. The species responsible were:

Agaricus hortensis (2 cases)
Agaricus romagnesii (2 cases)
Agaricus xanthoderma (2 cases)
Agrocybe aegerita (1 case)
Amanita phalloides (17 cases)
Armillaria mellea (19 cases)
Boletus edulis (9 cases)
Boletus satanas (6 cases)
Calocybe gambosa (1 case)
Clitocybe candicantes (4 cases)
Clitocybe nebularis (9 cases)
Entoloma lividum (33 cases)
Hygrophorus penarius (1 case)
Hypholoma sublateritium (2 cases)
Leccinum scabra (4 cases)
Lepiota brunneoincarnata (2 cases)
Lepiota lilacea (1 case)
Leucoagaricus leucothites (2 cases)
Macrolepiota procera (1 case)
Omphalotus olearius (17 cases)
Pleurotus ostreatus (1 case)
Ramaria formosa (4 cases)
Russula olivacea (5 cases)
Xerocomus chrysenteron (1 case)

The reasons why toxicity varied could have been that some of these fungi could have been in poor condition or without adequate cooking when they were eaten (Bocchi *et al.* 1996, Uip *et al.* 1996).

Bacterial toxins

Escherichia coli

The coliform bacterium *E. coli* O157 : H7 produces toxins and has the ability to grow on salad vegetables. This was demonstrated in a study by Abdul Raouf *et al.* (1993), where raw salad vegetables were subjected to minimal processing and storage conditions simulating those routinely used in commercial practice. Behrsing *et al.* (2000) found that packaging vegetables in an atmosphere containing 3% O_2 and 97% nitrogen had no apparent effect on populations of *E. coli* O157 : H7, psychrotrophs or mesophiles. During storage for 14 days, populations of viable *E. coli* O157 : H7 declined on vegetables stored at 5 °C and increased on those stored at both 12 and 21 °C. The most rapid increases in populations of *E. coli* O157 : H7 occurred on lettuce stored at 21 °C. They found that an unknown factor or factors associated with carrots may inhibit the growth of *E. coli* O157 : H7. The reduction in pH of vegetables was correlated with initial increases in populations of *E. coli* O157 : H7 and other naturally occurring microflora. Eventual decreases in *E. coli* O157 : H7 in samples stored at 21 °C were attributed to the toxic effect of accumulated acids. Changes in visual appearance of vegetables were not influenced substantially by growth of *E. coli* O157 : H7, which in itself is a potential danger to consumers.

E. coli O157 : H7 is also a potential problem contaminant on fresh fruit. Dingman (2000) found that four of five apple cultivars tested (Golden Delicious, Red Delicious, McIntosh, Macoun and Melrose) inoculated with *E. coli* O157 : H7 promoted growth of the bacterium in bruised tissue independent of the degree of ripeness or whether they were harvested from the tree or collected as dropped fruit. When fruit was stored for 1 month at 4 °C before inoculation with *E. coli* O157 : H7, all five cultivars supported growth of the bacterium. Yu *et al.* (2001) found that two strains of *E. coli* O157 : H7 survived externally and internally on stored strawberries at 23 °C for 24 h and at 10, 5 and −20 °C for 3 days. The bacteria inside the fruit either survived as well or better than bacteria on the surface.

In order to control infections, hygiene and carefully handling must be paramount. Other supplementary methods have been studied. A coating on Ruby Red grapefruit and Valencia oranges prepared from a shellac formulation with morpholine to achieve pH 9 was toxic to *E. aerogenes* and *E. coli* in storage at 13 °C. The addition of the preservative paraben in the basic shellac had further inhibitory effects (McGuire and Hagenmaier 2001). Dipping inoculated fresh lettuce leaves and broccoli florets in hypochlorite solutions of 50 or 100 mg L^{-1} for up to 2 min was not effective in eliminating *E. coli* populations although they significantly reduced the *E. coli* counts compared to those inoculated and not dipped (Behrsing *et al.* 2000). Yu *et al.* (2001) found that 3% hydrogen peroxide was the most effective chemical treatment in reducing the bacterial population. However, other work has shown that this treatment can be phytotoxic.

Inoculated apples, oranges and tomatoes that had been submerged in sterile deionised water containing 1.5% lactic acid plus 1.5% hydrogen peroxide for 15 min at 40 °C had reduced bacterial pathogens compared to those in deionised water only. Furthermore, substantial populations of the pathogens survived in the control wash water, whereas no *E. coli* O157 : H7, *S. enteritidis* or *L. monocytogenes* cells were detected in the chemical treatment solution. The sensory and qualitative characteristics of apples treated with the chemical wash solution were not adversely affected by the treatment (Venkitanarayanan *et al.* 2002). Guan *et al.* (2012) showed that UV-C doses of 0.45–3.15 kJ m^{-2} resulted in 0.67–1.13 log CFU g^{-1} reduction of *E. coli* O157 : H7 inoculated on mushroom cap surfaces. Graça *et al.* (2012) tested ecoinnovative sanitizer techniques on minimally processed apple slices. Their results showed that acidic electrolysed water at 100 mg L^{-1} of free available chlorine had high bactericidal activity against *E. coli*, *L. innocua* and *S. choleraesuis*.

Salmonella

The bacterium *Salmonella typhimurium* produces toxins. Samples were taken from healthy and decayed portions of 341 fruits and vegetables collected in local supermarkets in the United States. Suspected *Salmonella* occurred in 20.2% of healthy and in 26.4% of decayed portions. The fungi *Alternaria* spp. and *Botrytis* spp. caused two-thirds of the fungal infections resulting in the decay. In a similar analysis of 121 samples with mechanical injuries, in which some two-thirds were gouges, cuts and bruises, there were no significant differences in *Salmonella* incidence between injured and non-injured portions. Of 332 suspected *Salmonella* randomly isolated from healthy and decayed or injured portions, 5.1% were confirmed as *Salmonella* by physiological and serological testing (Wells and Butterfield 1999).

Botulism

In the United States, Draughon and Mundt (1988) investigated 34 cases of acid food botulism, of which 17 involved home-canned tomatoes. They concluded that obviously mouldy tomatoes should not be used in preparing juices and home-canned tomatoes, as mouldy tissue had a high pH and may harbour *Clostridium botulinum* and botulinal toxin. Church and Parsons (1995) mentioned that there was a theoretical potential fatal toxigenesis through infections by *C. botulinum* in the depleted O_2 atmospheres in modified atmosphere packed fresh vegetables. It was claimed that this toxigenesis had not been demonstrated in vegetable products without some sensory indication, that is they have an 'off' taste (Zagory and Kader 1988, quoted by Church and Parsons 1995). However, Roy *et al.* (1995) showed that the optimum in-package O_2 concentration for suppressing cap opening of fresh mushrooms was 6% and that lower O_2 concentrations in storage are not recommended because they could promote growth and toxin production by *C. botulinum*. Sugiyama and Yang (1975) showed that with mushrooms prepacked in plastic film, *C. botulinum* not only grew on the mushrooms but also produced toxins.

Betts (1996) in a review of hazards related to modified atmosphere packaging of food indicated that vacuum packing of shredded lettuce had been implicated in a botulinum poisoning outbreak. Toxin was not formed when the inoculated packages were kept at temperatures as low as 6 °C. The results indicate that fresh vacuum-packaged enoki mushrooms do not present a botulism hazard when cultured aseptically and stored refrigerated. In the United States, botulinal toxin was not detected in 148 packages from 14 independent lots by Malizio and Johnson (1999) when spores were added to the packages. Spoilage was evident before toxin formation (Johnson

and Montecucco 2008). Hauschild (1989) found that some strains of *C. botulinum* could grow at temperatures as low as 3.5 °C. CO_2 levels of over 20% can retard bacterial growth such as *Erwinia carotovora* on potatoes. The degree of retardation increases with increasing concentrations of the gas, but at these high levels, *C. botulinum* may survive (Daniels *et al.* 1985).

Measures to reduce the microbiological contamination of ready-to-use fruits and vegetables were recommended by Hguyen-The (1991) and included good manufacturing practices, disinfections of the product, standards and specifications to control the finished product. A use-by-date that limits the shelf life to a week and a storage temperature of 4 °C was recommended. These measures will reduce the multiplication of microorganisms during transport and retailing.

Listerosis

The food-borne human pathogen, which is a gram-positive bacterium *Listeria monocytogenes* can cause the disease listerosis, which normally has only mild influenza-like effects on healthy people, but can be fatal to pregnant women, the elderly and those suffering from chronic diseases. The bacterium can grow well in the low acid conditions found in vegetables. Problems of listerosis are not commonly associated with stored fruit and vegetables. However, in a survey by Heisick *et al.* (1989) for the USA Food and Drugs Administration, *L. monocytogenes* was found in 21% of the samples of potatoes, 14% of radishes and 2% or less in cucumbers and cabbages. Berrang *et al.* (1989) found that it grew well on asparagus, broccoli and cauliflower in storage at 15 °C, and it even grew on asparagus in storage at 5 °C. They also found that under controlled atmosphere conditions, *L. monocytogenes* grew just as well as in air, but as vegetables can be stored for longer periods under these conditions, this can result in a longer time for the bacteria to grow. Conway *et al.* (2000) found that *L. monocytogenes* survived and its populations increased on Delicious apple slices at 10 or 20 °C in air or controlled atmospheres of 0.5% O_2 and 15% CO_2, but did not grow at 5 °C.

Shigellosis

This is a disease caused by infection with the bacteria *Shigella* spp. It is also called bacterial dysentery and the symptoms are diarrhoea, fever and abdominal pains. Contamination has been shown to be from many sources including that of shredded lettuce (Davis *et al.* 1988).

Aeromonas hydrophila

A. hydrophila is a gram-negative bacterium that is widely spread in nature. Callister and Agger (1987) found it on virtually every type of vegetable they analysed from a grocery store. They also showed that it could grow on vegetables at temperatures of 5 °C or even lower. The bacteria can cause diarrhoea, which is usually quite mild, but more severe cases have been reported. In experiments with 'ready-to-use' lettuce and mixed salads, Guerzoni *et al.* (1996) found that the inclusion of red chicory was an inhibiting ingredient to *A. hydrophila*. They also found an antagonistic effect of *Lactobacillus plantarum* against *A. hydrophila*, but the presence of *L. plantarum* appeared to negate the inhibiting effect of red chicory.

Safety in controlled atmosphere stores

Oxygen and carbon dioxide

Low O_2 and high CO_2 can have a direct lethal effect on human beings working in those atmospheres. In Britain, the Health and Safety Executive (HSE 1991) showed that work in confined spaces, such as controlled atmosphere stores, could be potentially dangerous and entry must be strictly controlled preferably through some permit system. Also, it is recommended that anyone entering such an area should have emergency breathing apparatus as well as proper training and instruction in the precautions. Stringent procedures need to be in place, including a person on watch outside and the formulation of a rescue plan. When a store is sealed, anyone entering it must wear breathing apparatus. Warning notices should be placed at all entrances to controlled atmosphere stores and an alarm switch located near the door inside the chamber in the event that someone may be shut in. There should be a release mechanism so that the door can be opened from the inside. When the produce is to be unloaded from a store, the main doors should be opened and the circulating fan run at full speed for at least an hour before unloading is commenced. In

the United Kingdom, the areas around the store chamber must be kept free of impedimenta in compliance with the appropriate Agricultural Safety Regulations.

O_2 levels in the atmosphere of 12–16% can affect human muscular co-ordination, increase respiration rate and affect their ability to think clearly. At lower levels, vomiting and further impediment to co-ordination and thinking can occur. At levels below 6%, human beings rapidly lose consciousness, and breathing and heartbeats stop (Bishop 1996). The limits for CO_2 levels in rooms for human occupation was quoted by Bishop (1996) from the Health and Safety Executive, publication EH40-95 *Occupational Exposure Limits*, as 0.5% CO_2 for continuous exposure and 1.5% CO_2 for 10 min exposure.

Great care needs to be taken when using modified atmosphere packaging containing 70 or 80% O_2, or even higher levels, because of potential explosions.

Ethylene

Ethylene is used in fruit ripening rooms. It is a colourless gas with a sweetish odour and taste, has asphyxiate and anaesthetic properties and is flammable. Its flammable limits in air are 3.1–32% volume for volume and its autoignition temperature is 543 °C. Care must be taken when the gas is used for fruit ripening to ensure those levels in the atmosphere do not reach 3.1%. As added precautions, all electrical fitting must be of a 'spark-free' type and warning notices relating to smoking and fire hazards must be displayed around the rooms.

Toxicity of packaging material

Another safety issue is the possibility that the plastic films being used in modified atmosphere packaging are toxic. Schlimme and Rooney (1994) reviewed the possibility of constituents of the polymeric film used in modified atmosphere packaging migrating to the food that they contain. They showed that it is unlikely that the polymerised constituents would be transferred to the food because of their high molecular weight and insolubility in water. All films used can contain some non-polymerised constituents, called monomers, that could be transferred to the food and the Food and Drugs Administration in the United States, and also the European Community have regulations related to these 'indirect additives'. The film manufacturer must therefore establish the toxicity and extraction behaviour of the constituents with specified food simulants.

Packhouse safety

Clarke (1996) reviewed safety and hygiene of the workforce and suggested the following. In some countries, there are strict laws to control the health and safety of all workers and these of course must be adhered to. In the absence of such laws, one could use the principles laid down here as a guide.

'Clothing should be provided that is suitable for each task. Most workers would be engaged on the inspection or packing, which would require simply an overall, a cap or hat and probably rubber gloves if preferred. The overall would often be of the coat type with a zip or button front. Trousers or skirts would often be open to personal choice, although some employers may well provide trousers for work in what is often a cool, draughty and elevated position. Shoes would also commonly be provided with a firm, non-slip sole and sturdy foot support. Boots would not normally be necessary but the lighter, ladies fashion shoes and high heels, etc. must clearly be avoided.

The start-up of all machines is a procedure that can lead to danger if not done to a set order. Firstly, a warning hooter should sound, giving everybody ample chance to stand clear of the machines, and then after a few seconds of the hooter, the whole line should start-up. This could however lead to unnecessary overloads on the electricity supply system as all motors would reach peak demand at once. It is just as acceptable to start all motors in reverse order with the last machines in the production line starting up first and the others in progression going back down the line to the first machines. The first motors are likely to be loaders and feeding belts, so it is important that all the line is fully operational before any crop arrives.

In case of danger on any part of the process line, there should again be close adherence to the regulations that govern all electrical machines for health and safety at work. For example, 'Every electrical motor shall be controlled by an efficient switch for

starting and stopping so placed to be easily worked by the person in charge of the motor. In every place in which machines are being driven by any electric motor, there shall be a means at hand for either switching off or stopping the motor/machine if necessary to prevent danger'. This would normally be achieved by the provision of a large red button placed at convenient positions all along the process line. All other regulations such as insulation should be taken care of by the machine manufacturer.

Walkways and forklift truck ways should be clearly marked on the floor in bright yellow paint so that both workforce and visitors are not likely to stray into the danger zone. Forklift trucks should be equipped with a hooter to warn anyone in danger and should be powered by gas to avoid fumes.'