Invited article

Institute of Brewing & Distilling

Received: 1 October 2012 Accepted: 12 October 2012 Published online in Wiley Online Library: 6 November 2012

(wileyonlinelibrary.com) DOI 10.1002/jib.47

125th Anniversary Review: Advances in analytical methodology in brewing

Chris Boulton*

Comprehensive sets of chemical, microbiological and sensory methods have long been available to characterize individual beers and explore the relationships between raw materials, process conditions and the outcome of the brewing process. Although the majority of major brewers are increasingly using quality assurance as opposed to quality control as the basis of operation, the need to use chemical analyses is still perceived as an essential prerequisite to brewing. The requirement to meet the various legislative codes and the need to manage the consistency of international brands arising from several individual breweries make the possession of robust analytical procedures essential. In many breweries there is a trend towards devolving traditional analytical tasks from central quality control laboratories manned by dedicated technicians to satellite stations, where the analyses needed to support production are performed directly by process workers. Parallel to these changes is a desire to achieve a greater understanding of the complex relationships between beer analysis and overall quality, in particular, the identification of markers that allow for the identification of processes such as beer ageing. This review summarizes the ways in which brewing analytical methods and the suppliers of analytical apparatus are evolving to meet the needs of the current modern industry.

Keywords: methodology; instrumentation; brewing; analytical methods; apparatus; quality

Introduction

The introduction into brewing of the thermometer by Combrune and the saccharometer by Richardson, both in the eighteenth century, is evidence that the industry pioneered the use of analytical techniques as a means of improving process control and ensuring consistency of product *(1)*. These early uses of physical measurements mark the beginning of a continuum, which eventually resulted in the development of an underpinning comprehensive set of procedures that when used in an appropriate manner assure the fitness of raw materials and ensure that process and product adhere to pre-determined specifications. The current accredited procedures are enshrined in the methods manuals of the EBC *(2)* and the ASBC *(3)*. Similar sets of methods have been codified by the Central European Brewing Technology Analysis Committee (*Mitteleuropäische Brautechnische Analysenkommission*, www.MEBAK.org) and by the Brewers Convention of Japan (www.brewers.or.jp). The use of a common set of methods, whose efficacy has been assessed by the impartial bodies responsible for the compilation of these analytical manuals, has been essential in order to ensure that results obtained from different locations are reliable and consistent. The trend towards the adoption of common methods and nomenclature, as evidenced by the merging of the IBD and EBC manuals, is welcome and hopefully represents an early stage in the journey towards full international harmonization.

The process of innovation and refinement is continuous as new methods are introduced and older redundant ones are archived. This is inevitable but the process must be one that is properly controlled. In this regard, coordination of the assessment of potential new methods by participating laboratories by independent analytical committees is essential.

There are several drivers for the development of new analytical techniques. External legislation may dictate the adoption of methods for analysis of compounds that have not heretofore been considered. More precise and repeatable procedures may be adopted to replace current but inferior methods. Process innovations or new product development may produce a need for new analyses. The intention of this review is to describe the ways in which developments, both within and external to the brewing industry, have provided the impetus for changes in approaches to the analysis of beer and related materials and the ways in which methods have been adapted or new techniques developed to meet these needs.

Proficiency schemes

Brewing companies must adhere to the legislative codes that govern the markets in which they operate. This makes it mandatory to have a quality system that meets internationally agreed specifications and that has been accredited by a recognized

* Correspondence to: Chris Boulton, University of Nottingham, Nottingham, UK.
E-mail: christopherboulton@gmail.com

University of Nottingham, Nottingham, UK

external body. Current codes applicable to the brewing industry are: ISO 22000:2005, food safety management; ISO 9001:2008, quality management; ISO 14001:2004, environmental management; and OHSAS 18001:2007, occupational health and safety management. In addition, there is an optional code, ISO IEC 17025, that governs the competence of laboratories in testing and instrument calibration.

The Brewing Analytes Proficiency Scheme provides a means whereby brewing laboratories can assess their performance. It is jointly administered by Campden BRI and LGC Standards (www.bri-advantage.com/services). At the present time it is used by more than 200 brewing companies representing 60 countries. Identical samples, usually of commercial beers or lyophilized microbial samples, as appropriate, are supplied regularly to participating laboratories and the anonymized results of in-house analyses can be compared with those obtained from the whole group. In this way the competence of the laboratory can be subject to continuous assessment, as can the abilities of individual operators. Demonstration of competence provides a useful monitor for external accreditation bodies.

The range of analyses encompasses chemical composition, assessment of the physical properties of beer, microbiological and sensory analyses (Tables 1–3). This list forms a useful benchmark for this review, as it shows the range of analyses that a typical brewery is required to undertake.

Table 1. Analyses included in the Brewing Analytes Proficiency Scheme (BAPS) proficiency scheme

Analyte	Method	Units
Beer analysis		
Alcohol by volume	All methods	% abv
Original gravity	All methods	°P
Present gravity	All methods	°P, °Sacch.
Bitterness	Spectrophotometric	BU
Colour	Spectrophotometric, A430	EBC
pH	Meter	pH units
Haze (0 °C)	Hach, LG Auto, Monitek, Dr Lange, Haffmans/VOS,	EBC
Haze (20 °C)	Sigrist, Optek	
CO_2	Volume expansion	g/L
	Pressure corrected	
Total gas pressure	Haffmans, Zahm, Nagel (pressure measurement)	g/L
	Hach, Orbisphere (thermal conductivity	
Refractive index	Refractometer	RI
SO_2	All methods	mg/L
Free diacetyl	GC	µg/L
Free 2,3-pentanedione		
VDK as diacetyl	Distillation	mg/L
Chloride, phosphate, sulphate, nitrate	Various	mg/L
FAN	All	mg/L
TSN	All	mg/L
Foam stability	Rudin, NIBEM	seconds
Acetaldehyde	GC	mg/L
Ethyl acetate		
n-Propanol		
Iso-Butanol		
2-Methyl butanol		
3-Methyl butanol		
Iso-amyl acetate		
Ethyl hexanoate		
Iso-α-acids	HPLC	mg/L
Tetra-iso-α-acids		
Total polyphenols	All methods	mg/L
Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, Fe^{2+}, Cu^{2+}, Zn^{2+}	All methods	mg/L
Dimethyl sulphide	Various	µg/L
Methylthioacetate		
Hydrogen sulphide		
Methanethiol		
Glucose	Various	% w/w
Maltose		
Maltotriose		
Total carbohydrate		

FAN, Free amino nitrogen; TSN, total soluble nitrogen.

Table 2. Microbiological analyses included in the BAPS proficiency scheme

Analysis	Method	Units
Total aerobic count	Plate count with or without membrane filtration	Cfu/unit volume
Total anaerobic count		
Total aerobic bacterial count		
Wild yeast		
Lactic acid bacteria		
Identity of provided organism	All	NA

Table 3. Beer sensory analyses included in the BAPS proficiency scheme

Descriptor
Fruity/estery
Alcoholic/solvent
Fruity/citrus
Hop
DMS
Cereal
Malty
Caramel
Burnt
Other sulphur
Oxidized/old
Sweet
Bitter
Sour
Astringent
Body
Linger
Other (diacetyl, rancid, cheesy, lactic acid, acetic acid, phenolic, chlorophenolic)

Drivers for change

Siebert *(4,5)* describes a successful analytical method as one that must determine an analyte in its sample matrix with adequate sensitivity and minimal interference. In other words, the detection system must be sufficiently sensitive to provide desired levels of precision and repeatability. Preferably this is achievable without separating the analyte from the complex mixture of other chemicals that are likely to be present. If this is not possible, it is necessary to use a separation process and remove the analyte from other sources of interference. Accredited analytical methods have been designed to meet these requirements, although with many there will be some element of compromise. The development of new methods, which are more finely tuned to the particular needs of brewers, explains why the contents of the analytical method manuals are in a continual state of flux. This may be via the development of totally new methods of separation, detection and quantification. Perhaps, more commonly, the method may be essentially unchanged, but the method of detection might be changed. Thus, suppliers of apparatus to brewing laboratories adapt existing methodologies and provide these in the form of a ready to use package that fits into a particular niche that suits the needs of the modern industry.

The fiercely competitive nature of the modern industry has resulted in reductions in the sizes of workforces, including skilled laboratory staff. Many routine analyses are performed in satellite laboratories by relatively unskilled operatives. This development has been a major driver for the introduction of equipment that requires little input from the operator. An additional gain is more consistent performance as a result of minimizing human intervention. An example is the assessment of foam performance of a beer via timing of the foam collapse time. This can be achieved manually, but it is now likely that an automated procedure would be used. For example, the Haffman Nibem foam stability tester (www.haffmans.nl/engineeredProduct_P_foam_measurement.aspx) requires no manual operations other than loading the sample. The foam is generated automatically, the collapse time measured and the result tagged and either printed or exported to a computer. Similarly, the Steinfurth foam meter (www.Steinfurth.de/page,beer-analysis-instruments.htm) uses an auto-sampler, which automatically transfers and loads the beer into the instrument, generates the foam and via optical sensors measures the collapse time. After exporting the result, the instrument is automatically washed in preparation for the next test.

In-process analyses

Continuing the theme from the preceding section, several companies have introduced equipment designed to perform off-line routine analyses with a minimum of operator involvement. In the case of colorimetric procedures, the company will typically provide a suitable spectrophotometer that is pre-programmed with a number of calibrations that correspond to accredited methods taken from the analytical manuals. The necessary reagents may also be provided in the form of kits and after analyses are completed, dedicated software generates labels and stores results. The Spectroquant® Pharo software package (www.merckmillipore.co.uk/chemical/spectroquant-pharo-software-beer-analysis) supplies software in the form of a USB stick that contains calibrations suitable for 21 commonly used beer analytical methods, including total carbohydrates, free amino nitrogen, bitterness and iso-α-acids. Mancherey-Nagel adopt an almost identical approach (www.mn-net.com) using methods based on the MEBAK manual and a system described as the Nanocolor® system.

Flow injection analysis is a suitable technique for use in automated procedures. Liquid samples are conveyed down a line via a peristaltic pump, mixed with reagents and conveyed to a detector *(6)*. The approach has been adapted for analysis of glucan in beers and malts *(7)* and sulphite levels in beers *(8)*.

Microplate readers, a relatively old technique, based on the use of multi-well disposable plates in which samples and reagents are mixed and subsequent reaction products detected

and quantified by absorbance, luminescence, fluorescence or light scattering, are suitable for routine high-throughput assays *(9)*. The procedure has been recommended as an alternative to flow injection analysis for the semi-automatic analysis of β-glucans, free amino nitrogen, total soluble nitrogen and diastatic enzymes *(10,11)*.

Headspace sampling, followed by separation via GC and subsequent detection and quantification, can be automated using an auto-sampler and remains the method of choice for diacetyl and other vicinal diketones. In addition, the technique is commonly used for beer sulphur compounds (DMS, SO_2, H_2S) and organic contaminants of water such as trihalomethanes.

In-line analyses

It is logical to progress from automatic off-line analysis to use sensors that are suitable for in-line or in-tank use. Apart from eliminating the requirement for manual input, other than routine maintenance and calibration, this approach confers the additional advantages of providing timely and possibly continuous results with the possibility that the output can be used in a control loop. The requirements of ideal sensors are as follows: sensitivity within the desired range of operation; responsive only to the chosen analyte; providing a stable output requiring minimum or no calibration; not affected by any other component present in the medium; have no effect on the analyte or any other component of the medium; provide an output suitable for use in a control system; and finally be robust and able to withstand the rigours of the brewery environment including cleaning-in-place (CIP) regimes. In addition to the usual probes for measuring physical parameters such as temperature, pressure, conductivity and flow, in-line sensors are available that are suitable for determining the concentrations of the majority of the major beer process variables, including solids content (haze), colour, specific gravity, original gravity, alcohol content and dissolved gases (O_2, N_2, CO_2) *(12–15)*. Opportunities for the use of in-line measurements at various stages of the brewing process are shown in Table 4.

Developments in in-line sensors have concentrated on improvements in sensitivity and stability, especially in terms of reducing the need for maintenance and calibration. This is particularly evident in the case of in-line gas analysis. Older polarographic oxygen electrodes have been superseded with those that utilize the quenching by oxygen of fluorescent dyes. These require little maintenance, as they do not have membranes or require electrolytes and they do not consume oxygen. Operating ranges are typically 0.05–20 mg/L dissolved oxygen and they can be used over a temperature range of 0–50 °C. Sensors for dissolved N_2 and CO_2 are based on membrane diffusion thermal conductivity *(16)*. CO_2 can be measured accurately over a range 0–15 g/L and N_2 over a range 0–250 mg/L. Probes are supplied pre-calibrated.

As with laboratory equivalents, in-line apparatus capable of multiple analyses is available. The Anton-Paar Company (www.anton-paar.com) provides a single monitor capable of determining real, apparent and original extract, alcohol (via combined damping of oscillation in a u-tube and ultrasonic analysis) and CO_2 using a volume expansion method based on the gas laws.

The majority of analyses used in the fermentation stage of brewing tend to be based on off-line samples apart from those

Table 4. In-line analyses throughout the brewing process

Stage	Sensor type	Process
Brewhouse	Light scatter turbidity	Control of wort solids in lauter tun run-off and trub ex-kettle
	A_{430} light absorption + light scatter	Wort colour corrected for solids
	Ultrasonic densitometer + flow meter	Extract ex-lauter or mash press
		Total extract
	pH	Wort acidification
Cooled wort	Dissolved oxygen (amperometric or optical fluorescent quenching)	Check of pre-pitch wort oxygen
	Aber capacitance meter	Yeast viable pitching rate
	Dual beam NIR light scattering	Yeast pitching rate with correction for trub content of un-pitched wort
Yeast propagation	Dissolved oxygen,	Yeast growth
Yeast disinfection	pH	Acid washing
Fermentation	Dissolved oxygen	Recovered carbon dioxide
End fermentation	Aber capacitance meter	Yeast cropping
Green beer transfer	Light scatter turbidity	Continuous centrifuge operation
Conditioning	Light scatter turbidity	Green beer yeast count
Filtration, blending, bright beer tank, pre-filler	Light scatter turbidity	Filter powder dosing
	Haze	Filter operation
	Oxygen	Dissolved gases
	Carbon dioxide (thermal conductivity)	
	Nitrogen (thermal conductivity)	
	A_{430} light absorption	Colour
	Ethanol (NIR)	Beer specification
	Specific gravity	

used to control wort oxygenation and yeast pitching during fill. This is understandable since the cost of fitting hygienic sensors in multiple fermentation vessels is considerable. Early attempts to fit automatic gravity sensors to allow monitoring of fermentation progress *(17–21)*, usually based on differential pressure measurements, have not seen widespread adoption. It would be useful if this could be rectified, although of greater practical utility would be the ability to measure diacetyl concentration in-tank. Early and precise detection of the achievement of the diacetyl specification would provide a means of removing a significant proportion of overall residence times and remove the need for lengthy laboratory sample preparation and analysis. Early approaches proposed the use of a manifold system of capillary tubing, in which samples could be removed from the headspace of individual fermenters, and after a heat treatment conveyed to a gas chromatograph for analysis. Undoubtedly this approach could be made to work; however, the need to maintain hygienic conditions in the extensive tubing system is challenging. More recently, Freshman *et al*. *(22)* have described a portable analyser that uses field asymmetric ion mobility spectrometry as being suitable for diacetyl determination. This technology, which is used for military applications such as the detection of volatiles from explosives, could be adapted for in-line use. It is highly specific and is capable of the simultaneous measurement of several volatile analytes that would perhaps lend itself to on-line flavour assessment of both fresh and stale beers.

It is likely that other opportunities for in-line measurement will be introduced. In particular, some of the rapid microbiological techniques offer the possibility of real-time assessment of the status of beers on route from the bright beer tank to packaging (see below).

Data handling

Breweries generate large quantities of analytical data and organized systems are required to ensure that it is recorded, stored and processed in a logical manner. The universal adoption of computers has made this relatively easy. Once the data is stored in electronic form it can be processed as desired. Thus, it may be used for trend analysis, confirmation of achievement of specification, detection of non-standard behaviour, etc. The data can be fed into higher quality systems to provide basic information for underpinning scheduling, raw material supply, distribution and maintenance. Analytical data can be manually entered into brewery computer data acquisition systems, but now more typically, apparatus designed for routine quality analyses is fitted with communication systems for automatic data collection via commonly used data transfer protocols such as USB, Ethernet, RS485 or Profinet.

Product safety

A diverse range of organic materials deriving from pollution, whose presence in beers would be viewed as a source of concern, may have to be analysed either in finished beers or in raw materials. Many of the compounds are likely to arise at very low concentrations and detection and identification would be challenging for most brewery quality laboratories. For this reason, analyses where required are usually performed by accredited third party organizations. A variety of separation and detection technologies is used. Nitrosamines in malt and beers have been analysed using HPLC and detection via photoconductivity *(23)*. Scanlan *et al*. *(24)* used a chemiluminescent system to detect *N*-nitrosodimethylamine in commercial beers. These early methods have been largely superseded by the use of mass spectrometry. The technique of GC-MS is very powerful, comprising sample separation via gas chromatography followed by identification of the resolved peaks using mass spectroscopy. The technique has been applied to the determination of nitrosamines in beer *(25)*. Hengel and various co-workers in a series of reports describe methods for the analysis of various insecticides in hops by GC MS *(26–28)*.

The presence of the so-called biogenic amines is considered a health risk for consumers who for medical reasons are receiving treatment with monamine oxidase inhibitory drugs *(29–32)*. For this reason several authors have reported concentrations arising in various beers and analytical methods used to generate the data. These include separation using reverse-phase liquid chromatography and detection via fluorescence after treatment with orthophthalaldehyde *(33,34)*, separation of N-substituted benzamide derivatives by capillary zone electrophoresis using a UV–vis scanning detector *(35)* and the same method of separation as in the latter report, but in this case with detection via laser-induced fluorescence. In the last report detection limits of less than 20 μg/L were obtained *(36)*.

The presence of SO_2 in beer is desirable because of its ability to function as an antioxidant. However, it presents a risk to human health and in most legislative codes a maximum concentration in the region of 20–25 mg/L is permitted in beer *(37,38)*. Since in most countries the SO_2 content must also be declared on labels, suitable analytical methods are required. Analytical methods must detect free SO_2 and that bound to aldehydes such as acetaldehyde, as adducts. Accredited methods include that of Monier-Williams, which requires a distillation step and quantification via conversion of SO_2 to sulphuric acid and titration with sodium hydroxide *(39)*, and colorimetric procedures involving reaction with ρ-rosaniline and formaldehyde to form a complex with an absorption maximum of 550 nm or reaction with 5,5-dithiobis(2-nitrobenzoic acid) to form a complex with an absorption maximum of 415 nm. The colorimetric procedures can be adapted for use in automated flow injection analytical methods. A voltammetric approach for free and total SO_2 has been reported *(40,41)* involving purging of volatile aldehydes with nitrogen of a beer sample diluted with alkali and after trapping with an electrolyte solution and derivatization with hydrazine detection with a hanging mercury drop electrode. A duplicate beer sample is acidified and purged and analysed in the same way. The difference between the two analyses represents the free SO_2 fraction. A more recently published procedure *(42)*, which reportedly correlates well with accredited methods, uses acidification of the beer sample to release all SO_2, which is separated using a semi-permeable membrane and analysed by stripping chronopotentiometry.

Microbiological analyses

Hygiene testing via the use of ATP bioluminescence, in which ATP presumed to have been derived from contaminating microorganisms or residual soil is detected as light emitted from the enzymatic reaction of firefly luciferin, luciferase, has largely replaced classical microbiological testing *(43,44)*. Nearly 100 individual pieces of commercial apparatus for hygiene testing have been designed *(45)*. Currently, the most successful of these are based on the use of kits, which comprise self-contained dipsticks, in which the bespoke swab is applied to the surface to be

tested and then mixed with the included reagents and the result is obtained by placing this within the apparatus. An example is the Biotrace unit, now part of the 3 M company (www.3m.com).

Much routine microbiological analysis continues to use the classical techniques of sampling, plating on suitable selective and differential media, followed by incubation and enumeration of the resultant colonies. Results typically take a few to several days to obtain and therefore these methods are of historic value only. Warehousing costs are such that there is significant financial advantage to be gained from releasing finished product to trade as soon as possible.

In the interests of beer freshness, the use of cold sterile filtration as a means of ensuring microbiological stability is becoming increasingly common. This removes the safety net of pasteurization and this coupled with very-high-speed packaging lines places great demands on microbiological analyses that classical techniques are unable to satisfy. The use of micro-colony techniques, an enrichment step in which a short incubation period allows small microbial counts to be increased, shortens detection times, but in most cases still requires 24 h for results to be obtained. Filterable samples can be passed through 0.44 μm membrane filters to concentrate low counts.

It would be preferable if results could be obtained in real time, with detection of very low counts, possibly in the presence of high viable yeast counts and with positive identification of contaminating potential spoilage organisms with no interference from non-spoilers. These are very exacting requirements.

Rapid microbiological techniques have been subject to review *(45–47)*. There are currently no methods where potential spoilage organisms can, in real time, be positively identified and distinguished from dead cells using samples removed automatically from a process stream, for example, as might be needed to obtain results throughout a packaging run.

The use of the polymerase chain reaction in which nuclear material is extracted and the copy number multiplied allows detection and positive identification in 1–2 h *(48–50)*. This is too slow for real-time analyses; nevertheless, kits are available that allow detection of all common brewery spoilage organisms *(51,52)*. The method includes detection and discrimination of obligate anaerobic beer spoilers such as *Pectinatus* and *Megasphaera (53)*. The ChemScan® RDI system (www.aeschemunex.com) uses a laser scanning system and detects microorganisms, also within a few hours, in filtered samples stained with specific proprietary fluorophores. It distinguishes viable from non-viable cells and specific probes are available for some beer-spoilage organisms. The Microstar rapid microbiology system, (http://www.millipore.com/catalogue/module/c10711) uses detection of microorganisms via ATP bioluminescence. Organisms are recovered from membrane filters and after washing and staining with appropriate reagents, bioluminescence owing to the presence of viable cells is detected by automatic image analysis. A single yeast cell or approximately 100 bacteria can be detected within 24 h.

Analyses applied to yeast

Analyses are applied to yeast in order to assess quantity, viability, physiological condition and identity. Determination of viable yeast concentration is required in order to control pitching rates. The Aber biomass meter (www.aber-instruments.co.uk) quantifies yeast mass by measuring the capacitance of intact cells when placed within a radio-frequency field. Both laboratory and in-line versions are available. The instrument is used widely in in-line pitching and cropping control systems *(54–57)*. By definition the instrument does not assess viability since it is not responsive to non-viable cells (those with a disrupted membrane). However, its ability to quantify the viable fraction of yeast populations places its utility above those in-line sensors based on light scattering, since the latter do not distinguish living and dead cells.

A plethora of methods, the so-called yeast vitality tests, have been proposed for the analysis of yeast physiological state. These include viability tests the results of which are themselves a measure of vitality *(58–60)*. These analyses are used to assess fitness of individual batches of yeast to pitch, either in a go, no-go approach, or as predictors of subsequent fermentation performance, which preferably allows selection of optimum pitching rates and/or wort oxygenation. The most commonly used analyses, not including viability staining tests, are based on the ability of yeast to acidify the external medium *(61–64)* and the release of magnesium ions by yeast *(65)*. These methods produce results that are to some extent predictive of fermentation performance. However, there is little evidence that they provide more information than a simple viability test such as the usual counting of unstained and stained cells treated with the vital dye methylene blue. Perhaps the results are more repeatable, but in any case they are unsuitable for automation. In this regard, the approach that shows the most promise is flow cytometry. The device introduces cells into a stream of rapidly moving fluid and in single file they are made to pass through an orifice with an aperture of 50–100 μm. Individual cells are passed through a laser beam light source. In the presence of suitable dyes, usually fluorescent types, the resultant scattered radiation is detected. A unique feature of the instrument is that it detects the responses of individual cells and if desired subpopulations can be sorted and recovered *(66)*. The power of the approach is that a multitude of aspects of cell physiology can be probed by the choice of staining agent. These may be physiological markers, or via the use of fluorochromes linked to antibodies the potential beer spoilers can be identified. The current disadvantages are cost and the requirement for skilled operatives. This will change and it is likely that devices will be developed suitable for routine in-line use for both assessment of brewing yeast and detection and enumeration of beer spoilage microorganisms *(67–69)*.

Definitive identification of yeast is most efficiently accomplished using techniques that analyse the genome. A variety of analytical techniques have been used including karyotyping, polymerase chain reaction (PCR) and restriction fragment length polymorphism *(45)*. Current best practice for genetic finger printing of brewing yeast strains suggests that the best method (recommended by ASBC) is PCR of interdelta regions of chromosomal DNA (regions of DNA which flank DNA associated with retrotransposons). This method can be used for testing stability, strain identification and detection of mutants *(70–72)*.

Beer quality

Assessment of sensory attributes of beer remains largely the province of trained taste panels and chemical analyses of flavour compounds tend to be restricted to a few esters, higher alcohols, selected sulphur compounds such as dimethyl sulphide and the vicinal diketones, especially diacetyl. However, there are some areas where efforts are being made to correlate sensory properties of beer with chemical composition.

A current area of interest relates to beer staling. In several major brewing companies beer flavour stability is routinely assessed using electron spin resonance spectroscopy. The method assesses the endogenous anti-oxidant properties of beer measured as a lag time in forced resistance to oxidation using a spin trapping method *(73–75)*. The approach is used to assess the flavour stability of finished beers and also the effects on beer ageing of the conditions used in various steps in manufacture. It is useful in the sense that the technique produces a numerical value that appears to correlate with real-time ageing as assessed by more conventional sensory techniques.

The complex chemical composition of beer requires equally complex methodologies to determine the true relationships with sensory attributes. So-called electronic noses have been designed to provide a chemical fingerprint of beers with the aim of exploring processes such as ageing or as a quality control method for providing assurance of conformance to trueness-to-type of individual batches *(76–79)*. Electronic noses and tongues [see Baldwin et al. *(80)* for a recent review of the topic] typically use an array of sensors such as metal oxide semi-conductors, which provide an output that is influenced by the presence of volatile organic compounds, to generate complex composite signals. In more recent approaches, techniques such as nuclear magnetic resonance spectroscopy have been used. This is a rapid and non-invasive technique that provides spectra that shed light on a wide range of compounds. The complex data sets are subject to multivariate analysis. Using this technique several different types of beers – ale, lager and alcohol-free – could be distinguished *(79)*. However, a great deal of further development work is needed to produce a practical tool with the capability to replace the taste panel.

Conclusions

A huge body of the literature is devoted to the various analytical methods used in brewing, for which it is not possible to provide a comprehensive review. Methods are in a constant state of flux, as improvements to existing procedures are identified, or entirely new approaches are introduced. Modifications to existing methods are often limited to improvements in the sensitivity of detection or the introduction of procedures that allow greater automation, either off- or on-line. Greater reliance on the latter is likely to become ever more important, as will the need to provide efficient and rapid analytical support, preferably in real-time, to the meet the demands of processes such as very high speed packaging lines using cold sterile-filtered beers. Completely new procedures are commonly imported from 'main-stream' science, often making use of new methods of separation and detection systems capable of greater discrimination and sensitivity. Techniques such as mass spectroscopy coupled to separation methods such as gas or liquid chromatography are immensely powerful and ideally suited to the analysis of complex matrices such as beers or its constituent raw materials. Similarly, in the realms of microbial spoilage and yeast handling, the great precision afforded by methods capable of analysing the genome offer great potential, especially when linked to techniques such as flow cytometry that have the ability to bring these analytical techniques to bear on a single cell. With regard to external factors, the threats to the process from pollutants are likely to grow as will the need to meet more stringent labelling legislation. For all of these reasons, the need to maintain a robust armoury of analytical methods that is flexible enough to respond rapidly to changing marketing conditions is essential.

References

1. Anderson, R. J. (2005) The transformation of brewing: an overview of three centuries of science and practice. *J. Histor. Soc.*, *121*, 5–24.
2. European Brewery Convention (1998) *EBC-Analytica*, 5th ed., Hans Carl Fachverlag, Nurnberg.
3. American Society of Brewing Chemists (2009) *Methods of Analysis*, 14th ed., The Society, St Paul, MN.
4. Siebert, K. J. (2006) Brewing control systems: chemical analytes, in *Brewing: New Technologies* (Bamforth, C. E., Ed.), pp. 372–390, Woodhead Publishing, Cambridge.
5. Siebert, K. J. (2011) Elements of analytical measurements in brewing. *J. Am. Soc. Brew. Chem.*, *69*(2), 100–107.
6. Baptista, M. S., Chan, T. D. and Gao, G.-H. (1996) Near infrared detection of flow injection analysis by acousto-optic tunable filter-based spectrophotometry. *Anal. Chem.*, *68*, 971–976.
7. Jorgensen, K. G. (1988) Quantification of high molecular weight $(1 \rightarrow 3)$ $(1 \rightarrow 4)$-β-D-glucan using Calcofluor complex formation and flow injection analysis 1. Analytical principle and its standardization. *Carlsberg Res. Commun.*, *53*, 277–285.
8. Ruiz-Capillas, C. and Jimenez-Colmenero, F. (2009) Application of flow injection analysis for determining sulphite in food and beverages: a review. *Food Chem.*, *112*, 487–493.
9. Bacoes, M. and Ashour, A. (1987) Use of a 96 well microplate reader for measuring routine enzyme activities. *Anal. Biochem.*, *166*, 353–360.
10. Scmidt, M. R. and Budde, A. D. (2008) Fluorescent micro plate readers as an alternative to flow injection analysis for determination of wort β-glucans. *Proc. World Brewing Congr.*, Honolulu, p. 81.
11. Nischwitzi, R., Rigoni, P., Marker, C., and Evans, E. (2012) The use of micro plate readers in the malting industry. *Proc. 32nd IBD Convention, Asia Pacific Section*, Melbourne, Australia, Presentation, Session 15, http://www.ibdasiapac.com.au
12. Buckee, G. K. (1986) Automated methods of analysis available in the brewing industry for in-line analysis, quality control and assurance purposes. *J. Inst. Brew.*, *92*, 323–329.
13. Besford, R. P. (1990) In-line and on-line analysis. *Ferment*, *3*, 46–47.
14. Daoud, I. S. (1991) New techniques for in-line measurement. *Ferment*, *4*, 40–46.
15. Forrest, I. S. (1996) In-line instruments, the way towards brewing efficiency? *Ferment*, *9*, 273–280.
16. Robert, E. and Klein, C. (2007) Improved method for CO_2 measurements. *Brew. Beverage Int.*, *5*, 21–24.
17. Moller, N. C. (1975) Continuous measurement of wort/beer extract in a fermenter. *Tech. Q. Master Brew. Assoc. Am. 12*, 41–45.
18. Cumberland, W. G., MacDonald, D. M. and Skinner, E. D. (1984) Automated fermenter control at Moosehead Breweries Ltd. *Tech. Q. Master Brew. Assoc. Am.*, *21*, 39–44.
19. Dutton, J. (1990) FV control with real-time SG monitoring. *Brew. Distill. Int.*, *May*, 20–21.
20. Sugden, S. (1993) In-line monitoring and automated control of the fermentation process. *Brew. Guardian*, *June*, 21–32.
21. Hees, M. and Amlung, A. (1997) Automation of fermentation using continuous extract content measurement in a cylindroconical fermenter. *Brauwelt*, *37*, 1549–1551.
22. Freshman, S., Wilson, T. W., Laine, C., Allsworth, M. and Angove, J.. (2012) The Lonestar™ portable analyzer for flavour quality control: detecting diacetyl, 2,3-pentanedione and acetaldehyde. *Proc. World Brewing Congr.*, Portland, OR, p. 104.
23. Jasinkski, J. S. (1984) Liquid chromatographic determination of nitrosamines in malt and beer with a photoconductivity detector. *Anal. Chem.*, *56*(12), 2214–2218.
24. Scanlan, R. A., Barbour, J. F., Hotchkiss, J. . H. and Libbet, L. M. (1980) *N*-Nitrosodimethylamine in beer. *Food Cosmet. Toxicol.*, *18*(1), 27–29.
25. Tipler, A. (2010) Determination of low levels of nitrosamines in beer using the Clarus 680 GC/MS and D-Swafer system. Perkin Elmer application note, www. perkinelmer.com/CMSresources/Images/44-74150APP_GCMSbeer.pdf
26. Hengel, M. J., and Shibamoto, T. (2000) Gas chromatographic-mass spectrophotometric method for the analysis of dimethomorph fungicide in dried hops. *J. Agric. Chem.*, *48*(12), 5824–5828.
27. Hengel, M. J., and Shibamoto, T. (2002) Method development and fate determination of pesticide-treated hops and their subsequent usage in the production of beer. *J. Agric. Food Chem.*, *50*(15), 4430–4439.

28. Hengel, M. J., and Miller, M. (2007) Analysis of flonicamid and its metabolites in dried hops by liquid chromatography–tandem mass spectrometry. *J. Agric. Chem.*, *55*, 8033–8039.
29. Murray, J. A., Walker, J. F., and Doyle, J. S. (1988) Tyramine in alcohol-free beer. *Lancet*, *1*(8595), 1167–1168.
30. Lippman, S. B., and Nash, K. (1990) Monamine oxidase inhibitor update – potential adverse food and drug interactions. *Drug Saf.*, *5*, 195–204.
31. Tailor, S. A. N., Shulman, K. I., Walker, S. E., Moss, J., and Gardner, D. (1994) Hypertensive episode associated with phenelzine and tap beer – a re-analysis of the role of pressor amines in beer. *J. Clin. Psychopharmacol.*, *14*, 5–14.
32. Shulman, K. I., Tailor, S. A. N., Walker, S. E. and Gardner, D. (1997) Tap (draft) beer and monamine oxidase inhibitor dietary restrictions. *Can. J. Psychiat.*, *42*, 310–312.
33. Izquierdo-Pulido, M., Hernandez-Jover, T., Marine-Font, A. and Vidal-Carou, M. C. (1996) Biogenic amines in European beers. *J. Agric. Chem.*, *44*, 3159–3161.
34. Loret, S., Deloyer, P. and Dandrifosse, G. (2005) Levels of biogenic amines as a measure of the quality of the beer fermentation process: data from Belgian samples. *Food Chem.*, *89*, 519–525.
35. Kalac, P., Krizek, M., Pelikanva, T. and Prokopova, M. (2002) Biogenic amine formation in bottled beer. *Food Chem.*, *79*, 431–434.
36. Cortacero-Ramirez, S., Arraez-Romain, D., Segura-Carretero, A. and Fernandez-Gutierrez, A. (2007) Determination of biogenic amines by capillary electrophoresis coupled to laser-induced fluorescence detection. *Food Chem.*, *100*, 383–389.
37. Directive 2003/89/EC of the European Parliament. *Offic. J. EU*, http://ec.europa.eu/food/food/labellingnutrition/foodlabelling/fl_com2003-89_en.pdf
38. Ilett, D. R. (1995) Aspects of the analysis, role and fate of sulphur dioxide in beer, a review. *Tech. Q. Master Brew. Assoc. Am.*, *32*(4), 213–221.
39. Monier-Williams, G. W. (1927) Determination of sulphur dioxide in foods. *Br. Food J.*, *29*, 51–53.
40. Barros, A. A., Rodriguez, J. A., Almeida, P. J., Guido, L. F., Golcalves, P., Santos, J. R., Machado-Cruz, J. M. and Fereira, A. (2001) Application of voltammetry in beer analysis. *Proc. EBC Congr.*, Budapest, p 84.
41. Almeida, P. J., Rodriguez, J. A., Guido, L. F., Santos, J. R., Barros, A. A. and Fogg, A. G. (2003) Voltammetric determination of free and total sulphur dioxide in beer. *Electroanalysis*, *15*, 587–590.
42. Dvorak, J., Dostalek, P., Sterba, K., Cejka, P., Kellner, V., Culik, J. and Beinrohr, E. (2006) Determination of total sulphur dioxide in beer samples by flow-through chronopotentiometry. *J. Inst. Brew.*, *112* (4), 308–313.
43. Hysert, D. W., Kovecses, F. and Morrison, N. M. (1976) A firefly bioluminescence ATP assay method for rapid detection and enumeration of brewery microorganisms. *J. Am. Soc. Brew. Chem.*, *34*, 145–150.
44. Simpson, W. J. (1999) Developments in ATP-bioluminescence technology in the brewing industry. Brew. Guardian, March, 21–25.
45. Priest, F. G. (2003) Rapid identification of microorganisms, in *Brewing Microbiology* (Priest, F.G. and Campbell, I., Eds), 2nd ed., pp. 305–328, Chapman and Hall, London.
46. Storgards, E., Haikara, A. and Juvonen, R. (2006) Brewing control systems: microbiological analysis, in *Brewing: New Technologies* (Bamforth, C. W., Ed.), pp. 391–426, Woodhead, Cambridge.
47. Boulton, C. A. (2012) Microbiological methods and the challenge of the modern brewing process. *Brew. Distill.*, *May*, 13–16.
48. Brandl, A. and Geiger, E. (2003) Microbiological quality control in breweries by PCR. *Proc. 29th EBC Congr.*, Dublin, pp. 1094–1104.
49. Braune, A. and Eidtmann, A. (2003) First experiences of using real-time PCR as a rapid detection method for brewery process control at Becks. *Proc. 29th EBC Congr.*, Dublin, pp. 1128–1135.
50. Wold, K., Bleken, A. and Hague, T. (2005) Practical experiences on the use of TaqMan real-time PCR as a microbiological routine quality control for fermenters at Ringnes brewery, *Proc. 30th EBC Congr.*, Prague (CD ROM), pp. 1085–1087.
51. Vogeser, G. and Dahmen, M. (2004) Improvement of microbiological analysis by the use of real-time PCR. Proc. World Brewing Congr., San Diego, CA, p. 69.
52. Vogeser, G., Heidereich, B. and Hertel, C. (2005) Identification of new beer spoiling bacteria species enrichment and specific detection by PCR. *Proc. 30th EBC Congr.*, Prague, pp. 1107–1108.
53. Juvonen, R., Koivula, T. and Haikura, A. (2008) Group-specific PCR-RFLP and real-time PCR methods for detection and tentative discrimination of strictly anaerobic beer-spoilage bacteria of the class *Clostridia*. *Int. J. Food Microbiol.*, *125*(2), 162–169.
54. Harris, C. M., Todd, R. W., Bungard, S. J., Lovitt, S. J., Morris, J. G. and Kell, D. B. (1987) Dielectric permittivity of microbial suspensions at radio-frequencies: a novel method for the real-time estimation of microbial biomass, *Enzyme Microbial Technol.*, *9*, 181–186.
55. Boulton, C. A., Maryan, P. S., Loveridge, D. and Kell, D. B. (1989) The application of a novel biomass sensor to the control of yeast pitching rate. *Proc. 22nd EBC Congr.*, Zurich, pp. 653–661.
56. Boulton, C. A. and Clutterbuck, V. J. (1993) Application of a radiofrequency permittivity biomass probe to the control of yeast cropping. *Proc. 24th EBC Congr.*, Oslo, pp. 509–516.
57. Carvell, J. P. (2000) Developments in using off-line radio-frequency impedance methods for measuring the viable cell concentration in the brewery. *J. Am. Soc. Brew. Chem.*, *58*, 57–62.
58. Mochaba, F. (1998) Practical procedures to measure yeast viability and vitality prior to pitching. *J. Am. Soc. Brew. Chem.*, *56*, 1–6.
59. Lentini, A. (1993) A review of the various methods available for monitoring the physiological status of yeast: yeast viability and yeast vitality. *Ferment*, *6*, 321–327.
60. Boulton, C. A. (1996) A critical assessment of yeast vitality testing. *Ferment*, *9*, 222–226.
61. Kara, B. V., Simpson, W. M. and Hammond, J. M. (1988) Prediction of fermentation performance of brewing yeast with the acidification power test. *J. Inst. Brew.*, *94*, 153–158.
62. Opekarova, M. and Sigler, K. (1982) Acidification power: indicator of metabolic activity and autolytic changes in *Saccharomyces cerevisiae*. *Folia Microbiol.*, *27*, 395–403.
63. Sigler, K., Mikyska, A., Kosaf, K., Gabriel, P. and Dienstbier, M. (2006) Factors affecting the outcome of the acidification power test as a measure of yeast quality: critical reassessment. *Folia Microbiol.*, *51*, 525–534.
64. Gabriel, P., Dienstbier, M., Matoulkova, D., Kosai, K. and Sigler, K. (2008) Optimized acidification power test of yeast vitality and its use in brewing practice. *J. Inst. Brew.*, *114*, 270–276.
65. Mochaba, F. M., O'Connor-Cox, E. S. and Axcell, B. C. (1997) A novel and practical yeast vitality method based on magnesium release. *J. Inst. Brew.*, *103*, 99–102.
66. Petit, J.-M., Denis-Gray, M. and Ratinaud, M.-H. (1993) Assessment of fluorochromes for cellular structure and function studies by flow cytometry. *Biol. Cell*, *78*, 1–13.
67. Riis, S. B., Pedersoen, H. M., Sorensen, N. K., and Jakobsen, M.(1995) Flow cytometry and acidification power test as rapid techniques for determination of the activity of starter cultures of *Lactobacillus delbrueckii* spp. *bulgaricus*. *Folia Microbiol.*, *12*, 245–250.
68. Novak, J., Basarova, G., Teixeira, J. A. and Vicente, A. A. (2007) Monitoring of brewing yeast propagation under aerobic and anaerobic conditions employing flow cytometry. *J. Inst. Brew.*, *113*, 249–255.
69. Kurec, M., Baszczynski, J., Lehnneut, R., Mote, A., Teixeira, J. A. and Branyik, T. (2009) Flow cytometry for age assessment of a yeast population and its application in beer fermentation. *J. Inst. Brew.*, *115*, 253–258.
70. Naumov, G. I. (1996) Genetic identification of biological species in the *Saccharomyces sensu stricto* complex. *J. Ind. Microbiol.*, *17*, 295–302
71. Hammond, J. R. M. (2003) Yeast genetics, in *Brewing Microbiology* (Priest, F.G. and Campbell, I., Eds), 3rd ed., pp. 67–112, Kluwer Academic/Plenum Publishers, New York.
72. Quain, D. E. (2006) Yeast genetics in brewing: new insights and opportunities, in *Brewing: New Technologies* (Bamforth, C. W., Ed.), pp. 149–166, Woodhead, Cambridge.
73. Uchida, M., Suga, S. and Ono, M. (1996) Improved flavour stability of beer – rapid predictive method for beer flavour stability by electron spin resonance spectroscopy. *J. Am. Soc. Brew. Chem.*, *54*, 205–211.
74. Uchida, M. and Ono, M. (2000) Technological approach to improve beer flavour stability: adjustments of wort aeration in modern fermentation systems using the electron spin resonance method. *J. Am. Soc. Brew. Chem.*, *58*, 30–37.
75. Barr, D., Heiss, A., Kamlowski, A., Maler, D., Erstling, J. and Melling, H. (2001) Shelf life analysis of beer using an automated lag-time EPR system. *Spectroscopy*, *December*, 16–19.
76. Chmielewski, J., Sikorska, E., Gorecki, T., Khemlinski, I. and Sikorski, M. (2007) Evaluation of beer ageing using an electronic nose. *Polish J. Food Nutr. Sci.*, *57*, 91–93.
77. Ghasemi-Varnamkhasti, M., Mohtasebi, S. S., Siadat, M., Lozano, J., Ahmadi, H., Razavi, S. H. and Dicko, A. (2011) Ageing fingerprints

characterization of beer using a electronic nose. *Sensors Actuat. B: Chem.*, *159*, 51–59.

78. Duarte, I. F., Barros, A., Belton, P. S., Righelato, R., Spraul, M., Humpfer, E. and Gil, A. M. (2002) High-resolution nuclear magnetic resonance spectroscopy and multivariate analysis for the characterization of beer. *J. Agric. Food Chem.*, *50*, 2475–2481.
79. Duarte, I. F., Barros, A., Almeida, C., Spraul, M. and Gil, A. M. (2004) Multivariate analysis of NMR and FTIR as a potential tool for the quality control of beer. *J. Agric. Food Chem.*, *52*, 1031–1038.
80. Baldwin, E. A., Bai, J., Plotto, A. and Dea, S. (2011) Review: Electronic noses and tongues: Applications for the food and pharmaceutical Industries. *Sensors*, *11*, 4744–4766.