

Microbial update

water & soft drinks

by Dr Roy Betts, Head of the Microbiology Dept, Campden-BRI, Chipping Campden, Gloucestershire, GL55 6LD, UK. Produced as a service to the food industry by Oxoid, part of Thermo Fisher Scientific.

Drinks form a very large and increasingly diverse market ranging from 'tap' water, through bottled spring and mineral waters, to fruit drinks and juices, soft carbonated flavoured drinks and a much newer market of sports and energy drinks. The complexities of this range with respect to their microbiology, are large. Some products, for example mineral waters, have production methods defined by legislation and cannot be processed or preserved in any way.

Other products can be heat processed or may contain preservatives. Some, for example citrus juices, will be naturally acidic, whilst vegetable juices, for example carrot, are not. Manufacturers may wish to retain a 'healthy' product image for some (no preservatives), whilst for others their popularity is not diminished by containing many artificial colours, flavours and preservatives.

All of these variations can affect product microbiology, and a very full and detailed understanding of the different product parameters is required in order to devise recipes and procedures that will deliver stable and safe drinks to the consumer.

Water

Water is, by weight, the most important constituent of any living cell, and is an absolute requirement for life. Water has been and indeed is still a most important vector of illness in both developing and advanced countries around the world.

To start to consider the importance of water microbiology, we must look at how water is used and how it comes into contact with our bodies.

When considering water, it is easy to confine discussions to drinking water, but water is used in many other places with our food industry, and can come into contact with foods in many ways.

Potable water

Potable water is a term used widely, and this simply means water that is fit to drink. Legislation and regulation in different coun-



tries throughout the world will give different definitions of potable water, but essentially it must be free from human pathogens and hazardous chemicals.

Water sources that are used as a supply of potable water have to be picked carefully, there must be a year round supply, protection from pollution and an ability to be suitably treated. Surface waters (reservoirs, lakes, rivers) offer a good supply, but can be at risk from contamination. Ground waters potentially offer a better quality supply, as they are more protected from external factors. These are often used without any form of treatment, over those used to reduce hardness.

Water can contain a wide variety of innocuous micro-organisms such as acinetobacter, chromobacterium, flavobacterium, moraxella, and pseudomonas.

However, contamination of waters with animal or human faecal material can introduce large numbers of pathogenic micro-organisms, many of which have a low infective dose. Pathogenic bacteria linked

to waterborne outbreaks include, *Campylobacter* spp., *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Vibrio cholera*, *Yersinia enterocolitica*, and *Aeromonas hydrophila*.

Distribution of these in drinking water can result in large outbreaks. Additionally, viruses such as enterovirus, adenovirus, hepatitis A, rota virus and Norovirus may be found in sewage contaminated water, whilst the protozoan parasites *entamoeba*, *giardia*, *cryptosporidium* and *cyclospora* may be found in some raw waters.

Water that is a threat to health, may be contaminated before treatment, and the infective agent then not effectively eliminated; or contamination can occur after effectively treated water has been distributed. Whichever the case, such water has been the cause of large outbreaks of illness.

Processing of raw water

Raw waters will usually receive a pre-treatment to clear large debris, and then be stored in storage tanks or ponds. Ponds can increase water quality by allowing sedimentation, and antimicrobial action due to the effects of sunlight.

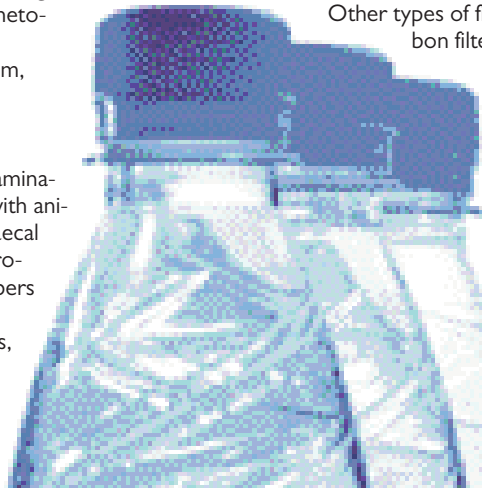
Sedimentation can be improved by use of various metal salts or clays, which can cause a flocculation of suspended solids. It has been reported that up to 99% of the pathogens present in raw water can be eliminated at this stage.

Filtration can be used to further reduce microbial levels. This is often through natural or man made sand beds and can be efficient enough to effect a 1000 to 10,000 fold microbial reduction, as well as a removal of some chemical contaminants.

Other types of filtration, for example carbon filters, micro, ultra or nano-filtration can also be used.

As a final phase of water treatment, a disinfection step is often employed. This step is aimed at microbial inactivation, and is often required to maintain a bactericidal effect for some time throughout distribution. The most commonly used disinfecting agent is chlorine,

Continued on page 12



Continued from page 11

but others can be used (bromine, ozone, UV light).

Following treatment, water is distributed to its point of use. The distribution system must protect the clean water supply from recontamination, as well as preventing growth of injured organisms present in treated waters.

Complex, very long or old distribution systems give the highest risk of microbial contamination or development, and it is at this stage that concerns over pathogens such as legionella, aeromonas and mycobacterium arise.

Overall, the water supply systems in developed countries treat and supply very large amounts of water to large populations and it is done very safely and effectively.

A great deal of care goes into this as by its very nature a minor contamination issue at a treatment works can supply poor quality water to large numbers of people and cause large outbreaks of illness. This is, however, a rare occurrence.

Bottled waters

There has been a trend in recent years, toward the consumption of bottled water rather than 'potable tap water'. This has been accelerated by health alerts that we should drink larger volumes and stay



hydrated, together with concerns over consumption of drinks containing large amounts of caffeine or sugar.

Bottled waters come in many forms and they are highly regulated (Full details of regulations in individual countries should be obtained).

● Bottled water or 'table water' is any water placed in a sealed container and sold

for drinking. Such water can be treated to remove micro-organisms.

● Spring water is bottled water derived from an approved underground source. Spring water can be treated to remove microbiological contaminants.

● Natural Mineral Water is bottled water obtained from an approved underground source, and may not be treated to remove micro-organisms.

Bottled waters are usually available in either carbonated or non-carbonated forms. The addition of carbon dioxide can reduce microbial levels in bottles, as it is an antimicrobial and additionally reduces the pH of the water.

The effects of storage on the bacterial levels in bottled water can be varied. The natural flora in water will tend to remain at a low level, whilst the water is in its natural environment. This can, however, change once the water is bottled.

Storage of bottled waters at room temperature is reported, on occasion, to allow multiplication of a bacterial flora to levels of $>10^6$ /ml. The reason for this increase in numbers may include an increase in oxygen level during the bottling process, trace amounts of nutrients in the bottle and an increase in temperature of the bottled water over the source water.

As the organisms in the bottle grow and subsequently die and lyse, this may provide a second source of nutrients for other

species, including human pathogens, if they were present.

There has been considerable work done on the survival of various human pathogens in bottled waters, and results suggest that salmonella could survive for over one year and *E. coli* O157 for over 300 days. This shows the importance of ensuring the absence of human pathogens from bottled waters.

It is important for users to understand that bottled waters are not sterile, they will contain a varied microflora of generally innocuous organisms, but this should be kept in mind when handling bottled waters.

They should not be stored continuously at elevated temperatures; once opened they should be consumed relatively quickly; and there should be good stock rotation to minimise storage of old bottles.

Fruit juices

Fruit juices, as differentiated from fruit drinks, must consist of almost 100% pure juice, with only a very few additional ingredients permitted.

Fruit juices may be squeezed directly from the fruit, or may be prepared from crushed material. The resulting juice can be clarified, often by the use of enzymes, or can be left with much suspended fruit solids within it.

The shelf life of the long and short life products is dependant on any process the juice receives. Some are unpasteurised 'fresh' juices and will be directly from

crushed/squeezed fruit.

These products will have the freshest taste but the shortest life, due to the presence of a large diverse microbial flora and a lack of any form of preservative.

The microbiological issues with fresh unprocessed juice will depend on the natural flora and the physical and chemical parameters of the juice, for example pH. Many fruit juices will have a low pH. This will tend to inhibit bacterial growth but will select for the growth of yeasts and moulds.

If the juice is not stored at low temperature or is stored for too long, then fermentation is likely to occur. With respect to safety, whilst most bacterial pathogens will be inhibited at the low pH of fruit juices, some can survive and indeed may be present on the outer surface or skin of some fruits. There have been outbreaks of food poisoning linked to such products as fresh pressed orange juice (salmonella) and apple juice (*E. coli* O157).

Vegetable juices can have a higher pH than fruit juices and can be more open to attack from bacteria. In the past pasteurised carrot

juice has been the cause of a small outbreak of botulism in North America due to incorrect (non-chilled) consumer storage.

Pasteurised fruit juices, should be relatively free from the risk of bacterial pathogens, however spoilage by yeasts and mould can still occur and is controlled through use of chilled storage with a short shelf life.

Some juices will be commercially sterile (UHT processed) and such products can be stored at ambient temperatures (until opened) and have a long life.

A final point to note when considering juices is the potential for mould toxins to be present. In fruits like apples, the growth of mould on fruit can produce toxins such as patulin.

This is not greatly affected by heat processes and, if present in the fruit, may be present in a juice prepared from that fruit even though a heat process has been applied. This can be avoided by use of good quality, non-mould containing fruit.

Carbonated soft drinks

Over a thousand years ago, people drank and bathed in mineral waters which were naturally carbonated. The appeal of this effervescent water led scientists in the 18th Century to make artificial 'fizzy water', but with limited success.

The Rev. Joseph Priestly conducted various experiments and

managed to produce an artificial mineral water in 1768 by the production of carbonic acid using the action of vitriolic (sulphuric) acid on chalk. The carbonic acid was then absorbed in water held under pressure.

In 1771 a Swedish chemist, Tobern Bergman attempted to commercially produce carbonated water, but the process was too expensive. Success was finally achieved by Jacob Schweppe, who developed a condensing machine which could infuse water

with gas. He patented the process in 1783, and so began the carbonated drinks industry.

Microbiologically, carbonated drinks have, as noted previously, an additional 'safety feature' as carbon dioxide is antimicrobial, but additionally it is soluble in water and further reduces the pH of the drink, thus making microbial growth less likely.

Many carbonated soft drinks also contain 'weak acid' preservatives (sulphur dioxide, sorbates or benzoates are often used), which can prevent microbial spoilage of acidic beverages.

Generally weak-acid preservatives inhibit growth but do not kill contaminating organisms.

They are much more effective at low pH values where solutions contain increased concentrations of undissociated acids. Microbial inhibition by weak-acids involves diffusion of undissociated preservative molecules through the microbial membrane; the dissociation of these molecules inside the cell liberates protons, thus acidifying the cell cytoplasm and preventing growth.

It is, however, important to note that some yeasts can be preservative resistant and can grow in some preserved drinks.

A number can even break down sorbate into pentadiene, a chemical responsible for kerosene-like taints in some drinks.

Conclusions

Drinking water and soft drinks are a very varied product grouping and any short article can only briefly consider some of the microbiological issues involved with these products.

Good quality raw materials, an understanding of the chemistry of the product, the use of pH control, heat processes and preservatives all come together to produce stable and safe drinks. It is important to note that drinks are not devoid of pathogen risks. Waters and juices have all been responsible for outbreaks of illness.

An understanding of potential hazards and the use of suitable controlling factors will minimise pathogen risk and allow consumers to enjoy soft drinks in the future. ■

FaxNOW +44 1256 329728

✉ val.stroud@thermofisher.com

References

- The International Commission on Microbiological Specifications for Foods. (1998) Micro-organisms in Foods, Book 6. Microbial Ecology of Food Commodities. Blackie Academic & Professional. London.
- Lund, B. M., Baird-Parker, T. C. and Gould, G. W. (Eds) (2000) The Microbiological Safety and Quality of Food. Vol 1. Aspen Publishers Inc. Gaithersburg, Maryland.
- Robinson, R. K., Batt, C. A., Patel, P., (Eds) (2000) Encyclopedia of Food Microbiology. Vol 3. Academic Press. London.
- Gaze, J. E. (2006) Pasteurisation: a food industry practical guide. Campden BRI Guideline No. 51. Campden BRI, Chipping Campden, Glos, GL55 6LD.
- Photographs copyright Shutterstock.