



**Diploma in Beverage Packaging (Beer)**  
**Unit 2.6 Carbonated Soft Drinks**

**Product Integrity**

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## DIPLOMA IN PACKAGING (BEER) - MODULE 2

### UNIT 2.6: Carbonated Soft Drinks

#### ELEMENT 2.6.4: Product Integrity

##### SYLLABUS.

###### 2.6.4.1 Microbiological testing:

- Requirements and results

###### 2.6.4.2 Analytical testing:

- Requirements and results

###### 2.6.4.3 Full line inspection:

- Differences with beer operations

###### 2.6.4.4 Legislative declarations:

- Ingredients / allergens / colours
- Nutrition labeling on soft drinks

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### 2.6.4.1 Microbiological Testing

Please cross-reference to Unit 2.1 Quality and Unit 3.2 Process Gasses in the Pack Diploma material

Before we look at microbiological testing, we need to consider what hygiene is all about and its importance in the manufacture of soft drinks.

Good hygiene is essential to ensure that the drinks we manufacture and sell are safe to consume and free from contamination. Contamination from micro-organisms, chemicals and foreign matter can cause harm, injury and considerable distress.

The soft drinks industry prides itself on producing drinks to the highest standards. These guidance notes, from one of the top trade associations in the world, give practical advice on food hygiene as it relates to soft drinks, including carbonated drinks, still and dilutable (or cordial) drinks, fruit juices and bottled waters. They outline how excellent hygiene standards can be maintained through good manufacturing practice.

#### The Importance of Good Hygiene

Good hygiene means:

- Compliance with Food Law
- Reduced consumer complaints
- Consumer confidence
- Enhanced reputation
- And for those involved in production, good working conditions

Poor hygiene can cause:

- Consumer complaints
- Injury to our consumers
- Illnesses e.g. food poisoning
- Unwillingness by the consumer to purchase again
- Bad publicity
- Loss of reputation and business

Hygiene can be effectively controlled through Good Manufacturing Practice and **involves everyone**.

#### Personal hygiene

Production staff must maintain a high standard of personal cleanliness and appearance, be in good health and adopt hygienic manufacturing principles.

- Cleanliness – wash hands thoroughly with soap and warm water. Wear protective clothing and head covering, if required.
- Appearance – e.g. keep hair clean and use hair nets.
- Health – report diarrhoea, vomiting, heavy colds and discharge from eyes and ears. Only return to work when medically cleared. Always cover cuts and abrasions with a clean, waterproof dressing.
- Hygienic manufacturing principles – identify possible sources of contamination, do not wear jewellery, do not eat or smoke in production areas, avoid unhygienic habits
- No chewing of gum

## Hygiene cleaning

Cleaning is an essential part of hygiene activities.

Why clean ?

- To ensure that cross contamination does not occur between products
- To remove matter on which micro-organisms might grow
- To allow effective disinfection/sterilization to reduce levels of micro-organisms
- To reduce the risk of contamination of drinks
- To prevent infestation by pests
- To promote a good image to visitors, including customers
- To provide a pleasant and safe working environment

Methods range from cleaning by hand or machine, foam cleaning, cleaning out-of-place (COP) and Clean In Place (CIP). Instructions and procedures must be followed to achieve good results and to be in line with cleaning schedules and plans. Reduction of cleaning time is not allowed, nor is it allowed to mix chemicals or transfer them to unmarked drinks containers. Cleaning problems or defects must always be reported.

## Housekeeping

Effective systems must be in place to ensure production is operated in a clean and tidy manner. Waste and rubbish need to be hygienically stored and disposed of. The following actions are essential:

- Keep work areas clean and tidy
- Use the correct location for storage
- Clear away waste before receptacles get full
- Regularly remove packaging waste
- Prevent pests from gaining access – keep doors and windows shut
- Don't eat or drink in production areas
- Report signs of pests immediately
- Don't disturb bait boxes
- Roll up hoses when not in use

## Micro-organisms – the unseen enemy

Soft drinks can be contaminated by micro-organisms, which are tiny living things, not plants or animals. They are found everywhere, in the air, in soil and water, in and on us and sometimes in the food we eat and the drinks we consume.

They are normally viewed using a microscope.

Examples are:

### Bacteria

These are very small micro-organisms (1-5 microns) and are present everywhere on earth, in the air, in soil, in water and in food. Most bacteria are harmless and some are beneficial such as those used for cheese and yoghurt manufacture. Some bacteria can cause illness, where large numbers have grown in the food e.g. *Salmonella*, or small numbers are carried on the food and grow inside us to cause illness e.g. *Campylobacter*. They divide by binary fission and grow very rapidly. They have no true nucleus (prokaryotic) and can be grouped into **Gram +ve** and **Gram -ve** types by difference in cell wall type.

Bacterial spores are produced by *Bacillus* and *Clostridium* within the cell (endospores). They are very small (< 1 micron) and are always heat resistant, as well as being resistant to chemicals and irradiation. They often need a 'heat shock' to germinate.

Some bacteria cause spoilage of food, making food 'go off' and some can cause the drinks we make to 'go off'.

## **Moulds**

Most moulds are harmless and in the same way as bacteria, some are beneficial such as penicillin. However, some can cause illness, but the main cause for concern is spoilage of food and they will also attack the drinks that we manufacture.

They grow as filaments or 'hyphae' that often form a visible mass (mycelium). There are many morphological varieties and require oxygen for growth. Moulds have a true nucleus (eukaryotic) and their spores are <10 microns in size.

## **Yeasts**

As far as we know, nearly all yeasts are harmless and some are beneficial like the yeasts we use for baking and for making wine and beer. However, there are many different types of yeast that spoil the drinks we make and cause them to 'go off'.

They are very small (< 10 microns in diameter) and grow as single cells, which divide by budding. They have a true nucleus (eukaryotic) and often grow in a mould-like form. Some produce spores (ascospores) that confer some resistance.

## **Viruses**

These are incredibly small and measured in nanometers. Being parasitic, they never grow in foods and are rarely seen in foods, rather in patients' specimens. However they are a common cause of food poisoning.

## **Protozoa**

These are small single celled animals (e.g. *Cryptosporidium*, *Giardia spp*) and are usually found in animal sources that contaminate water supplies. They are highly resistant to chemical treatment but do not grow in foods. They cause food poisoning and are controlled by filtration.

*Cryptosporidium spp* is excreted in the faeces of infected hosts (human and animal). Oocysts are transmitted through water and agriculture. Food-borne transmission is rare but it has been recently found in freshly prepared apple drink. Cysts are resistant to chlorination of water supplies and are controlled by excluding agricultural slurries from water supplies. 'Boil water' warning notices are used during outbreaks.

## **Algae**

These are small single celled plants (*Dinoflagellates*) and are photosynthetic. They are typical water-borne organisms and cause 'algal blooms'. Some produce powerful food-poisoning toxins and are concentrated by filter-feeding shellfish. They are controlled by elimination from the food chain.

## **Growth of micro-organisms**

We have to understand how micro-organisms grow that we can control them and prevent spoilage and maintain food safety of soft drinks.

Bacteria, moulds and yeast need food and water to grow and live just as we do. They also produce waste products. Sometimes they produce chemicals and gases in the drinks that can change the taste and make the drinks 'fizzy'

Yeasts can produce alcohol and carbon dioxide through fermentation.

Sometimes the chemicals can be poisonous to us and these are called toxins.

In order to grow, micro-organisms require the following: warmth, moisture, time, food, the right pH and sometimes oxygen.

**Warmth** – micro-organisms can grow in a range of temperatures from cold to hot. They grow fastest at the temperature they favour the best. They grow slowly at low temperatures and not at all in freezing conditions. They grow more and more slowly as the temperature increases and eventually will die. In certain conditions, bacteria can form spores so that they can survive high temperature. Like us, they wish to live as long as possible. The bacteria thicken their cell walls to prevent being destroyed by heat

**Moisture** – Micro-organisms need some water to grow. We supply the water very well in the drinks that we manufacture, plus we use water for cleaning.

**Time** – Bacteria are nature's fast breeders and some typically multiply by dividing in two every 10-20 minutes. Yeasts are slower and grow by making another one of itself by budding. Moulds are ever slower growing than yeasts and grow by producing more and more threads (mycelium) and reproduce by producing spores.

**Food** – most bacteria like food that high in protein and there are only a few types that actually like our soft drinks. Moulds attack all sorts of different food. They normally cannot grow in our carbonated drinks, because of the carbon dioxide content, but sometimes they grow on the surface of our drinks. Yeasts usually prefer food that is slightly sweet and can sometimes grow within our soft drinks.

**pH** – how acid or alkaline the food or drink is also very important and the micro-organism cannot grow if the soft drink is too acidic or too alkaline for them. Yeast does not mind acidic drinks and can grow in fruit juices. Other drinks are mainly neutral so we sometimes have to use preservatives in the formulation using certain chemicals.

**Oxygen** – like us, some micro-organisms need the oxygen in air for growth. Some may grow without oxygen (anaerobically) and there are some that do not mind growing with or without.

### **Beneficial and Detrimental Changes**

From the earliest times, people have been suffering or benefiting from the presence and activities of micro-organisms in foods and beverages.

Beverages may become contaminated with a variety of micro-organisms, some of which may grow and bring about a change in the product.

Two kinds of change may result:

- Beneficial
- Detrimental

Beneficial changes include alcoholic fermentation, a preservative effect and the development of flavour and aroma.

Detrimental changes are those where beverages become a hazard to health through the presence of pathogenic organisms or toxins. High numbers of harmless organisms may bring about unacceptable observable changes giving rise to spoilage.

### **Spoilage of soft drinks**

A fruit juice, soft drink or concentrate may be judged to be of good microbiological quality when

- it does not constitute a HEALTH HAZARD through harbouring pathogenic micro-organisms or their toxins
- it is not likely to deteriorate rapidly through the presence of high numbers of spoilage organisms – QUALITY HAZARD

Soft drinks rarely present health risks, although ingredients used in their manufacture may be contaminated. A more realistic hazard may exist through the growth of certain toxin-producing moulds (mycotoxin) on the fruits used in these products. Many mycotoxins are carcinogenic and the possibility of their presence is therefore of great concern, for example **PATULIN**.

Yeasts, moulds and bacteria all occur in the raw materials used in the manufacture of soft drinks, although they are mainly spoiled by the growth of yeasts and moulds.

Many different yeasts and moulds may be present – possibly the most important of which are *Zygosaccharomyces bailii* (yeast) and *Byssoschlamys* (mould)

Bacteria include mainly lactic acid bacteria e.g. *Lactobacillus* and acetic acid bacteria e.g. *Gluconobacter* and a few species of spore-forming bacteria e.g. *Bacillus coagulans*. A relative newcomer is *Alicyclobacillus acidoterrestris*

One aspect of hygiene in the soft drinks industry is about preventing spoilage of product. However, mistakes do occur and it is all about investigating how this has happened and endeavours to prevent further re-occurrence.

Spoilage is caused by micro-organisms and sometimes by incorrect processing and storage. The effect of spoilage in soft drinks can lead to a change in:

- Appearance
- Taste
- Colour
- Texture
- Smell

Spoilage can occur in many different forms.

**Clearing** – a fruit juice product can lose its cloudiness and become visually clear.

**Lumps** – a clear product can develop lumps or 'floc' in suspension within the liquid drink

**Slime** – a slime or mucous substance appears in the drink

**Fermentation** – alcohol and gas (CO<sub>2</sub>) are produced and can cause the drink container to fail.

**Off-flavours** – an unpleasant or off flavour can be produced and sometimes unpleasant smelling gases.

**Cloud production** – Instead of lumps, the drink becomes cloudy. This is only apparent in clear drinks.

**Mould growth** – moulds grow and become visible as a white or coloured mass of furry growth on the surface of the soft drink. Sometimes the mould may grow inside the product and look like cotton wool balls.

**Neck ringing** – a ring of colour appears around the 'neck' of the drink. This is not always caused by micro-organisms but by an ingredient in the drink separating from solution and floating to the surface.

**Chemical changes** – changes can occur where the ingredients and drinks have not been correctly processed or stored correctly – not the result of micro-organism spoilage.

Colour of soft drinks can change with incorrect pasteurisation or 'overcooking'. Also long storage of a product can cause a colour and flavour change as well as formation of a sediment.

### Process Control

All ingredients, packaging, drinks in process and finished products must be stored and handled carefully to prevent contamination and the growth of micro-organisms.

The following rules apply:

- Areas should be kept clean and dry, be well lit and ventilated
- Adequate space should be available and storage areas not over-loaded
- Stock rotation procedures must be strictly adhered to and products not kept longer than necessary
- Correct temperatures maintained where necessary and temperatures/times for defrosting checked regularly.
- All raw materials and packaging must be kept clean and covered i.e. lids kept on and bags intact and kept off the floor
- All cleaning materials and chemicals must be stored away from the raw material, ingredients and finished product storage areas

## Methods of Preservation

Each drink has been specifically designed and formulated to prevent micro-organism growth by using some, or all of the following methods of preservation.

**Pasteurization** – heat treatment process. Some soft drinks need to be pasteurised to kill off unwanted spoilage micro-organisms. Pasteurization does not sterilise a soft drink. If there are too many micro-organisms in the drink i.e. heavy contamination, then some spoilage organisms may survive and grow. The setting of the pasteurisation process is important in respect of time and temperature. Incorrect and unstable settings could result in failure and therefore spoilage occurs.

**Carbon Dioxide** – some micro-organisms need oxygen to grow and therefore by adding carbon dioxide to a soft drink, growth is prohibited. Too little CO<sub>2</sub> may allow growth to occur.

**Acidity level** – most micro-organisms do not favour acidic conditions and the more acid the drink (i.e. the lower pH) the better at preventing microbial growth.

**Chemical preservatives** – preservatives prevent growth of micro-organisms. We must use the correct amount in the formulation for preservation to take effect.

## Cleaning and disinfection

Cleaning is a critical part of a soft drinks company's operation, and for it to be effective, it has to be done in a controlled and organised fashion.

There are two areas that need to be defined:

**Cleaning** – removal of grease, dirt and food particles

**Disinfection** – this is carried out after or during the cleaning stage and will reduce micro-organisms down to a safe level.

Cleaning should not be carried out on an ad hoc basis but as part of the overall production plan to ensure that the building structure, production equipment, machines and utensils are cleaned to an acceptable standard. Product containers, returnable and non-returnable, are also cleaned before they are filled to ensure that they are completely cleaned – this allows for efficient filling as any minute specks of dust can cause frothing or 'fobbing' of carbonated soft drink during the filling process.

Cleaning can take place during the process, between product runs, at the end of processing, or 'as we go'. The methods of cleaning, the frequency, the amount and type of chemical used are planned, taking into account:

- The level of dirt, remaining food and drink residues
- The risk to the drinks being made
- The build-up of micro-organisms

Possible types of cleaning are:

**CIP (clean in place)** – pipework and tanks are cleaned in a totally enclosed system and cleaning chemicals are circulated through the system. Typically a hot or cold solution of caustic soda is circulated for a specific time period, followed by several rinses of water.

**Manual** – cleaning chemicals are applied to surfaces of production line equipment, using machines and utensils plus 'elbow grease'.

**Ultrasonic** – using high frequency sound in a water bath, for filling valves and other small items.

## Basic steps for cleaning and disinfection

1. **Pre-clean** – get the area ready, dismantle or prepare the equipment, remove any dirt or drink residues by scraping or rinsing in cold water
2. **Main clean** - wash items with a cleaning agent
3. **Rinse** – rinse off with clean water
4. **Disinfection** – disinfect using either a disinfectant or sterilant, very hot water at 82°C and above, or steam
5. **Rinse** – if necessary, using clean water. Some disinfectants may be left on the items being cleaned.
6. **Dry**- leave to dry
7. **Clean equipment and put away** – clean and disinfect any hygiene utensils and equipment and put away in correct storage areas.

## Chemicals for cleaning and disinfection

- **Cleaning agents** – there are many types of cleaning agents available. Detergents remove grease, oil and general dirt. Strong alkalis are very abrasive and can remove heavy soiling and caked-on deposits.

All cleaning agents suspend the dirt in number so that it can be rinsed away easily. Sometimes hot water is needed to help the dirt to dissolve – it depends on the chemical used.

- **Disinfecting and sterilising agents** – these are chemicals used for killing off micro-organisms and reducing the numbers on the equipment to a safe level. Dependent upon the chemical chosen and how it is used, 100% of the micro-organisms can be killed.
- **Detergent-sterilisers.** These are a combination of detergent and disinfectant, sometimes called a sanitiser, so they can clean and disinfect in one step.

All chemicals can be dangerous, so manufacturers' instructions should always be followed. Assessment of use should be formally prescribed by the soft drinks manufacturer in accordance with supplier safety precautions and specification.

The correct amounts should always be used and chemicals should never be mixed. Using too little means that cleaning and disinfection may be ineffective – using too much may not achieve a better result, it just wastes money !

The cleaning and sanitising processes used should be validated to ensure consistent efficacy.

## Microbiological analysis and testing

We must firstly consider which micro-organisms can be studied in the laboratory, and which cannot.

Bacteria and yeasts grow to produce visible colonies and also produce colour changes or turbidity in liquids. Their growth may be analysed by chemical by-products or examined by genetic composition. Moulds also grow to produce visible colonies and can be identified using microscopy.

Viruses and protozoa are usually only found in low numbers in food. Viruses are sub-microscopic (an electron microscope is needed to view them)

Taking this into consideration, and also looking at the factors affecting microbial growth, whilst many micro-organisms may contaminate drinks, not all will be capable of growing, or will grow equally well. Only those organisms that tolerate the conditions presented by the product and its storage environment will be able to grow. Environmental conditions will lead to selection of one or more members of any initial contamination microflora which may then grow and possibly bring about spoilage.

## Methods used

Two basic methods are used in food microbiology:

- Growth to produce a visible effect in liquids or on solids
- Analysis of microbial components or products to achieve detection or identification

An **aseptic technique** is employed of which the key principles are:

1. Avoidance of contamination to the analyst/colleagues
2. Protection of the original material or culture
3. Do the test or operation

The objective is to prevent contamination by

- use of sterile equipment
- use of sterile growth media
- working cleanly and quickly
- flaming manipulation equipment and containers
- minimising aerial contamination (closed windows)
- not touching organism contact surfaces

### Equipment used

Let us consider the types of equipment used in micro testing.

Swabs, loop and wires – these are used to pick up and transfer samples from ingredients and any surfaces to be examined. They may be pre-sterilised, disposable or flame-sterilisable and re-usable. Loops have a ring-shaped end and are used transfer liquids e.g. cultures and reagents. Wires are straight-ended and used to stab into solid growth media or to pick up very small quantities of test materials. If metal loops or wires are used, they must be thoroughly cooled after flaming to prevent killing the microbial culture.

Pipettes – various types are used. Pasteur pipettes are used for transferring small quantities of liquids – they may be glass or plastic and are usually disposable. Graduated pipettes are used for measured volumes (typically 1ml or 10ml) They may be disposable or re-usable. So-called ‘broken pipettes’ are NOT broken/damaged – they have a wide aperture for picking up particulate suspensions. **IT IS NEVER PERMITTED TO ‘MOUTH PIPETTE’ IN A MICROBIOLOGY LABORATORY – A TEAT MUST ALWAYS BE USED !**

The above-mentioned equipment and many other items may be disposable or re-usable.

Re-usable equipment may cost less but uses more manpower for cleaning etc Disposables cost more and use less manpower, but are considered ‘non-green’

### Media used

Liquid media – often called broths, this is used to amplify microbial numbers to useful detectable levels. A number of different ingredients are used for this type of medium e.g. meat extracts, vegetable extracts, sugars, salt, acids, they can be tailored for specific requirements – known as selective media.

Solid (or semi-solid) media – these are broths to which agar (agar agar) has been added. Agar is a 1-2% suspension of a polysaccharide extracted from seaweed and it makes a firm gel in water. The gel remains solid at temperatures up to 95-100°C, whilst molten agar stays liquid down to 40-45°C. This means that organisms may be suspended in molten agar which is allowed to set and then incubated to produce visible growth. Suspensions of organisms can be spread on the surface of pre-made agar and incubated until visible growth occurs.

***General purpose media – is used to isolate as many organisms as possible, although it cannot isolate all as some have special requirements. They typically contain meat and vegetable extracts as a source of nitrogen. They also contain sugars such as glucose as a carbon source, as well as buffers to prevent build-up of inhibitory acids. They are used for making general purpose colony counts.***

Elective media – usually a liquid growth medium that may contain a specific nutrient to encourage growth of the target population in a culture of mixed micro-organisms. The technique is generally known as **enrichment** e.g. ethanol in media for acetic acid bacteria, lactose for spoilage yeasts in dairy environments.

Selective media – can be liquid or solid, this is the opposite of elective media. It contains chemicals to inhibit the growth of the unwanted part of a mixed population of micro-organisms in a sample. Examples are sodium biselenite to suppress non-Salmonellae in *Salmonella* enrichment media, or potassium tellurite in *Staphylococcus aureus* media

Diagnostic media – usually solid media which gives reactions that are diagnostic for the target organism. They often contain selective agents e.g. xylose lysine desoxycholate agar (XLD) for *Salmonella (salmonellae)* appear as black-centred red colonies on red agar)

### Test results and controls

Microbiological media are rarely 100% selective. In practice, we must consider that

- ‘false negatives’ (i.e. failure to detect) is never acceptable. Failure to detect a pathogen could be disastrous
- ‘false positives’ (test not selective enough) are preferable to ‘false negatives’ but the results must be confirmed
- positive results are therefore considered to be ‘presumptive’ until validated by a confirmatory test.
- To check that a test has worked, **positive and negative control cultures must be used.**

### Sterilisation and disposal

Sterilisation of all equipment and media is **essential** as numbers of organisms are being amplified from low to high levels – otherwise target organisms will be outgrown.

Wet heat e.g. steam in an autoclave or oven is very effective for liquids. 121°C for 15 minutes is usual. Dry equipment e.g. pipettes is less effective than steam. 160°C for 2-3 hours is needed for sterilization.

Disposal is extremely key and **must** be controlled. Incubated tests grow huge numbers of micro-organisms so discarded materials must be thoroughly sterilised by autoclave or sealed for professional disposal by incineration.

### Microbiological Sampling

It is important to consider the basic requirements for obtaining and handling samples. These are :

- that samples are taken by trained staff
- samples are taken at the time required
- samples are taken from the place required
- samples are taken representatively
- samples are taken and handled aseptically
- samples are stored correctly until use
- all samples are labelled appropriately

As regards the size and number of samples to be taken, they must be as large as possible in order to be representative. The size must be practicable to handle in the laboratory, typically 25g for presence or absence tests on pathogens. NB ‘absence’ means ‘not detected by the method used’.

Also the size and number of samples depends on the history of the product and the plant being tested. One sample per day is usually meaningless – HACCP standards must be used/followed.

In terms of sampling plans, two can be applied or considered:

- A ‘2-class’ plan, where presence & absence type tests are taken with a Yes/No result
- A ‘3-class’ plan, where colony count type tests are taken with a variable result.

In the microbiological control of soft drinks, samples are typically taken in the following areas :

- Raw materials
- Plant swabs – internal pipework & filling valves, essentially all surfaces that come directly into contact with product and always after line hygiene/CIPs **before** start of production runs.
- Storage trials – organoleptic and initial determination of micro stability
- End product testing – will only detect severe defects
- By customer requirements

## Colony counting and isolation

The principles of counting colonies of micro-organisms are such that it must be assumed that organisms may be present at varying levels in a sample. They may be embedded in the substance being tested and must be extracted. They may be in clumps and be separated as far as possible. Some small clumps may remain, so we must use the term 'colony forming unit' rather than number.

The objective is to extract organisms from a sample. The sample must be diluted quantitatively so that organisms are separate enough to grow as individual colonies. The organisms must be immobilised either on or in the agar medium. It is then possible to count the number of colonies that have grown, and to calculate the concentration of organisms in the original sample.

For isolation, there are a number of methods - these are :

- Membrane filtration procedure
- Agar pour plate procedure
- Agar spread plate procedure
- Agar spiral plate method

The choice of method is determined by both the anticipated number of organisms in the sample AND the clarity of the sample.

Membrane filtration – this is suitable for samples containing small numbers of micro-organisms. A large volume (usually 100 ml) is filtered through a membrane with a pore size of either 0.45 microns or 0.8 microns. The microbial cells are retained on the surface of the membrane filter itself.

The membrane is aseptically transferred to an agar plate and incubated at the appropriate temperature.

Agar pour plating – empty sterile petri dishes and molten agar (45°C) are used. If the agar is too hot, it sterilises the sample ! The bases of the petri dishes are labelled in pairs with the corresponding dilutions. Then 1.0ml of diluted sample is pipetted aseptically into each corresponding pair of plates (total of 2.0mls).

Then approximately 15 ml of agar is poured into each dish. The sample must then be mixed with the agar by gently sliding the petri dish in four directions. Once the plates have been allowed to set, they are inverted and then incubated.

Agar spread plating – agar plates that have been pre-poured and dried are used. After ensuring that the plates have been labelled in pairs on the dish bases with corresponding dilutions (e.g.  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ ) 0.1ml of diluted sample is aseptically transferred by pipette onto each pair of plates.

Sterile spreaders are used to spread the sample evenly across each plate. Disposable spreaders can be used for each plate or the spreader can be sterilised between each operation. It is sensible and important to work from the most dilute to most concentrated sample.

The plates are then allowed to dry before inversion and subsequent incubation. NB. Mould plates should be incubated in an upright position.

Agar spiral plating – to introduce a varied concentration of organisms to different sectors of a petri dish, to reduce the number of dilutions used and thus save on media usage, spiral plating can be adopted.

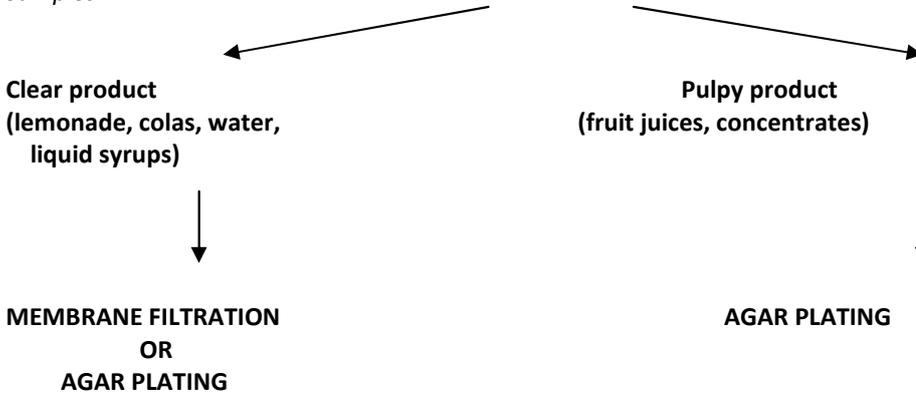
Pre-poured level agar plates are used – the plates are placed on a rotating turntable and a stylus delivers a measured volume of suspension on to the rotating turntable. Whilst moving from the centre to the edge of the plate, a spiral inoculum is formed. The stylus delivers more sample to the centre than to the edge of the dish.

After incubation, colony density will decrease from the centre to the edge.

A calibrated template is used to count colonies on countable sectors of the petri dish and results are calculated.

## Method selection chart

Samples



## Calculation of Results

The general method of counting colonies can be done either manually or mechanically on countable pairs of incubated plates ('countable' usually means between 30 and 300 colonies)

The average is then calculated as the arithmetic mean i.e.

$$\frac{A + B}{2}$$

The average is then multiplied by the dilution factor in order to obtain the concentration of organisms in the original sample. NB Spread plates only use 0.1ml inoculum so the result is the count x dilution x 10.

The lower limit of detection for 1 colony = 10/g for a pour plate

1 colony = 100/g for a spread plate

## Expression of Results

For individual samples, the exponential or log notation is used to reflect logarithmic microbial growth i.e. not 30,000,000 but  $3.0 \times 10^7$  or log 7.48

When comparing results, geometric means are used e.g..

$$\frac{\text{Log}_{10}(A) + \text{Log}_{10}(B)}{2}$$

**Weighted Mean Counting** is a technique to make use of plates at all countable dilutions:

Example 1

| <u>Dilution</u> | <u>Counts</u> |
|-----------------|---------------|
| $10^{-2}$       | 315, 286      |
| $10^{-3}$       | 46, 29        |
| $10^{-4}$       | 3, 7          |

$$\text{The weighted mean count} = \frac{315 + 296 + 46 + 29 + 3 + 7}{2.22} = 309$$

Example 2

If the count of 46 was missing from 1, then the weighted mean would be :

$$\frac{315 + 286 + 29 + 3 + 7}{2.12} = 302$$

### Alternative methods of analysis

Those available are:

- Direct examination
- Dye reduction tests
- Electrical methods
- ATP content of cells
- Immunological methods
- DNA/RNA hybridisation method

**Direct examination** – under a microscope, the lowest concentration of bacteria that can be seen is around 1 million per ml sample. If they can be concentrated by filtration and suitably stained, then a direct count can be made. The **Direct Epifluorescent Technique** is an example – it can be used for raw milk and other liquid foods.

The sample is clarified if possible and filtered through a polycarbonate filter. Bacteria retained on the filter are stained with a dye (acridine orange) and counted directly under an epifluorescence microscope (fluorescent light from above the sample). Counting can be manual or automated using image analysis.

**Dye reduction tests** – some bacteria metabolise rapidly in the presence of some dyes e.g. methylene blue or resazurin and de-colourise them. Tests using these principles were widely used in the past for assessing milk quality and are still used now, though less frequently. The limitation of dye reduction testing is that it detects the entire microbial population rather than potential spoilage agents e.g. psychrotrophs.

Samples are incubated in the presence of the dye. If de-colourisation occurs within a given time limit then bacterial numbers are high and the sample fails.

**Electrical Methods** – micro-organisms cause conductivity changes when growing in liquids, often by the production of acids. These changes can be detected between electrodes when a threshold conductivity is reached. Time to detection is inversely proportional to numbers of organisms initially present.

The sample is incubated in a broth containing a pair of electrodes connected to a central analytical unit. When conductivity changes are detected, the detection time is compared with a calibration curve and contamination levels are assessed.

**ATP Determination** – Adenosine triphosphate (ATP) content of microbial cells is usually proportional to their numbers. ATP also fuels light emission in fire flies. If the firefly ‘luciferin/luciferase’ chemicals are extracted from fireflies and exposed to microbial ATP then light emission is proportional to the quantity of microbial ATP.

A photomultiplier is used to measure light emission, which is proportional to microbial concentration.

**Immunological Methods** – the basic principle is the adhesion of an antigen (a micro-organism or its constituents) to a specific antibody (immunoglobulin) raised in animals. The simplest reaction when the two are combined is **clumping** (e.g. slide agglutination tests). Several sophisticated adaptations are used in modern microbiology.

**DNA Probes** – copies of DNA material identical with unique parts of DNA from bacteria can be prepared artificially. These copies (or probes) can be coupled to detection systems. DNA is released from the bacteria under investigation (lysed) and ‘mixed’ with

the probe. Modern technology enables the DNA to be amplified from small numbers of bacteria by a technique known as polymerase chain reaction (PCR). DuPont have a system called 'BAX'.

## Culture Media

General purpose culture media such as nutrient agar and yeast extract agar support the growth of many different bacteria, yeasts and moulds. They are used to obtain a general picture of microbiological condition of a raw material or product.

### Bacteria

- For bacteria, generally plate count agar and nutrient agar are used
- For acetic acid bacteria, YE agar, wort agar, universal beer agar and chalk agar can be used.
- For lactic acid bacteria, MRS agar, orangeserum agar and tomato juice agar are used.

### Yeasts and moulds

- Dichloran rose-Bengal Choramphenicol agar (DRBC) can be used but the rosebengal becomes inhibitory after two hours of exposure to light. DRBC is used for  $a^w > 0.95$
- Dichloran 18% glycerol agar (DG18) can be used if the sample has an  $a^w < 0.95$  (e.g. concentrates)
- Oxytetracycline glucose yeast extract agar (OGYE) is also used for yeasts.
- Malt extract agar can be used (if only yeasts are expected)
- Acetic dichloran yeast extract sucrose agar (ADYS), malt extract agar with 0.5% acetic acid, YM media - for acidophilic species (including preservative-resistant species of yeasts and moulds), which, when they do grow, cause big problems in specific food industries e.g. soft drinks. All are able to grow in at least 0.5% acetic acid.

### Yeast enrichment techniques

Low numbers are difficult to detect but present a serious spoilage potential. Optional techniques for enrichment include:

- Membrane filtration for clear liquids with direct plating of filter or into broth covered with oil.
- Centrifugation can be used but it may mean that only small volumes are screened.

Enrichment techniques can be used for viscous or pulpy material, where the product itself is diluted at least 1:1 with sterile water – this increases the water activity to allow spoilage yeasts to grow and also dilutes preservatives.

Half the product is decanted from the sample container & replaced with sterile water. The cap is left loose & then incubation at room temperature or 25°C, shaking daily to look for fermentation (bubbles). It may be useful to add ethanol and/or acetic acid. Large volume growth can be examined in large diameter plates.

Long incubation at low temperature (up to 14 days @ 25°C) is suggested, observing broths. Over this period, plating out onto general and selective agars is carried out, with observation under microscope.

### Confirmatory tests

The microscope is not used nearly enough for helping with confirming 'presumptive positives' – for example, are colonies on yeast and mould count actually yeast or bacteria? Are presumptive *Listeria* on an 'Oxford plate' actually Gram-positive rods?

To be effective, a microscope must be correctly aligned.

Some confirmatory tests are used for general identification, some for specific organisms.

**Gram staining** is a general test for confirmation. Bacterial cell walls are of two types

- Gram-negative – cell walls are rather thin and complex. They contain lipoprotein
- Gram-positive – walls are rather simple and thick. They contain relatively simple proteins

The Gram staining technique distinguishes between the two. It is important in food microbiology as Gram-negative bacteria thrive in moist and often cool conditions but Gram-positives thrive in preserved conditions.

A smear of young bacteria on a microscope slide is stained with an aniline dye, usually crystal violet. All cells take up the stain, which is a deep purple in colour. The stain is fixed in the cells as a complex using an iodine solution.

A solvent is then applied. Gram-negative bacteria lose the stain complex whilst Gram-positive retain it.

Gram-negative bacteria are counter-stained with a contrasting colour e.g. safranin. The stains are examined by microscope and Gram reaction and shape are recorded.

Gram-positive cells are purple whilst Gram-negative cells are red.

**The Catalase Reaction** – is used as some bacteria produce the enzyme ‘catalase’ that breaks down toxic peroxides.

One of a number of methods sees a small quantity of growth being placed on a slide and covered with a cover slip. Some hydrogen peroxide solution is then pipetted under the cover slip. The glassware used must be very clean; media containing blood should not be used as blood contains catalase. Some bacteria produce pseudo-catalase in low levels of glucose; the media should contain 1% or zero glucose.

**The Oxidase Reaction** – bacteria with a respiratory metabolism produce cytochrome c oxidase. This produces a rapid purple reaction with tetramethyl p-phenylene diamine dihydrochloride.

A solution of the reagent is placed on filter paper and the organism is added. Commercial reagent ‘dip-sticks’ are now available – they can be applied directly to a colony of the organism.

### Use of Basic Tests for Identification by Profile

It is too costly to do tests on all isolates. Simple identification tests can be used to develop a ‘profile’ for a bacterial isolate. This narrows down the number of possible names. Final identification can then be made by relatively few tests.

#### Use of profiles

##### Example 1

|                   |          |
|-------------------|----------|
| Gram reaction     | Negative |
| Cell shape        | Rod      |
| Catalase reaction | Positive |
| Oxidase reaction  | Positive |
| Spore production  | Negative |
| Mobility          | Positive |

- **Profile**  
Typical of *Pseudomonas*
- **Confirmation**  
Oxidative use of glucose

##### Example 2

|                   |          |
|-------------------|----------|
| Gram reaction     | Negative |
| Cell shape        | Rod      |
| Catalase reaction | Positive |
| Oxidase reaction  | Negative |
| Spore production  | Negative |
| Mobility          | Positive |

- **Profile**  
Typical of *Enterobacteriaceae*
- **Confirmation**  
Oxidative and fermentative use of glucose  
Reduction of nitrate to nitrite  
Resistance to bile salts

Commercially available kits can sometimes be used effectively. They contain a series of tests in a strip but need some knowledge of what organism is likely to be isolated. The isolate must be pure. These kits save on labour but are relatively expensive, plus interpretation can prove to be difficult.

### Preservatives and preservative-resistant organisms

Preservatives are chemicals that are used to prevent or delay the spoilage of foods and drinks. A large number of chemicals have been described that show potential as food preservatives, but only a small number are permitted in food and drink manufacture. Preservative addition should **NOT** be seen as a substitute for good manufacturing practice, since poor hygiene will encourage development of resistant micro-organisms.

Preservatives permitted in Soft Drinks manufacture include:

Sodium benzoate, benzoic acid  
Potassium sorbate, sorbic acid  
Sodium bisulphite, sulphur dioxide  
Sodium metabisulphite  
Dimethyl dicarbonate

**Benzoic acid/sodium benzoate** – was the first chemical preservative permitted in foods by the US FDA. Its antimicrobial activity is related to pH, the greatest activity being at low pH values. In acidic foods, benzoate acts as a yeast and mould inhibitor although it is effective against some bacteria in the 50-500ppm range. Sodium benzoate is used as it is more water soluble – benzoic acid has a tendency to precipitate. One drawback is that it can impart a slight flavour at high levels of usage.

**Sorbic acid/potassium sorbate** – is employed as a food preservative usually as the potassium salt. Levels must not exceed 0.2%. It is more effective in acid foods and is effective at lower acidities than sodium benzoate due to its dissociation properties.

Sorbates are primarily effective against moulds and yeasts, whilst less effective against bacteria. They are less soluble in cold water and may precipitate out. Also they are pretty expensive.

**Sulphur Dioxide** – used in its gaseous or liquid form, or in the form of one or more of its neutral or acid salts. It is a most effective and wide acting preservative. Concentrations of 100-200ppm are effective in fruit juices and beverages. However it cannot be used in conjunction with aluminium, it can bleach deep colours and can be detected by some consumers.

**Dimethyl dicarbamate (DMCD)** – acts as a sterilant, killing micro-organisms. It hydrolyses when in contact with water, so is not present when the consumer opens the product container. It forms methanol + CO<sub>2</sub>. Maximum permitted level is 250 mg per litre. Advice on application indicates it may not be active against ascospores.

### Problem Organisms

Some organisms can and have become resistant to some preservatives. These include both bacteria and yeasts. Some bacteria have been shown to be resistant to pasteurisation and capable of growth at low temperatures. Some organisms produce acetaldehyde which binds sulphur dioxide.

**Zygosaccharomyces bailii** – is one of the most problematic organisms in the soft drinks industry. Contamination has been known to shut a manufacturing plant down for days whilst a full hygiene programme was initiated.

It grows over pH range <2 and >7, is tolerant to acetic acid, can grow in high sugar levels of higher than 70% v/v and is heat tolerant at temperatures greater than 75°C. It has a benzoic acid tolerance of >1000ppm, a sorbic acid tolerance of >800ppm and an alcohol tolerance of > 20% v/v.

**Alicyclobacillus acidoterrestris** – this thermophilic spore-forming bacterium has been implicated in juice spoilage incidents in the UK and Germany. It is resistant to pasteurisation, is capable of growth at a wide range of temperatures and also produces taints.

Drinks and concentrates which are most susceptible are either fresh (not heat-treated) or pasteurised (but not UHT heat-treated) products that have been stored unpreserved at ambient temperatures.

## Which preservation system to use ?

Points to consider include:

- product parameters
- the microbial population of the plant
- organic acids
  - which are bacteria sensitive, yeast and mould resistant ?
  - which organisms have a variable activity ? acetic>lactic>citric
  - which organisms are resistant to >0.5% acetic acid ?  
*Penicillium roqueforti*, *Monascus ruber*, *Candida krusei*  
*Schizosaccharomyces pombe*
  - which are resistant to > 4% acetic acid ?  
*Moniliella acetoabutans*
- acid preservatives
  - used for controlling yeast and moulds but resistant organisms exist
  - activity depends on pH (dissociation)
- heat
  - will the product withstand a heat process ?
  - which organisms are resistant to heat ?  
*Bsyssochlamys fulva*, *Alicyclobacillus acidoterrestris* are heat resistant in different environments e.g. high sugar
- combined systems, hurdle (barrier) technique
- legal maximum concentrations
- organoleptic considerations
- economics of use
- change in formulations/recipes
- finally, should preservatives be used when there is rarely a problem ?

**NB Please refer to your own company specifications as regards which methods of sampling, identification and rectification are used in your business as the information detailed above is general to the manufacture of soft drinks and may be non-specific as regards your own company requirements.**

## 2.6.4.2 Analytical Testing

### Analytical testing of carbonated soft drinks

#### Pre-carbonation

In the preparation of soft drinks, analytical testing is carried out on batches of prepared syrup or on finished product prior to carbonation. Carbonated samples must be de-gassed prior to analysis for brix and acid testing to remove the effect that CO<sup>2</sup> can have on the product characteristics. The tests will confirm whether or not the product is within customer specification and include:

- quantitative brix testing using refractometer or density meter to gauge sugar level in syrup or final product
- quantitative acid testing (for non-sugar drinks) using titration
- qualitative taste testing by comparing a production sample of carbonated product with a standard (usually from a sample from the previous production run for the same product)
- quantitative testing for specific ingredients e.g. caffeine, aspartame using chromatography analysis, where a sample is injected into a gas or liquid chromatograph column & the effluent from the column is analysed to provide a 'fingerprint' result that can be compared to a standard, from which the amount of ingredient can be measured to ensure compliance with product formulation and legislated limits.

Technical training of operators must be carried out to ensure that these key areas of analysis are conducted with precision and accuracy, with recording of results held for traceability purposes.

Sensory testing of product in pre-carbonated form must be carried out to ensure conformance to specification – this is usually in the form of taste testing and visual appearance against standard samples or samples from previous production runs.

Further analytical checks are also conducted on treated water being used for product make-up. These include measurement of dissolved O<sub>2</sub> following the de-aeration process in water treatment.

#### Post-carbonation & filling

Some modern production lines in soft drinks manufacturing feature in-line automated measurement of product characteristics such as carbonation and brix. Where this instrumentation is used, it is **essential** that regular calibration of instruments be conducted as part of a formal quality plan.

Even when this has been seen to be in place, there is always a risk that a pressure gauge may drift away from the calibrated standard and that subsequent readings may be outside of specification, leading to production of sub-standard product..

Essentially the key areas of quantitative testing with carbonated soft drinks after filling are:

- Carbonation
- Brix/acid testing
- Check-weighing of packs
- Closure conformance

#### Carbonation determination

Whilst this has already been covered in the product preparation module, it is worth re-capping to aid understanding.

It is necessary to consider the physics associated with carbonation. CO<sup>2</sup> is soluble in water or a soft drink, the degree of solubility increasing as the liquid temperature decreases. Equilibrium is reached when the gas leaving solution equals the amount entering. The amount of carbon dioxide that a solution can maintain depends on temperature and pressure. There is an equilibrium pressure at a given temperature at which this occurs. If the pressure is decreased, or the temperature increased, the carbon dioxide will come out of solution. The laws of physics, which apply to this are:

- **Henry's Law**, which states,

'The amount of gas dissolved in a given volume of solvent is proportional to the pressure of the gas with which the solvent is in equilibrium'

and

- **Charles Law**, which states,

'The volume of an ideal gas at constant pressure is directly proportional to the absolute temperature'.

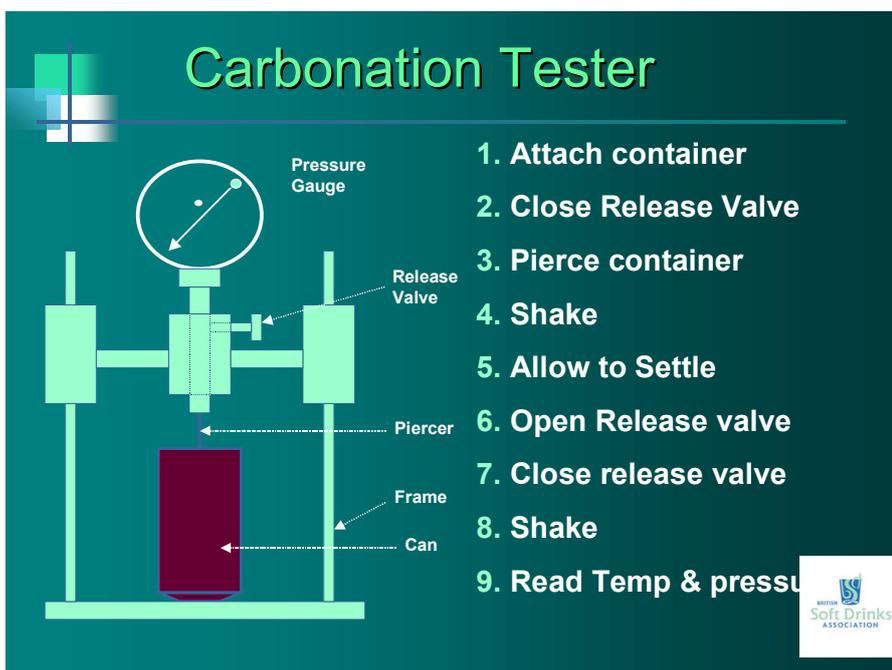
If the temperature and equilibrium pressure of a product are known, then there must be a fixed carbonation level. The amount of CO<sup>2</sup> in terms of volumes. It is the number of times the total volume of dissolved gas can be divided by the volume of liquid in the container. For example, a 4 volume carbonated product will contain CO<sup>2</sup> to the extent of 4 times the volume of the beverage. If this were a 2 litre bottle, this would be 8 litres of CO<sup>2</sup>. In mainland Europe, the unit of **grams per litre** is often used, where 1.92 gms per litre equals 1 volume CO<sup>2</sup>.

Carbonation is determined by :

1. Taking a sample filled & sealed container from the production line or from warehouse stock, shaking it to equilibrium and noting the internal pressure and product temperature.. There are several variations of this procedure but the following methods are recommended by the American Bottlers of Carbonated Beverages and have been widely adopted elsewhere in the world.

- Containers direct from production line. The container is punctured with a pressure gauge device and shaken until maximum pressure is attained. It is then allowed to settle for 30 seconds and the container internal pressure released. The release valve is closed and the container is shaken again to maximum pressure. This pressure is recorded from the gauge, the product temperature is read and then the gas volume is read from standard graph, table or through use of calculator.
- Containers from stock. The container is punctured gently with no agitation – the pressure is released, valve closed and the container re-shaken to maximum pressure. Again, after recording the temperature, the gas volume is read from graph, table or calculator.

Compliance with the above procedures, sometimes known as the 'second-shake methods', minimises the misleading effect of a high air content in the beverage. Below is a diagram of the traditional stationary carbonation tester – this has now been modified by connecting the apparatus to a revolving motor, in order to standardise the 'shake' motion for repeatability purposes.



2. Laboratory test. The CO<sub>2</sub> is boiled off from a sample of product and the volume of CO<sub>2</sub> measured at atmospheric pressure. This is converted to 32°F and the liquid volume measured, the ratio of gas/liquid gives carbonation in Volumes Bunsen.

Where in-line instrumentation is used, sensors may be fitted to alarm any out-of-specification condition that in turn prevents filling sub-standard product by stopping the filler.

Some typical product carbonation levels are as follows:

| Product type      | Carbonation (in volumes of CO <sub>2</sub> ) | Carbonation in g/litre | Comments                                                                                                       |
|-------------------|----------------------------------------------|------------------------|----------------------------------------------------------------------------------------------------------------|
| Lightly sparkling | 2.0                                          | 4                      |                                                                                                                |
| Fruit carbonates  | 2.5                                          | 5                      | Relatively low levels in comparison to other drinks in order to enhance the fruit flavour                      |
| Lemonades         | 3.0 to 3.5                                   | 6-7                    |                                                                                                                |
| Colas             | 4.0                                          | 8                      |                                                                                                                |
| Mixer drinks      | 4.5 to 5.0                                   | 9-10                   | Relatively high levels as when mixed with spirits e.g. whisky, gin, vodka, the level of carbonation is diluted |

The above levels of carbonation will vary depending on the manufacturer specification for a particular drink.

Levels of carbonation will also vary according to the packaging container. Products filled into PET bottles will be 'over-carbonated' at the filling stage by approximately 10% as the level will reduce somewhat in the period between filling and eventual consumption by the consumer and this needs to be counteracted by slightly higher levels of CO<sub>2</sub> in the product during manufacture. The unstable nature of PET will allow CO<sub>2</sub> to leech through the walls of the bottle in the initial 3 months from filling. Also, a large bottle of Cola e.g. 2litre may not be consumed at one time so the carbonation level and pack will need to be capable of retaining a reasonable level of gas during the period of ullage.

The loss of CO<sub>2</sub> through the product container is not seen when product is filled into aluminium cans, so filling carbonation varies between canning and bottling of carbonated product.

**You must familiarise yourself with the varying carbonation specifications of the product range filled by your manufacturer and the reasons why.**

### Brix/acid testing

As with carbonation, if in-line instrumentation for brix control is used, regular checks must be in place to ensure that all product is within specification. In the absence of in-line automation, and where product mixers are used, regular samples must be taken, at start-up of production and during a run, and brix measurements taken using a refractometer to measure the sugar level or 'brix'. With the common Mojonnier mixer, fine control of brix can be achieved by vernier adjustment of the variable water orifice

Typical brix and acid readings for product ranges are as follows:

| Product type                                 | Typical brix reading | Typical acid reading |
|----------------------------------------------|----------------------|----------------------|
| Colas                                        | 10.5 degrees         | n/a                  |
| Lemonades                                    | 4.0 to 4.5 degrees   | n/a                  |
| Diet or non-sugar lemonades                  | n/a                  | 0.2 to 0.3%          |
| Fruit carbonates                             | 5 degrees            | n/a                  |
| Non-fruit mixer drinks e.g. tonics, ginger   | 5 to 9 degrees       | n/a                  |
| Non-fruit, non-sugar mixer drinks            | n/a                  | 0.15 to 0.4%         |
| Fruit mixer drinks e.g. bitter orange, lemon | 8 to 9 degrees       | n/a                  |
| Diet or non-sugar fruit mixer drinks         | n/a                  | 0.3 to 0.4%          |

As with carbonation, you must know and understand the differing brix and acid specifications for your company's product range, along with the consequences of any variation during both the preparation of the drink and the filling stage into containers.

### Check-weighing of packages

The function of the filling machine, whether it be for cans or PET/glass bottles, is to transfer the prepared product into the container in the correct volume and without affecting the product quality in any way.

To check the delivery of the correct quantity and quality of product, samples should be taken ideally from each filling head in sequence over an agreed period of time.

Regular samples of product in container are taken after filling and the contents measured to ensure that they meet the average weight/volume legislation requirements. By law, filling containers at a level less than specified on the product label is not permitted, whilst over-filling is wasteful and costly. It is therefore crucial to measure and control the filling operation as keenly as possible.

Soft drinks are commonly checked for contents by weighing samples ex-filler against an empty container & closure. The weights are converted to volume taking into account the specific gravity of the drink being checked.

Records of measurement must be retained for external audit by law to ensure compliance.

**You must familiarise yourself with the adopted legislation or codes of practice relating to declared filling volumes in your country as this is vitally important in terms of consumer trading practice.**

To help understand some of the terms used in volume declaration, here are some useful definitions:

- Nominal Quantity ( $Q_n$ ) is the amount stated on the pack label
- Negative Error is the amount below the nominal quantity i.e.  $Q_n$  - net fill

The following table lists Nominal quantities and what is termed the Tolerable Negative Error

| Nominal Quantity ( $Q_n$ ) (g or ml) |       | Tolerable Negative Error |         |
|--------------------------------------|-------|--------------------------|---------|
| From                                 | To    | % of $Q_n$               | g or ml |
| 5                                    | 50    | 9                        |         |
| 50                                   | 100   |                          | 4.5     |
| 100                                  | 200   | 4.5                      |         |
| 200                                  | 300   |                          | 9       |
| 300                                  | 500   | 3                        |         |
| 500                                  | 1000  |                          | 15      |
| 1000                                 | 10000 | 1.5                      |         |
| 10000                                | 15000 |                          | 150     |
| above 15000                          |       | 1                        |         |

In many countries, an 'average-fill system' is used – this recognises the statistical nature of the filling process and provides safeguards to prevent under-filling below certain levels. The requirements placed on the manufacturer are as follows:

- The actual contents of the packages produced shall be not less, on average, than the stated nominal quantity (declared on the package label)
- Not more than 2.5% of the packages may be non-standard i.e. have negative errors larger than the Tolerable Negative Error, specified for the nominal quantity.
- No package may be inadequate i.e. have a negative error larger than twice the specified Tolerable Negative Error

Providing that the filler machine is cleaned & hygiene according to the recommended schedule as laid down by supplier & owner, no particular hygiene problems relating to filling of containers should be encountered. However, if complaints are received that suggest a microbial spoilage problem, by analysing the number and nature of complaints with the total volume produced in a batch of product, an indication may be obtained as to whether the issue relates to the bulk batch or to one or more filling valves or indeed capping heads associated with the filler.

## Closure conformance

Whether it's bottle caps or can ends, the efficiency of the closure operation is another key part of the manufacturing operation that requires constant attention and measurement to ensure compliance with specification.

Industry standards are in place to provide guidance for tolerances and, as with carbonation, brix and fill volume measurement, records of results of closure checks must be retained for audit trail purposes in the event of quality issues.

Seam checks and analysis for soft drinks can closing is identical to that used in beer canning. Cap torque measurement similarly is the same principle as used with screw cap application.

Qualitative checks on crowns where used in soft drinks manufacture are the very same as with the bottling of other beverages such as beer.

## HACCP

The soft drinks industry prides itself on producing high quality, safe drinks, The HACCP system is a best practice approach to preventing poor quality or unsafe drinks from reaching the consumer. HACCP is a system that identifies, evaluates and controls hazards, which are significant for food safety.

HACCP helps to comply with Food Legislation, as there is a need to implement and maintain a food safety management system. For HACCP to be successful, you must already good standards of hygiene through Good Manufacturing Practice, the pre-requisites of safety support measures.

A Food Safety Hazard is a potential cause of harm or injury to the consumer from contamination of a soft drink. There are three types of hazards:

- Physical – foreign bodies such as glass or metal
- Chemical – dangerous fluids or residues
- Biological – micro-organisms and their poisonous by-products

Hazards can occur at any stage in the preparation from receipt of raw materials through to product handling and consumer use.

HACCP requires a soft drinks company to map its individual processes and identify all possible hazards and their cause at every step in the manufacturing process and decide how to control them.

### Example:

|                    |                                                          |
|--------------------|----------------------------------------------------------|
| Process step       | – inline filter                                          |
| Hazard description | – physical contamination with metal because filter fails |
| Control measure    | – intact filter                                          |

## Action

Some points in the process are essential and are identified as Critical Control Points (CCPs) These are the last points in the process where a particular hazard will be reduced to an acceptable level eliminated – no subsequent process activity will make the product safe.

Vital actions at CCPs include control by specified procedures and equipment. These controls are supported by the underpinning pre-requisite systems of calibration, training, repairs and maintenance. To ensure the control activities happen effectively, CCPs must be monitored by either observation or measurement of control parameters such as time, temperature, pressure or chemical concentration, either on a continuous basis or at predetermined intervals.

Planned corrective action will need to be taken at a CCP when loss of control is detected to stop unsafe drinks reaching the consumer and to bring the CCP back into control. HACCP requires documented procedures, alongside efficient and accurate record keeping.

## Keeping HACCP going

HACCP is not just a 'one-off' project. It is a practical system in use all the time we manufacture. It is therefore essential to find out whether the HACCP plan developed is:

- Valid
- Happening in practice
- Corrected when changes in operation occur (e.g. when a new product is made or piece of equipment is introduced)

Regular reviews and audits are needed. Most of these will happen within the company but sometimes there may be audits from an external company or an enforcement body.

**The benefits of HACCP** can be best summarized as:

- Saves time and money in the long run
- Avoids harming or injuring the consumer
- Food safety standards are improved
- Ensures compliance with the law
- Develops a focussed system
- Requires everyone's involvement
- Improves teamwork and efficiency
- Not a project – a practical system
- Knowledge of process increases
- Supports a Due Diligence defence

Spelling out ..... **SAFE DRINKS**

For HACCP to be effective, everyone must be involved, follow procedures exactly at critical control points and receive good instruction and training.

### 2.6.4.3 Full Line Inspection

#### **Purposes and principles of inspection**

With high speed manufacture of soft drinks in bottles and cans, the inspection of materials and packaging is most important. Much of this is now carried out by automation and electronics although there is still the need for human intervention.

Much emphasis nowadays is placed on the responsibility of suppliers of packaging to ensure that it conforms with agreed specifications – it is the supplier who must ensure that quality is not impaired so as to produce defective goods. However, this does not remove the on-going need for all staff to be aware and vigilant for any problems.

Where automation is employed for inspection, manufacturers must have in place systems of calibration and regular maintenance programmes to ensure that all inspection equipment is working as it is intended.

The processes, equipment and procedures for soft drinks are almost identical to those used in the brewing industry, so reference at this point can be made to Unit 1.4.7.1 Full Line Inspection Procedures

This applies to glass bottling, PET bottling and canning lines.

#### ***Canning and PET Bottling lines – some general points***

**Visual and minor faults at depalletization** – human intervention is almost certainly required to cover off any specific faults that may escape automatic inspection.

**Vacuum bridge** – this is a simple transfer mechanism in air conveyors for empty cans where defects in can rims can be detected and affected cans rejected by means of gravity to waste collection beneath the conveying gap.

**Fill level inspection of both bottles and cans** – entirely carried out on closed containers by electronic means using radioactive sources or ultrasonic detection.

**Coding checks** – although much coding equipment is now fully or semi-automated, it is vital that checking of correct coding is carried out as part of the operator or technician job responsibilities. It is common for this to be a shared responsibility all the way along the production line so as to avoid errors. Maintenance and cleaning must be carried out as part of a regularly planned routine within the job parameters.

**General pack inspection** – visual inspection by line operators and technicians should ensure compliance with specification.

**PET closure and label inspection** – both these key checks are carried out electronically by use of sensors incorporated into the capper and labeller systems with regular calibration and maintenance both key to effective operation.

#### 2.6.4.4 Legislative Declarations

##### Why is there nutrition labelling on soft drinks?

Nutrition labelling helps the consumer make informed choices about the drinks they and their families enjoy.

In the UK, full nutritional information now appears on the labels of soft drinks and a new scheme GDA (guideline daily amount) which has been introduced progressively across the range of soft drinks makes such information even easier to use. **You must identify if there is a similar approach in your own locality or country – the following information is for guidance and applies to the UK.**

GDAs are a guide showing recommended daily levels of different nutrients in the diet and are based on a healthy adult of a normal weight. They are made up of five key ingredients

- calories
- sugar
- fat
- saturated fat
- salt

and put this information in context by relating it to a healthy diet, showing per portion information as a percentage of the GDA.

GDAs have been in use in the UK since 1998 and are generally used throughout the food and drinks industry. They are also becoming increasingly common in Europe and Australasia and similar 'benchmarks' called 'dietary values' are now widely used in the USA and elsewhere.

GDA labels can be found on carbonated drinks, still and dilutable drinks, fruit juices and flavoured waters.

More than 50 companies have committed to put consistent GDA labels on the front of 20000 products. Over £4million has been invested in advertising and educational campaigning that not only makes the public aware of the existence of the labels but encourages them to make more informed choices about the products they are consuming. The labelling scheme has been also been adopted by over 50% of the retail market with the major supermarket chains leading the programme, further increasing awareness and use of the labels.

By 2007, according to market research, the majority of consumers were already aware of GDA labelling and around half of consumers claimed to have actively used these food labels when making purchase decisions.

Consumers claim to use the label for controlling, comparing, counting and checking the content levels of particular nutrients. The research also showed that 84% of consumers said it was 'very' or 'quite easy' to understand the labels. 82% said they would like to see GDA labels used on even more brands.

The GDA scheme uses numbers instead of colours because it makes it easier for consumers to tell if a product contains a higher or lower amount of a particular nutrient, compared to another product. GDA labelling is based on realistic portion servings whereas the previously used 'traffic light labelling was applied per 100g regardless of how the food or drink was actually consumed.

### **Using as an example – UK Bottled water**

The information on the label of a bottle of water provides a lot of information for the consumer. It is important that this information is accurate in order to protect the consumer and ensure fair trade.

In the UK, the British Soft Drinks Association represents the industry interests and has worked with the Food Standards Agency to agree guidance for the benefit of consumers, the industry and the enforcement profession.

### **What is bottled water?**

There are three different denominations of bottled water:

- Natural Mineral Water – comes from a named source, has a consistent mineral composition and is untreated
- Spring Water – comes from a named source, may be subject to certain permitted treatments
- Bottled Drinking Water – any other packaged water

The regulations that cover them are The Natural Mineral Water, Spring Water and Bottled Drinking Water Regulations 1999 and subsequent amendments.

Bottled water may have carbon dioxide added to it. Minerals may be added to Spring Water or Bottled Drinking Water, but not Natural Mineral Water.

If organic materials such as sweeteners or flavourings are added to water, it becomes a soft drink and is not classified as water.

### **Sales description**

**Natural Mineral Water** – the sales description must be one of:

“Natural Mineral Water” referring to a still or non-effervescent product.

“Naturally Carbonated Natural Mineral Water” meaning an effervescent Natural Mineral Water whose carbon dioxide content is the same after bottling as it was at source.

“Natural Mineral Water Fortified with Gas from the Spring” meaning an effervescent Natural Mineral Water whose carbon dioxide content derives from the same ground water but the carbonation level after bottling is greater than that in the source.

“Carbonated Natural Mineral Water” meaning a sparkling Natural Mineral Water which has been carbonated at least in part with carbon dioxide from another origin.

**Spring Water** – for a Spring Water, the sales description is Spring Water with any reasonable qualifier such as “sparkling “ or “carbonated”. It should not include the words “natural” or “mineral” in order to prevent possible confusion with Natural Mineral Water.

**Bottled Drinking Water** – the following terms may be used in the sales description of a Bottled Drinking Water, with the meanings as described:

- Blended – a mix of more than one source
- De-ionised – water in which most of the major ions have been removed by de-ionisation.

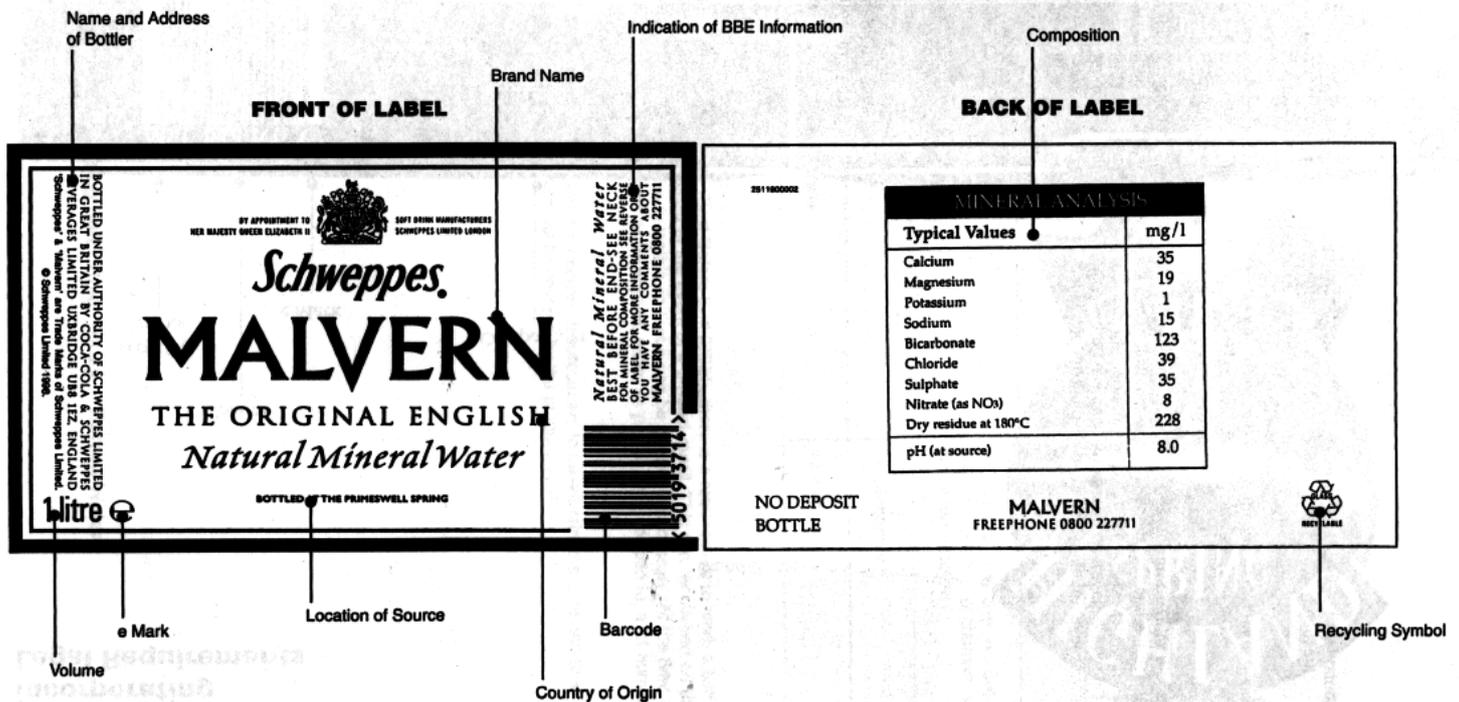
- De-mineralised – water which has been subject to distillation, reverse osmosis or deionisation
- Purified – water which has been treated to remove pollutants or disinfectants
- Re-mineralised – water which has been made up to a particular chemical composition.
- Sparkling – can be used where the product is carbonated
- Still – can be used to indicate a non-carbonated product.

### Prohibited terms

**Natural, Mineral and Spring** – these words all have specific meanings in the context of the denominations of water to which they apply and should therefore not be used in any other way.

**Pure** – the UK Food Standards Agency advises against use of this word on bottled water.

**Organic** – no water may be called “organic” but the agricultural land in the catchment area may have organic approval.



### Example of a bottled water label

### Other Information on the Label

**Name of the source** – both Natural Mineral Water and Spring Water must be drawn from a named source. The name of the source must appear on the label in type at least 50% bigger than any part of the trade description. It is not permitted to market Natural Mineral Water or Spring Water from any one source under more than one trade description

**Chemical analysis** – Natural Mineral Water must have a consistent mineral composition; this must appear on the label in a standard format prescribed in the regulations. Other waters may carry a chemical analysis in the standard format if their mineral composition is consistent.

**Infant feeding** – while it may be permitted in some European countries, no reference to infant feeding is currently permitted on bottled waters in the UK.

**Language of the label** - all food and drink, including bottled water, must be labelled in a language easily understood by the consumer.

In the UK, this is taken to mean English. Regulations specify that certain information must be given including details such as ingredients, name of the food, best before/use by date and directions for use. The brand name alone is not sufficient. The retailer has a responsibility under the law to ensure that products are not sold that contravene the relevant legislation relating to food labelling. Any retailer that does could face a fine up to £5000.

The information on a label provides the consumer with essential information. This protection is undermined if the label is in a language that the consumer does not understand.

Consumer satisfaction can be affected – a drink might not taste the way it was expected to due to poor storage or a different recipe – some brands have different recipes in different countries. Also it is difficult to contact a foreign help-line, either with a consumer complaint or to take up an on-pack promotion.

The risks with foreign language labelling are:

- If labelling is incomplete, a product recall would be extremely difficult if not impossible
- Wrongly or incomprehensively labelled products could have serious implications for diabetics and people with allergies and intolerances.

**Other claims** – any health and compositional claims must comply with the regulations.

### **Flavourings and legislation**

Most food ingredients have been well investigated in terms of use and effect and are categorised and registered on permitted lists as appropriate to local legislation.

Flavourings, however, by virtue of their complexity have always existed as a separate group. Distinction is made under various systems as to whether flavourings are of natural origin. This follows adoption by the UN Food and Agriculture Organization (FAO/WHO Food Standard Programme, the Codex Alimentarius Commission, of a proposal put forward by the International Organization of the Flavour Industry (IOFI) circa 1975, to divide flavouring substances into three groups:

- Natural flavours and natural flavouring substances – these are preparations and single substances, acceptable for human consumption, obtained exclusively by physical processes from vegetable, and sometimes animal, raw materials either in their natural state or processed for human consumption.
- Nature-identical flavouring substances are substances chemically isolated from aromatic raw materials or obtained synthetically. They are chemically identical to substances present in natural products intended for human consumption, whether processed or not.
- Artificial flavouring substances are, for the purpose of the Codex Alimentarius, those substances which have not yet been identified in natural products intended for human consumption, whether processed or not. They are hence only made available via synthesis.

The most frequently used register of flavouring substances appears in the GRAS listings compiled by the 'Flavour and Extract Manufacturers' Association (FEMA) of the USA. They comprise those substances 'generally recommended as safe' when used in the minimum quantities required to produce the intended physical (sensory) effect and in accordance with the principle of good manufacturing practice. Each substance is allocated a FEMA number to enable cross-referencing with other listings., e.g. those of the Council of Europe, US Food and Drug Administration (FDA) and Chemical Abstracts Service (CAS).

In Europe, flavourings have generally been considered to be compound ingredients, and concern has been expressed about the safety of their 'undeclared' components. Following extended interaction with representatives of the European flavour industry, trade associations and other bodies, a new list has been drawn up of all chemical substances currently in use for flavourings, in the member countries. This was published in 1999 and the register has in excess of 3000 entries for flavouring substances used in or on foodstuffs. Work continues in assessing the level of health risk that may be associated with these ingredients, with a view to limiting their use as necessary.

## **Colours**

The use of food colours is carefully controlled under various pieces of legislation. There is no universal listing of colours for use in soft drinks, and it is necessary to investigate the permitted list to ensure compliance for goods to be manufactured in, or exported to, a specific country.

Both the EU and the FDA have published lists that are regularly reviewed. Most concerns have been expressed over the use of azo colours, to which some people have an allergic reaction – most frequently to sunset yellow and tartrazine.

Food colours are broadly divided into two classes – natural and artificial. In the US, they are listed as either 'exempt from certification' or 'certified colourants'. The natural colours are botanical extracts, with the exception of carmine (a red colour) which should be termed perhaps as an entomological extract as it is obtained from the insect *Dactilopius coccus*, or cochineal beetle, which breeds and feeds on particular cacti indigenous to Central America.