# Module 2

## 2.5 Quality

2.5.1 Quality management
2.5.2 Laboratory analysis
2.5.3 Sensory analysis
2.5.4 Hygiene

DIPLOMA IN DISTILLING

# Contents

## Abstract

In this final Unit of the Diploma of Distilling module 2, 2.5 Quality, we will look at the considerations for quality assurance and product analysis.

We will start with an examination of Quality Management (2.5.1), including the various international standards. We will then look at Laboratory Analysis (2.5.2) and Sensory Analysis (2.5.3) before discussing plant cleaning and Hygiene (2.5.4)

## Learning Outcomes

On completion of this section you should be able to:

1. *Explain the requirements for ISO 9001 accreditation.*
2. *Describe laboratory testing for alcohol and congener concentrations.*
3. *Understand the principles of CIP systems.*

## Prerequisite Understanding

Basic scientific knowledge and terminology.



© The Institute of Brewing & Distilling 2017

## 2.5.1 QUALITY MANAGEMENT

### Introduction

Quality Assurance is the organisation and management of tests and audits to ensure that quality standards are defined and the product is prepared so as to meet or exceed these standards consistently. Typically the following factors are involved:

- Definition of requirements and responsibilities for quality of processes and materials
- Definition of the required properties of the product
- Definition of the materials and processes involved
- Definition of the number and qualifications of personnel
- Assessment of factors affecting quality
- Defnition of quality assurance actions: education, training and motivation; requirements for and methods of analyses (quality control); documentation of results
- Definition of responsibilities for quality assurance actions

### Quality Assurance standards

ISO 9000 is a family of standards for quality management systems maintained by the International Standardization Organization and administered by accreditation and certification bodies. ISO 9001 is the internationally recognised standard for an organisation's Quality Management systems, incorporating all the activities associated with quality. Basically there are two principal requirements (a) a well-planned and well-managed quality system and (b) documentary evidence that such a system exists and is operating properly A company that has been independently audited and certified with ISO 9001 has proof that formalized business processes are being applied, although this does not necessarily guarantee the quality of the company's products.

The requirements in ISO 9001:2008 include

- A set of procedures that cover all key processes in the company,
- Monitoring processes to ensure they are effective;
- Keeping adequate records;
- Checking output for defects, with appropriate and corrective action where necessary;
- Regularly reviewing individual processes and the quality system itself for effectiveness; and
- Facilitating continual improvement

The quality policy is a formal statement from management, closely linked to the business and marketing plan and to customer needs. The quality policy is understood and followed at all levels and by all employees. Each employee needs measurable objectives to work towards. Decisions about the quality system are based on recorded data and the system is regularly audited and evaluated for conformance and effectiveness. Records must show how and where raw materials and products were processed, to allow products and problems to be traced back to their source. ISO 9001 stipulates a need to determine customer requirements: that may not apply literally to the actual distilled spirits, but certainly it is necessary to create systems for communicating with customers about product information, enquiries, contracts, orders, feedback and complaints.

Regularly reviews of performance are required, through internal audits and meetings. It is necessary to determine whether the quality system is working and what improvements can be made. Records must be kept of the procedures and decisions for dealing with past problems, and for anticipating potential future problems, and monitoring their effective-ness. Also required are documented procedures for dealing with actual and potential problems involving suppliers or customers, or internal problems e.g. a faulty batch of product: how to deal with the cause of the problem and keep records to use in improve-ment of the system in future.



© The Institute of Brewing & Distilling 2017

ISO 9001 can provide the basis, and the stimulus, for Total Quality Management. This involves an understanding by all staff of the advantages of operating a strict documented quality assurance system at all levels, controlled by system auditing and management review. TQM includes the following principles:

- Quality must be managed. Management must be involved, and lead. Implementing TQM starts with top management and flows downward.
- Every employee has a customer and is a supplier
- Every employee is responsible for quality
- Problems must be prevented, not just corrected
- Quality must be measured
- The quality standard is defect-free
- Quality improvements must be continuous, and planned and organised accordingly

A quality control system involves a variety of quality assurance techniques and co-operation between all departments, to their mutual benefit. Although the scope of TQM is much wider, NAMAS and HACCP are briefly described below as examples of the application of documented procedures to maintenance and improvement of standards.

National Accreditation of Measurement and Sampling (NAMAS) by the United Kingdom Accreditation Service (UKAS) means that testing and calibration laboratories have been assessed against internationally recognised standards to demonstrate their competence, impartiality and performance capability. All aspects or company operation can be evaluated: not only the laboratory methods and equipment and the final product, but also management systems or organisation. Such accreditation forms part of the assessment for ISO 9001. Also, accreditation by UKAS confirms that external consultant laboratory, certification or inspection services meet international accreditation standards.

## HACCP

The concept of Hazard Analysis and Critical Control Points was invented to ensure the safety of processed food and drink products. It is not concerned with sensory quality being out of specification since that by itself is not a health hazard. It is true that similar principles can be applied to maintaining sensory quality, but that is not HACCP.

The original purpose of HACCP was to ensure as near as possible to 100% food safety on NASA space flights, but it quickly became adopted for food and drink processing in general. The important innovation of HACCP was that instead of quality checks on representative samples of the product, the manufacturing process itself was assessed to identify and eliminate hazards. It is not necessary to monitor every step. Only those steps where a significant hazard exists need to be considered as critical points for process control. For potable spirits, CCPs will be mainly associated with packaging, e.g. mcrobiological quality of the dilution water and the risk of broken glass or other foreign material in bottles are health or safety issues. Except for compounds like ethyl carbamate, methanol and NDMA, any faults earlier in the process, whatever their effect on sensory quality, do not make the product dangerous.

As a first step a team must be set up with collective expertise in all stages and aspects of the process, to decide the objectives of the plan and then set it in operation. Although the precise details of the system can be different in each company, and perhaps even for each product within the company, there is general agreement that the principles of HACCP are:

- Prepare a detailed flow diagram identifying every stage of the process. Use both that diagram and on-site inspection to conduct a hazard analysis of the entire process. Prepare a list of steps in the process where potential hazards exist, and specify the preventative measures which are required.



© The Institute of Brewing & Distilling 2017

- Then consider which of these steps have to be designated as Critical Control Points. Figure 2.8.1 explains the reasoning for choice of CCPs. A CCP for a later stage of the process may also be able to control a separate upstream hazard, in which case the earlier CCP is redundant. For operational efficiency and plant-friendliness, unnecessary CCPs must be avoided. Identify each CCP and the corrective action to be taken.

- Establish critical levels for preventive measures associated with each identified CCP. Each CCP will have one or more critical measures that need to be properly applied to ensure prevention or elimination of hazards. Each control measure must have critical levels as safety boundaries.

- Establish CCP monitoring requirements and procedures for using the results of such monitoring to adjust the process and maintain control.

- For each CCP, establish corrective action to be taken when monitoring indicates that there is a deviation from a critical limit.

- Establish effective record keeping procedures for the HACCP system. The HACCP plan and associated support documents, including corrective action records, must be on file and kept up-to-date.

- Establish procedures to verify that the HACCP system is working smoothly.

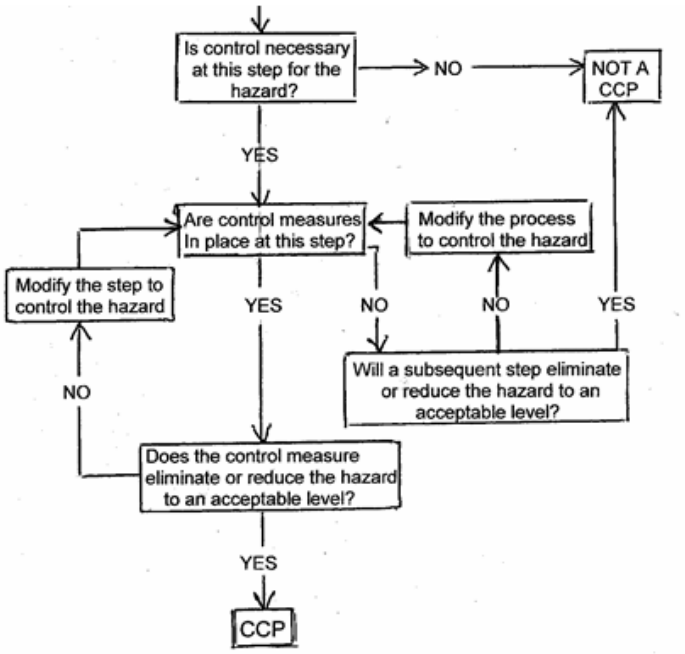



*Figure 1 "Decision tree" for specifying a Critical Control Point (CCP)*

## 2.5.2 Laboratory analysis

### Laboratory analytical techniques

With the exception of alcoholic strength of the final product, the analyses outlined below are not essential for the production and marketing of distilled spirit beverages. Although all could be useful in certain circumstances, they are not necessarily carried out routinely.

### Alcohol determination

Measurement of alcohol content is required at various stages of spirit production. Officially the exact measurement of alcoholic content of a spirit beverage is determined as the density of a sample distilled in the laboratory, to eliminate the effect of dissolved solids, but normally that procedure is required only occasionally for the calibration, or confirmation of the accuracy, of more convenient routine methods. The two methods in common use are hydrometers and electronic density meters, the latter based on resonant frequency of oscillation of the



© The Institute of Brewing & Distilling 2017

sample, which is related to density. Provided the correct hydrometer is used, i.e. calibrated for maximum accuracy in the range of alcoholic strength of interest, both methods provide the accuracy of +/- 0.1% which is required for production and revenue purposes. Also, an electronic density meter can be fitted in-line to provide a continuous record of alcohol strength.

## Gas-liquid chromatography

Depending on the analysis, the injected sample can be liquid spirit, head-space vapour or material extracted by a suitable solvent for the compound(s) of interest. Alternatively sample preparation could be by steam distillation, e.g. of the phenols associated with peated malt for some Scotch malt whiskies.

Although GLC can be used for some routine measurements of ethanol, its typical accuracy of +/- 1% is not precise enough for excise records or confirmation of bottling strength. However, GLC is suitable for a wide range of analyses of volatile compounds, and non-volatile compounds can also be measured if quantitative conversion to volatile derivatives is possible, but for the latter it is more convenient in practice to use.HPLC. The GLC column is normally narrow-bore glass or stainless steel tubing 2 or 3 m in length, coiled to fit the accurately attemperated oven of the apparatus. With specific packing, flow rate of carrier gas and oven temperature (or accurately programmed increase of temperature), when the system has been calibrated with known amounts of each compound of interest, the residence time between injection and detection at the far end of the column identifies the compound. The inert carrier gas is usually nitrogen, but for some purposes argon or helium is preferable. The size of the peak (to be strictly accurate, the area under the peak) of the detector response indicates the amount. An internal standard, a compound related to those being analysed but which would not be present naturally, must be added at a known amount to each sample to recognise and compensate for slight variations in residence time and/or detector response. As an alternative to inert packing, modern capillary tubing columns use the inner surface of the tube itself is the support. A flame ionization detector, which measures the effect of the congener vapour on burning hydrogen, detects the volatile acids, alcohols and esters from fermentation (although not all could be run in the same column if different packing is required). Specialized packings and detection systems, e.g. electron capture, are required for diacetyl and sulphur compounds, including dimethyl sulphide and polysulphides. The standard GLC system outlined above is suitable for most fermentation congeners, e.g. as back-up for sensory assessment of either new or mature spirit, but coupled to a mass-spectrometer (as GC-MS) can measure a much wider range of volatile compounds because of the greater versatility of the detection system.

## Non-volatile congeners by HPLC or TLC

The non-volatile compounds (e.g. sugars, tannins, lignin degradation products and other phenolic compounds) associated with maturation are more conveniently analysed by High-Performance (or High-Pressure) Liquid Chromatography or Thin Layer Chromatography. Both methods are more sophisticated versions of the original method of separation of coloured compounds by paper chromatography.. For HPLC the sample is chromatographed in the liquid phase through a column could be a miniature version of that used for GLC, or perhaps a small cylindrical column, and the separated components of the mixture are usually detected by UV fluorescence. As with GLC, identification is by elution time and quantification by the extent of detector response.

For TLC, metal foil or glass plates are coated with a thin layer of inert material, often silica. As with paper chromatography, samples are spotted on the start line. Then the plate is placed vertically in the solvent trough with the edge beside the start line just immersed (but, as in paper chromatography, not the start line



© The Institute of Brewing & Distilling 2017

itself). After elution, which is much faster than with paper, the plate is sprayed with reagent mixture (subsequent heating in an oven may be necessary) and the spots identified by their position and perhaps also by their colour reaction with the reagent. Although precise measurement is not possible, amount can be estimated reasonably accurately by intensity of colour and possibly also by the size of the spot, in comparison with standards run simultaneously,.
and preferaby on the same plate

It may happen that several components of a mixture run so close to each other that clear separation is impossible. Although only a single sample can then be analysed, one spot at one corner of the plate can be chromatographed as before, and its partly-separated components, at this stage still invisible, are then fully separated by chromatography with a different solvent running at 90º to the first, and then sprayed and heated as before. This description is necessarily vague since there are so many variations of plate coating, elution solvent and reagent, but one useful application of 2-dimensional TLC is the separation and measurement of the lignin degradation products associated with the maturation process.

### Nitrosodimethylamine

NDMA, a suspected carcinogen, became a problem in the brewing and malt whisky industries in the 1970s with the introduction of natural gas fuel for malt kilning. Its higher burning temperature created oxides of nitrogen (NOX) which in turn caused the formation of NMDA. Also, natural gas burns without formation of SO2, which inhibits NOX formation. . The problem could be solved by indirect heating, but if direct heating by flue gas had to be used, heavy fuel oil has sufficient sulphur content to inhibit formation of NOX. So NDMA analysis is seldom required now, but if necessary is by GLC of a directly injected sample of whisky and a thermal energy analyzer detector. Sensitivity < 1 Dg/litre is claimed, so ideally NDMA should not be detected. This is a particularly appropriate place to emphasise the COSHH requirements of chemical analyses. Although the small amount of NDMA that may be present in the samples is safe, the pure chemicals used for preparation of standards are hazardous.

### Ethyl carbamate

Also a suspected carcinogen, Ethyl Carbamate can occur in trace amounts in all distilled beverages, created by thermal decomposition during distillation of isobutyraldehyde cyanohydrin which is formed from naturally occurring cyanide glycosides of plant origin during fermentation. The problem was eliminated by (a) use of raw materials with low levels of CN precursors and (b) aeration during continuous distillation to prevent accumulation of cyanides in the system, this to be done in the presence of sacrificial copper in stainless-steel stills. GC-MS of a directly injected sample is an appropriate method of analysis.

## 2.5.3 Sensory analysis

### Introduction

Because of the high alcohol content of potable spirits, flavour analysis by tasting is not often used, and is not used at all in some companies. Sensory analysis is normally by nosing, i.e. by aroma only, so panelists must be selected on the basis of natural highly developed odour recognition, which is enhanced by regular training. Therefore trained panelists have a sensory threshold for aroma characters well below the average of the general public.

The basic requirement for sensory analysis is a trained panel working in a room of consistent ambient temperature and free from distracting odours. Whether communal assessment takes place round the table or panelists work in separate booths is the choice of individual companies and should not



© The Institute of Brewing & Distilling 2017

by itself have any effect on the significance of the results. Views on whether to provide computer terminals to record individual responses directly, or written forms with the option of giving more detailed impressions when appropriate, also vary between companies. For most types of test a minimum of ten trained panelists is necessary for statistical significance. Decisions on blending could well be based on one person's assessment, but that is a different situation from the use of a sensory panel as an analytical technique. Sensory assessment is believed to be most sensitive with potable spirit samples diluted to 20% abv with aroma-free water, perhaps distilled to achieve that ideal. Samples of 25 ml in tulip-shaped nosing glasses are diluted with an equal volume of water and left at ambient temperature for 30 min, each covered with a watch glass, to allow aroma to develop in the head space.

## Difference tests

Although there are many variations for specific purposes, there are only two basic types of sensory test: difference and descriptive. A difference test is precisely that: are the samples the same or different? For statistical validity a minimum 20 assessors is normally required, also there are various strategies for presenting the test samples.

The two most common types of difference tests for sensory analysis of spirits are (a) triangle, (b). A or not-A. Several other variations are widely used for food, beer and wine but because of the number of samples involved are unsuitable for spirits. Even when working only by nosing, an assessor is normally limited to a maximum of 10 samples in total (or 12, in some establishments) to avoid sensory fatigue.

**A or not-A**: A is a reference sample, and panelists are presented with that and a second which is either A again or one of the samples under test. And so on, with a succession of paired samples.

**Triangle test:** Each person is given three samples, two of which are identical (either 2 reference, 1 test or 1 reference, 2 test). For both methods, the intention is to determine if a statistically significant difference can be detected. It is also possible for panelists to comment on their perception of the aroma of the samples, thus providing a simple form of descriptive test, but the results can never be as significant as from a test designed specifically for that purpose. The triangle test is also ideal for assessing the sensory perception of panelists, by discriminating between accurately prepared samples.

## Descriptive tests

Such tests may be limited to describing the aroma components, but normally it is useful to estimate of the strength of specific characters in the assessment. A higher level of training is required than for difference tests: not only must the panelists have highly developed sensory perception but they also require the ability to recognise, and describe accurately, specific aroma components. Therefore assessors are trained to use a standard vocabulary of descriptive terms, as first used in the wine and brewing industries. The flavour wheel developed by the Scotch Whisky Research Institute is shown below as an example (Figure 2), but several more complex variations for whiskies have subsequently been proposed, e.g. to include "spicy" which did not appear in the SWRI flavour wheel.. Although there are numerous common congeners from the fermentation and maturation processes, the aromas of brandy, rum and whisky are sufficiently different to require specific "flavour wheel" standard terms for the various desirable and undesirable components of each. Equivalent wheels exist for brandy, gin and rum, and presumably also for other spirit beverages with sufficiently complex aroma and flavour to justify such a descriptive presentation of the component factors. Note that Figure 2.8.1 includes factors which are undetectable by nosing: the taste sensations sweet, sour, salt and bitter, and "mouthfeel" effects. Also, "metallic" in the "stale" sector and "glycerin" and "honey" in "sweet-associated" are



© The Institute of Brewing & Distilling 2017

probably taste rather than aroma sensations.

The flavour wheel for the mature final product is unnecessarily complex for new spirit, so a simplified version prepared within the company, perhaps with no more than 12 named characters, is adequate for sensory work before the start of maturation. For similar reasons, with neutral spirit or vodka samples, either a difference test or a simple list of the few likely aroma characters is sufficient for sensory analysis.

It is possible to relate most of the descriptive terms of the flavour wheel to specific chemical compounds. Accurately prepared solutions of such compounds can then be used for training and assessment of panelists.

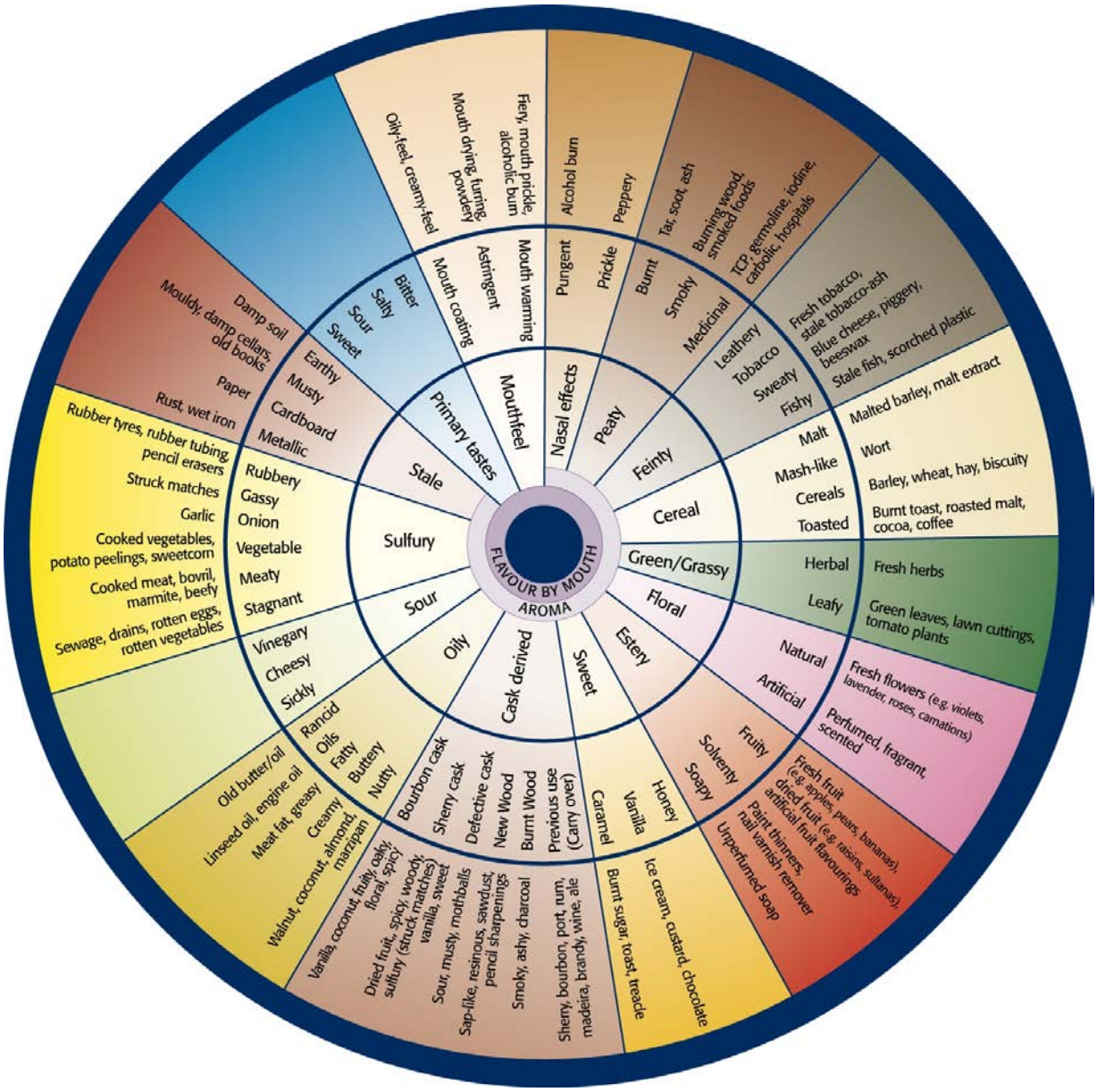



*Figure 2 Flavour wheel for mature whisky characteristics, kindly provided by the Scotch Whisky Research Institute (www.swri.co.uk)*



© The Institute of Brewing & Distilling 2017

## Flavour profile

In the simplest version, which originated with the wine industry, the assessor(s) describe(s) the character of each sample using standard terminology. The same system can be used with gin and matured spirits, but because of its limited precision and reproducibility it is used more by amateur enthusiasts than by professional assessors.

For a more accurate and reproducible assessment, a selection of sensory characters is scored on a 0 -10 scale. In practice it is difficult to operate with more than 8 in a single assessment, but with a suitable choice of characters appropriate to the product a precise profile can be prepared. The scores for a hypothetical Scotch malt whisky are presented in Figure 2.8.2, resembling a spider's web early in its construction, hence the term spider diagram. Even though there are differences between each distillation and even each cask, the shapes of spider diagrams of individual Scotch malt and grain whiskies are remark-ably consistent. Similar consistency applies to specific brands of brandy, gin and rum. To some extent, the characteristic spider diagram can be used for identification. However, legal disputes about the authenticity of a branded product are better resolved by GLC analysis, including ethanol content (to detect dilution), which gives a more detailed and precise profile of the flavour congeners of the genuine and allegedly fraudulent products.

| Descriptor | Reference compound |
|---|---|
| Buttery | Diacetyl |
| Cooked vegetable | Dimethyl sulphide |
| Earthy | Geosmin |
| Fruity | iso-Amyl acetate |
| Medicinal | o-Cresol |
| Musty (mouldy) | 2,4,6 Trichloroanisole |
| Nutty | Oak lactone |
| Pungent | Formic acid |
| Rancid | n-Butyric acid (or Ethyl butyrate) |
| Solvent | Ethyl acetate |
| Spicy (clove) | Eugenol |
| Sweaty | iso-Valeric acid |
| Vanilla | Vanillin |

*Figure 3 Typical flavour wheel descriptors and reference compounds*

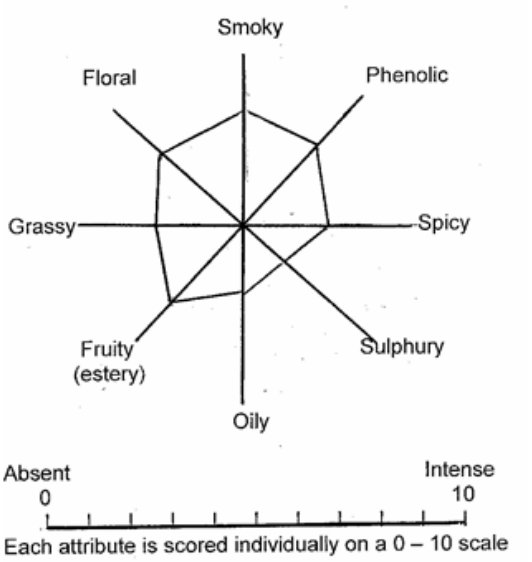



*Figure 4 "Spider diagram" of a hypothetical new-make Scotch malt whisky*

## Trueness to type

Trueness to type can be assessed in various ways, according to the product. GLC, as mentioned immediately above, could be used, but the trained human nose is a much more sophisticated analytical instrument and normally decides for routine quality analysis. The simplest assessment is "A or not A", a comparison with a standard sample shows identity, or not. Problems arise when it is "not A", what has gone wrong? A spider diagram may identify the discrepancy, but equally well may not, since only a limited number of characteristics is assessed. GLC or HPLC may be used, but many congeners are significant to spirit quality well below their detection levels by with these methods. Expressed another way, there are many congeners which are undetectable by laboratory instruments when present at or near aroma threshold levels. The company must rely on the expertise of the sensory panel or the blender to put things right.



© The Institute of Brewing & Distilling 2017

## 2.5.4 HYGIENE

### Spoilage micro-organisms and their effects

The alcohol content of distilled spirits is too high for microbial growth to occur, but being a food product, a high level of physical cleanliness is required at all stages of production. In addition, fermentation vessels and associated equipment must be free from microbial contamination in order to maintain product quality. In some brandy and rum distilleries microbial growth other than, or in addition to, culture yeast is encouraged for the flavour of the final product. That aspect, discussed in Module 1, is not contamination in the harmful sense that is the subject of this unit. The main microbial contaminants are listed here in alphabetical order rather than decreasing order of importance. The named genera are also important contaminants of the brewing industry, and the reading list at the end of this unit refers to brewing literature, but the spoilage effects are different in breweries and distilleries. It is important to be aware that the alcohol content of distilled spirits is too high for microbial growth to be possible, but contaminants introduced with the reduction (dilution) water at the time of bottling may be able to survive. Also, it is assumed that readers are already familiar with basic microbiological methods, including Gram's stain to distinguish between the two main types of bacterial cell-wall structure.

Acetobacter and Gluconobacter. The most important feature of these strictly aerobic Gram-negative bacteria is their ability to oxidize sugars or ethanol to acetic acid, produced in such quantity that the flavour of the acid, and ethyl acetate produced by subsequent esterification, would be objectionable. Also, yeast growth is inhibited by acetic acid, and not just by the low pH, since acetate ion also has an inhibitory effect. The difference between the genera is that Gluconobacter can oxidize only as far as acetic acid; whereas Acetobacter can eventually complete the oxidation to CO2. In addition to the well-known acetic acid spoilage of alcoholic beverages up to about 12% abv, they are common on plants, particularly sugary fruits. Being strictly aerobic, they are no longer able to grow after a blanket of $CO_2$ forms in the fermentor head space once fermentation is under way.

The normal habitat of the facultatively anaerobic Gram-negative genera Escherichia and Enterobacter is the human and animal intestine. Although some toxin-producing strains of Escherichia coli are capable of causing human disease, that is an unusual occurrence. The main significance of the normally non-pathogenic E. coli is that its presence in a water supply indicates contamination by domestic sewage or farm animals, so intestinal pathogens may also be present. If present as contaminants of a fermentation, Escherichia and Enterobacter are no longer able to grow when about 2% alcohol and/or pH below about 4.6 have developed, but in their short period of growth in fresh molasses or cereal wort they produce objectionable aromas which could taint the distilled spirit. Obesum-bacterium, named for its unusually fat cells, is related to Escherichia and Enterobacter, but is not known to have an intestinal habitat; it is assumed to live on plant surfaces. Obesumbacterium has similar off-flavour effects but is slightly more resistant to alcohol and low pH.

Lactobacillus is probably the most likely bacterial contaminant of brandy, rum or whisky fermentations. These Gram-positive non-sporing bacilli are common on plants in general and therefore on the three types of raw materials. Although tolerant of the presence of air, lactobacilli grow only by anaerobic fermentation to lactic acid, and possibly also to ethanol and CO2, depending on the species (see unit 2.2). Troublesome strains can grow throughout fermentation, and also in the charger vessel for the still, being unaffected by anaerobic conditions, ethanol or low pH, and able to utilise a range of simple sugars and dextrins. Some rum and whisky distillers regard moderate contamination by lactic bacteria as good for



© The Institute of Brewing & Distilling 2017

flavour development, but excessive growth, over about 106/ml, causes not only unacceptable flavour but also loss of spirit yield by utilisation of fermentable sugar. Pediococcus is also a possible contaminant of this group, with similar nuisance effect to Lactobacillus, but less common. Another coccus member of the lactic acid bacteria is Leuconostoc. Leuco mesenteroides is capable of growth in concentrated molasses or syrup stocks of rum distilleries, using the glucose half of sucrose molecules to synthesise the viscous polymer dextran which blocks pipework and pumps. However, Leuco. oenos, is not a harmful contaminant in the malo-lactic fermentation, but quite the opposite, improving cider, wine and therefore brandy flavour by converting malic acid with its two –COOH groups to lactic acid, with only one.. A similar type of reaction may take place in prolonged rum and whisky fermentations with Lactobacillus spp converting di- or tri-carboxylic acids to lactic, again resulting in a more mellow spirit.

The Gram-negative rods of the genus Zymomonas also live on plant surfaces as their natural habitat, and like the lactic bacteria are tolerant of atmospheric oxygen but grow only by anaerobic fermentation: of fructose or glucose to ethanol and CO2 (see Figure 2.2.6 of unit 2.2). Being unaffected by the final pH and alcohol concentration and more tolerant of the anaerobic conditions than S. cerevisiae, Zymomonas is capable of growth throughout fermentation. However, its production of flavour congeners is likely to be different from the culture yeast, and therefore unwelcome.

That problem of different congeners also applies to fermentative wild yeasts, although they may be a useful component of the natural inocula of some brandy and dark rum fermentations. Biochemically, there are two main types of wild yeasts. The facultative anaerobes can grow during part or all of a fermentation (depending on alcohol tolerance), certainly affecting flavour but possibly also spirit yield. Not only are their metabolic products important, being different from distillery yeast, but distillation of wild yeast cells of different chemical structure could also affect the flavour of the distillate. Other Saccharomyces spp., even different strains of S. cerevisiae itself, come into this category, as well as other fermentative genera, of which Hanseniaspora (Kloeckera if non-sporing) and Schizosaccharomyces are perhaps the most important. Aerobic wild yeasts are restricted to growth early in the fermentation, but some, particularly Hansenula and Pichia spp., are capable of producing significant amounts of esters in that time. Finally it is worth mentioning that a few wild yeasts which produce zymocin "killer factor" against culture yeast are a possible hazard of "natural" mixed culture grape and molasses fermentations but the risk is low.

## Detection of contaminants

It is most unlikely that microbial contamination of culture yeast or a fermentation could be detected by routine microscopical examination. Wild and culture yeasts have similar appearance, and contamination by bacteria is difficult to detect against the background of a much greater number of larger yeast cells. Since yeasts stain Gram positive, Gram- negative bacteria are more likely to be noticed, but only in the high numbers of really serious contamination. Specific immuno-fluorescent staining can detect bacteria or wild yeasts in the presence of 100 – 1000 times as many culture yeast cells depending on the expertise of the operator, but requires special reagents and equipment (unit 2.1).

So culture methods are more effective, but require 2 – 3 days' incubation at 25oC (1 – 2 days at 30oC). Different methods are required for testing (a) grape juice or wort before inoculation (seldom done, but possibly required to trace the source of contamination) or
(b) culture yeast or an active fermentation. Standard nutrient media are suitable for (a), and a spread-plate count with a 0.1 ml sample on malt extract agar (a useful medium for grape, molasses or cereal



© The Institute of Brewing & Distilling 2017

fermentations) can detect contaminants down to 10 cells/ml. Using an indicator medium, e.g. Wallerstein medium (basically a synthetic equivalent of malt extract agar with added pH indicator bromocresol purple) can distinguish by colour and shape.the colonies of different yeasts or bacteria, i.e. distinguish that they are different, it is impossible to give a definite identification by colony morphology. The same media can be used to confirm effectiveness of sterilization of a vessel, to grow contaminants trapped on a membrane filter of 0.45 om pore size after filtration of 250 ml of last rinse.

Samples (b) require a selective medium to allow any contaminants to grow but inhibit growth of the culture yeast that is known to be there. For detection of lactic acid bacteria and Zymomonas, and usually for the other bacteria listed above, “actidione agar”, i.e. Wallerstein agar + 100 og/ml of the anti-fungal antibiotic cycloheximide (also known as actidione) is most commonly used, but various other media on the same principle are also available. Yeasts are unable to grow in the presence of the antibiotic; but bacteria are unaffected and grow as normal colonies. Lactic bacteria and Zymomonas grow by anaerobic metabolism and may require a reduced-O2, high CO2 atmosphere for first isolation. That is most easily provided by incubating plates in a sealed can, with a lit candle added just before closing. Completely anaerobic conditions are required only for Clostridium spp, which may be of interest to rum distillers.

No selective medium exists to suppress culture yeast but allow all other yeasts to grow. “Lysine agar” is the most useful, a synthetic medium of glucose, lysine, salts and vitamins, which depends on the inability of Saccharomyces spp to grow on lysine as sole source of nitrogenous compounds (lysine taken in by the specific transport system can not be converted to other amino acids). Most other yeast genera, and certainly all of the common non-Saccharomyces contaminants listed in unit 2.3, are able to utilize lysine, and grow to normal colonies. One problem with this medium is that it depends on starvation of the culture yeast, but even after thorough aseptic washing and centrifugation of the sample, sufficient intracellular N remains to allow growth of small colonies. More seriously, wild Saccharomyces yeasts, probably more likely contaminants than other genera, can not be detected. However, a modified actidione agar often works, with only 5 – 10 og/ml of antibiotic (whatever amount is found to be just sufficient to suppress the culture yeast) since many wild yeasts have sufficient antibiotic resistance to grow.

## Rapid methods

Since traditional culture methods require several days’ incubation various rapid methods have been developed to perform urgently required microbiological checks. Accepted terminology.for these methods is: “instant”= no growth required; “rapid”= shorter incubation than the traditional method. Two examples of instant methods (both involving brief incubation, but not for growth of the micro-organisms concerned) are immuno-fluorescence for detection of contaminants in a yeast culture (unit 2.3) and the polymerase chain reaction (PCA) which is capable of amplifying minute amounts of DNA from specific contaminants. A disadvantage of most instant methods, and certainly of these two, is inability to distinguish dead from living cells. Although PCA can detect contamination after a cleaning cycle, it can not confirm the effectiveness of sterilization since the DNA may be from killed organisms, so its usefulness is limited.

Rapid methods do recognise viable micro-organisms, but possibilities for distinguishing different types are limited. The two most convenient and widely used are detection of ATP by bio-luminescence, and detection of microbial metabolism by conductance, impedance or micro-calorimetry. The principle of the bioluminescence test is that light emission by fireflies (“glow-worms”) requires ATP, and the light intensity from purified extract of the insects in commercial kits of the



© The Institute of Brewing & Distilling 2017

luciferin/luciferase system is proportional to the amount of ATP. Although the literature supplied with the kit must be consulted for full details, the principle is that ATP is extracted from the sample, and the light emitted by its reaction with firefly extract is compared with known amounts of ATP which can in turn be related to numbers of micro-organisms.

Conductance, and its reciprocal measurement impedance, change in culture media during the lag phase with ion efflux, and the change is proportional to the number of living cells. Since these electrical effects are temperature-sensitive, incubation of the samples requires a water bath accurately attemperated to +/- 0.1oC. The cells of the instrument (sample sizes vary from 5 – 50 ml according to intention of the test) are each fitted with a pair of probes to detect and record electrical activity. Comparing the graphs with those from calibration cells with known numbers of commonly encountered yeasts or bacteria gives a reasonably accurate measure of numbers. Commercially available selective media allow different types of bacteria or yeasts to be recognised. Alternative instrumentation is available to detect the heat produced by microbial growth, but for a rapid method to detect small numbers of micro-organisms.

## Cleaning and sterilization: definitions

**Physical cleanliness** - visually clean.

**Chemical cleanliness** – anything in contact with the cleaned surface suffers no contamination. In practice, clean water will completely wet the surface and drain as a continuous film without forming rivulets or droplets.

**Detergent** – a cleaning agent. By a combination of physical and chemical processes a detergent removes soil from a surface.

**Biocide or Disinfectant** – an agent for destruction of micro-organisms, but not necessarily 100%. In the food industries the words Sanitiser, Sanitisation are commonly used to imply both combined cleaning and disinfection.

**Sterilant** – an agent for complete destruction or removal of micro-organisms, which is unlikely to be achieved by chemical means, and so is often used wrongly as a synonym of disinfectant or sanitiser.

**Cleaning in Place (CIP)** – Cleaning (not necessarily automatic) without the need to dismantle equipment. Automatic CIP is now almost universal for cleaning and disinfection in the distilling industry, not least to avoid as far as possible the manual handling of hazardous chemicals.. For cleaning vessels, there are two principal methods of applying detergents (a) low-pressure spray balls placed to cover the entire surface and (b) rotating high-pressure jets. The powerful stream of liquid from (b) gives the most effective cleaning, but the cleaning cycle can not be shortened in the case of light soiling. The jet must rotate itself through at least one complete cycle of coverage of the internal surface. Also, some external indication is required that the jet is actually rotating. The gentler spray from the ball relies solely on the chemical effect of the detergent, but the entire inner surface is sprayed continuously during the cycle. So for lightly soiled surfaces a shorter cleaning cycle is acceptable. It is essential to keep the holes in the ball clear, e.g. by a filter to remove solids from a recirculating system

Cleaning of pipework is carried out by circulating detergent solution through the pipes. A flow rate of 2 m/s is accepted as providing the necessary turbulent flow for cleaning, without the risk of damage (e.g. water hammer) from higher flow rate (Table 2.4.1). Clearly, >100 mm requires impracticable pumping rates, although a lower flow rate would be acceptable for sterilization of an already cleaned line.



© The Institute of Brewing & Distilling 2017

| Pipe diameter (mm) | Flow rate (litres/min) |
|---|---|
| 25 | 62 |
| 50 | 220 |
| 75 | 550 |
| 100 | 890 |
| 150 | 1550 |

*Figure 5 Flow rate of detergent to achieve cleaning velocity 2 m/s in pipes*

There are three basic types of CIP system: (a) a total-loss system, (b) a partial recovery system and (c) a full recovery system. Total-loss is best for plant with heavy soiling, where recovered detergent would be too contaminated for re-use. Recovery systems not only save detergent, which can be topped up and re-used, they also save water by using the final rinse as the pre-rinse of the next cycle. In all cases, the cleaning cycle involves a pre-rinse to remove loose soil, and detergent re-circulation to clean the vessel and pipework. After discarding or recovering the dilute detergent, the vessel is rinsed with clean water. A disinfectant may be added to this rinse water. A pulsed rinse cycle ("burst rinsing") gives more efficient use of rinse water.

Health & Safety aspects of distillery operation constitute one of the units of Module 3, but is is important to mention here that detergent and biocide preparations are labelled as hazardous chemicals. Therefore the storage, preparation, use and ultimate disposal of such materials is subject to the UK Control of Substances Hazardous to Health (COSHH) regulations or their equivalent in other countries, and according to the risk assessment and safety precautions literature provided with the products.

## Cleanliness and sterility requirements

Fermentation is susceptible to microbial infection, so the risk is minimised by sterilization of the relevant equipment. Contamination at this stage is not a health hazard, since pathogenic micro-organisms or toxins are eliminated by the distillation process, but microbial contamination causes off-flavours and loss of product quality. Therefore vessels for preparation of pitching yeast, fermentation vessels, and pumps and pipework.associated with these items absolutely require both cleaning and disinfection. Sterilization of the still charger vessel and associated pipework and equipment could also be considered, to reduce the risk of further microbial growth in fermented liquid awaiting distillation. For all other stages of the process, the physical cleanliness that is obligatory for a food product is sufficient.

## How often is cleaning required?

For stills the reduction of reactivity of copper surfaces and effect of accumulated soil on steam coil heat transfer (pot stills) or blockage of sieve plates (analyser column of a continuous still) are generally regarded as the most important factors, although the heated deposits could also generate off flavours. These effects develop slowly, so occasional cleaning is sufficient. However, plant for preparation of fermentable extract is much more sensitive. The equipment for the cereal mashes of whisky and grain neutral spirit is most susceptible to soiling, and cleaning (but not sterilization) after each use is essential, or various bacteria grow producing persistent off-flavours. For molasses and grape equipment, daily cleaning rather than after each use may be sufficient.

Process plant surfaces have an important effect on cleaning and sterilization. Comparing the smooth surfaces of stainless steel and copper with not only the roughness of cast iron and wood, but also the joints between the plates or planks, it is obvious that the latter two are extremely difficult to clean and disinfect (and probably absolute sterilization is impossible).



© The Institute of Brewing & Distilling 2017

## Cleaning and detergent action

Four distinct stages are involved in the actual cleaning, (though easy rinsing is also important):

- Wetting of the surface to allow intimate contact between detergent and soil
- Chemical action on the soil, e.g. solution of mineral scale by acids, hydrolysis of protein by acid or alkali, saponification of lipid material by caustic detergents.
- Dispersion of large particles as finely-divided soil.
- Suspension in solution of any removed soil.

Also, a good detergent allows efficient and complete removal of detergent and suspended soil by final rinsing..

Sodium hydroxide meets many of these requirements, and 2% NaOH used hot (>70°C) is also an efficient disinfectant, but it has two main disadvantages.

- Precipitation of mineral salts, e.g. Ca, Mg associated with hard water, which is prevented by addition of sequestrants Na gluconate or Na hexametaphosphate to detergent formulations.

- Reaction with CO2, forming much less efficient Na2CO3, requires fermentation vessels to be drained of CO2 before cleaning.

So to avoid that lengthy delay, acid detergents have become common in the alcohol fermentation industries, with the additional advantage of action on mineral scale. Phosphoric acid with added surfactants makes a good CIP detergent.

Some formulations also include nitric acid for greater detergency, also for disinfectant effect, but HNO3 mixtures are destructively incompatible with copper or Cu-containing alloys. However, the stainless steels commonly used in the fermentation industries are resistant. Various organic acids are also possible, but have no advantages over H3PO3 and are more expensive.

Quaternary ammonium compounds and “amphoteric” detergents are "surfactants" which are now widely used in the food industries. In a single compound they combine detergent and disinfectant activity: their powerful surface-active effect lyses microbial, particularly bacterial, cell walls and membranes. However, they have serious disadvantages for the fermentation industries. Foaming during CIP is a problem, they are difficult to rinse off, and residues cause foaming during fermentation, therefore they are not ideal for distillery use. On the other hand, that residual biocide on food-processing surfaces is an advantage.

## Biocides (disinfectants)

All biocides are dangerous, since their purpose is to kill, so rigorous attention to safety is essential. With automatic CIP there should be no requirement for manual handling of biocides, except for preparing the working solution. Few of these agents kill all micro- organisms, but if fermentation vessels, yeast mixing vessels and associated pipework are cleaned and then treated to kill any remaining yeasts and the spoilage bacteria listed above, that is sufficient. Absolute sterility of plant is not justified when the fermentation medium itself is not sterile.

Killing of micro-organisms by a disinfectant is influenced by:

- Concentration of disinfectant
- Temperature of disinfectant
- Number of micro-organisms
- Time of contact between disinfectant and micro-organisms
- Amount of inert organic soil.

Disinfection is a chemical reaction between the agent and the target sites on the micro-organisms, therefore rate of reaction is increased by higher concentration and temperature. Longer time of contact can compensate for lower concentration and/or temperature. Disinfectant is diluted by increased microbial load, and for some disinfectants, halogens in particular,

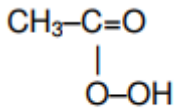


© The Institute of Brewing & Distilling 2017

inactivated by organic soil in general.

The two most useful biocides for distillery fermentation vessels and associated plant are steam and peracetic acid.
Saturated (wet) steam is very effective for sterilizing cleaned equipment only. Steam bakes some types of soil on to surfaces, making it more difficult to remove, and may protect embedded micro-organisms. Steam kills all types of micro-organism, although to achieve sterility at atmospheric pressure requires continuous steaming for 1½ hours. Steam is freely available in a distillery, and no subsequent rinsing is required. However, radiated heat from a vessel being sterilized could affect a nearby fermentation if the vessels are too close.

Peracetic acid is a fast-acting effective disinfectant, even at ambient temperature. The concentrated. acid has an unpleasant smell and is dangerous to handle because of its corrosive and strongly oxidising properties, so is unsuitable for manual use. However, it is a very effective final disinfectant in a CIP program.



**Peracetic acid**

Peracetic acid breaks down first to acetic acid and hydrogen peroxide (which also have anti-microbial activity) and then the latter to water and oxygen. The small amount of acetic acid formed at use-dilution is insufficient to cause flavour taint so a final rinse is not required.

Rinsing after sterilization is best avoided; or UV-sterilized water should be used. However, the other possible final disinfectants mentioned here must be rinsed, for obvious reasons. Chlorine (as hypochlorite NaOCl) or iodine (as iodophor, a solution of I2 in a surfactant, which must be non-foaming for CIP) are not widely used because of the risk of flavour taint from residue, but are very effective oxidising disinfectants. Most NaOH detergent formulations give effective disinfection when used hot, but the alkaline residue must be rinsed off; and they are incompatible with residual CO2 in fermentation vessels.

## Further reading

The following chapters in Brewing Microbiology, ed. F. G. Priest & I Campbell, collectively cover this unit: Gram-positive bacteria, Gram-negative bacteria, Wild yeasts, Rapid detection and identification of microbial spoilage, Cleaning and disinfection.

However, it is important to remember that (a) some of the spoilage effects described in beer do not exist in the distilling industry and (b) the problems caused by specific bacteria in beer and distilled spirits may be different.



© The Institute of Brewing & Distilling 2017