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Advanced Analytical Methods For The Analysis of Chemical And Microbiological Properties of Beer

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ABSTRACT

Beer is a worldwide consumed and universally popular beverage due to its pleasant sensory properties and nutritional/medicinal functions. Chemical (flavor, safety, nutritional and medicinal aspects) and microbiological (brewing yeast and contaminating microorganisms) properties of beer are among its key attributes. In this article, application of advanced instrumental methods for the analysis of chemical and microbiological characteristics of beer is discussed. Advanced instrumental techniques for the quick, reliable, selective and relatively sensitive analysis of food products including beer are widely used for research or quality evaluation purposes.

Key words: analysis, beer, flavor, hygiene, instrumental, microbiological, nutritional

INTRODUCTION

Beer, a brewed beverage made principally from malt (germinated barley), hop, water and yeast, is one of the most popular drinks worldwide. In 2004, the per capita consumption of beer around the world was 72.9 L (annually) on average, while in some countries this figure was higher than 130 L[(1)]. Popularity of beer arises from its pleasant sensory attributes and favorable nutritional and health (in light-to-moderate consumption) characteristics[(2)]. Investigations have shown that there is a high tendency for beer consumption among different levels of societies due to the above-mentioned criteria[(3-5)].

Most beers produced worldwide have alcohol content in the range of 3 - 6% (v/v)[(6,7)]. While a low-strength beer contains about 2 - 3% of alcohol, a medium/average-strength beer has about 5% and a high-strength/strong beer has about 6 - 12% of alcohol[(2)]. In recent years, there has been an increased market share for low-alcohol (< 2.5% alcohol content) and non-alcoholic (< 0.5% alcohol content) beers[(2,7-9)].

Chemical aspects include flavor, chemical hygiene (chemical safety) and nutritional and medicinal attributes. Flavor is by far the most important sensory attribute of beer. Chemical aspects of food materials comprise only the background level of perception. Among chemical aspects, flavor compounds (for example) are directly perceived as flavor; whilst, for example, chemical substances contributing to the foam formation are not perceptible unless they appear in the foreground state of visual foam. Microbiological aspect is associated to the analysis of type and viable counts of microorganisms including brewer's yeast as well as contaminating (invading) microorganisms.

Due to their efficiency and sensitivity, advanced instrumental methods of analysis are widely used for the assessment of different foods (including beer), for research or for quality evaluation purposes. The aim of this article is to review the most important advanced instrumental methods for the analysis of chemical and microbiological characteristics of beer.

I. *Chemical Analysis*

(I) *Flavor*

The typical beer flavor comprises a complex balanced mixture of numerous flavor agents, such as phenolics, proteins, carbohydrates, isohumulones (iso-alpha-acids), alcohols, tannins, lactones, aldehydes, unsaturated carbonyl compounds, vicinal diketones, ionones, methyl esters, fatty acids, essential oils, sulfur-containing volatile compounds, nucleotides, mineral ions and organic acids. More than 800

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flavoring agents have been found in beer. Although many of these compounds are not key flavor compounds, they introduce a background perception that plays an important role in the overall impression of the flavor of beer[10-13]. Table 1 presents several published articles on the flavor analysis of beer. Different methods for the analysis of various flavor compounds are expressed below:

1. *Xanthohumol, Isoflavons, Iso-alpha-acids and Other Phenolic Compounds*

Xanthohumol has been quantified in hops by HPLC using UV detection[14]. However, this technique offers insufficient sensitivity and selectivity for the quantitative analysis of the minor prenylflavonoids in beer. Tandem mass spectrometry (MS-MS), as a detection technique, can provide improved sensitivity[15]. Liquid chromatography (LC) coupled with (tandem) mass spectrometry has been successfully applied to the quantitative analysis of isoflavones in plasma[16], baby food and flour[17]. Stevens *et al.*[15] developed a method for the quantification of six prenylflavonoids (xanthohumol, isoxanthohumol, desmethylxanthohumol, 6- and 8-prenylnaringenins and 6-geranylnaringenin) in hops and beer by HPLC-tandem mass spectrometry (HPLC-MS-MS)[18-21].

Bitterness in beer is analyzed by assessing *cis* and *trans* isomers of hops iso-alpha-acids, such as isocohumulone, isohumulone and isoadhumulone, the major bittering agents of beer. They can be determined by using spectrophotometrical methods (at 275, 325 and 355 nm) after their extraction in toluene and dilution with methanol[22]. They can also be determined by direct titration with lead acetate and monitoring the electrical conductivity of the samples[22]. The amount of polyphenols has been quantitatively determined by applying non-specific spectrophotometric methods based on their absorption (at 600 nm) after their reaction with ferric ammonium citrate[23]. Catechin can be used as standard[23]. The polyphenols quercetin, rutin, catechin and epicatechin in beer can be determined by using a HPLC procedure[24]. Whittle *et al.*[25] analysed the polyphenols (20 procyanidin dimers and/or trimers) using a HPLC system equipped with an electrochemical detector. Light absorption (at 275 nm) of an *iso*-octane extract of acidified beer can be used as a routine method for the analysis of principal bittering agents in beer (isocohumulone, *iso*-humulone and isoadhumulone)[23].

Isoflavonoids of beer have been determined using gas chromatography (GC) as well as combined GC with mass spectrometry (GC-MS)[26]. Lapcik *et al.*[26] developed radioimmunoassays specific for daidzein and its 4'-derivatives (formononetin, 4'-sulfate and 4'-glucuronide of daidzein) and for genistein and its 4'-derivatives (biochanin A, 4'-sulfate and 4'-glucuronide of genistein) found regularly in beers. Compared to HPLC and GC procedures, the above method is less time-consuming and more convenient. Nardini *et al.*[27] determined free and bound phenolic compounds (especially phenolic acids) in beer by using a HPLC procedure after the addition of strong antioxidants and sequsterants (in order to protect phenolics from oxidation). They also used some releasing agents to extract phenolics from their bound state. Individual phenolic compounds have been analyzed by using thin layer chromatography (TLC)[28]. These compounds have also been analyzed by electrophoresis[29]. Cummings *et al.*[30] analyzed phenolic compounds in beer using amperometric screen-printed carbon electrodes. They reported that chromatographic procedures may require certain tedious sample preparation steps that can compromise sample integrity. According to Cummings *et al.*[30], phenolic biosensors are suggested as an alternative method of analysis that do not have the problems associated with the traditional analytical methods.

Biosensors made of carbon paste and plat tissue have been utilized in the analysis of complex flavanols in beer samples[31,32]. However, due to the poor mechanical stability of carbon paste and low sensitivity, these biosensors are not suitable for the brewing industry[33]. Cummings *et al.*[30] employed bio-electrodes made of three commercially available graphite-based printed electrodes. The enzyme tyrosinase was immobilized on the electrode using a straightforward polymerization step applicable for mass production purposes. Vanhoenacker *et al.*[34] analyzed *iso*-α-acids and reduced *iso*-α-acids in beer by direct injection into a liquid chromatography system equipped with a ultraviolet (UV) absorption or mass spectrometry detector. Such method was also reported for the determination of phenolic compounds of beer matrices[35-37]. However, a filtration step was necessary to avoid interference of fermentable sugars, dextrins and organic acids. De Pascual-Teresa *et al.*[38] proposed a HPLC separation system coupled to a diode-array spectrophotometric detector after a chemical reaction with *p*-dimethylaminocynnamaldehyde (DMACA). Recovery of phenolic compounds in beer can be performed using liquid-liquid extraction systems with organic solvents[25,39].

Solid phase extraction (SPE) is a common technique used for pre-concentration and purification prior to HPLC separation of phenolic compounds in wines[40-42]. Separation of phenolic compounds in beer has been performed commonly by reversed-phase liquid chromatography followed by ultraviolet[33,43], photodiode array[44], fluorimetric[39], electrochemical[33,43,45] or mass spectrometric detection[25]. Garcia *et al.*[46] presented a method based on SPE application followed by HPLC-UV analysis for quality control in the brewing industry for the determination of phenolic acids. The method was applied to the quantitative analysis of these compounds in alcohol-free beers. Montanari *et al.*[44] determined organic and phenolic acids in beer by using two different HPLC methodologies: HPLC-ECD (amperometric electrochemical detection) and HPLC-DAD (photodiode array detection). They found that the most common phenolic acid was *m*-coumaric acid, followed by ferulic, *o*-cumaric, *p*-coumaric and 3-OH-benzoic acids. Vanillic, chlorogenic, homovanillic, *p*-hydroxybenzoic, 2,6-dihydroxybenzoic, syringic, gallic, protocatechuic, caffeic and 3,5-dihydroxybenzoic acids were present in small quantities.

Vanbeneden *et al.*[47] quantified hydroxycinnamic acids and their corresponding aroma-active volatile phenols (simultaneously) in wort and beer by using a simple and rapid

Table 1. Several published articles on flavor analysis of beer.

Parameter	Method of analysis	Source
Bitterness	Assessing *cis*-and *trans* isomers of hops *iso*-alpha-acids using spectrometry (275, 325, 355 nm)	22
Amounts of bitter acids	HPLC with DAD or MS detection	55, 94, 95
	Electrophoresis	29
Amounts of different polyphenols	Non-specific spectrophotometry based on the color formed at 600 nm with ferric ammonium citrate	23
	HPLC methodology	24 - 26, 33, 36, 43 - 45, 48
	GC and combined GC-MS	50 - 54
	TLC	28
	Capillary zone electrophoresis (CAE)	29, 57, 58
	Radioimmunoassay	26
	Amperometric screen-printed carbon electrodes	30
	Phenolic biosensors/bioelectrodes	30 - 32, 43
	Liquid chromatography by direct injection with ultraviolet absorbance detection or mass spectrometry	25, 34 - 37, 43
	HPLC separation or liquid-liquid extraction (LLE) and on-line detection by diode-array spectroscopy after chemical reaction with DMACA	38, 3925
	Separation of phenolics by photodiode array, fluorimetry or electrochemical procedure	39, 43 - 45, 48
	HPLC coupled with NMR spectroscopy using both on-line and stopped-flow techniques	55
	Spectrophotometric and fluorometric methodologies	39, 59, 60
	Simple and rapid isocratic HPLC using amperometric electrochemical detection	47
	Solid-phase extraction followed by liquid chromatographic separation with ultraviolet detection	45
On-line monitoring of flavor profile during fermentation	Flow-injection analysis (FIA)	23
	HPLC	23
	Infrared spectroscopy	23
	GC-MS	23
Determination of volatile organic compounds (VOCs)	Membrane inlet mass spectrometry (MIMS)	61, 62
	Purge-and-trap GC-MS or static/dynamic headspace GC	63, 64, 73, 79
	Liquid-liquid extraction, simultaneous extraction and distillation, solid-phase extraction or supercritical fluid extraction (SFE)	69 - 72
	Headspace solid-phase microextraction with GC-MS analysis	78
Determination of stale flavor carbonyl compounds	Electronic tongue	79, 80, 111
	Liquid-liquid extraction	81, 82
	Low pressure or steam distillation	84
	Low-pressure distillation followed by purge and trap with Tenax TA	85 - 87
	Solid-phase microextraction (SPME)	
	Stir-bar sorptive extraction (SBSE) combined with GC-MS	88
	HPLC with UV detection	82
	Reversed-phase liquid chromatography using UV detection	70
	Spectrophotometry	90
	GC-MS	91
Carboxylic acids analysis	HPLC with DAD or MS detection	92
Sulphur compounds analysis	HPLC with DAD or MS detection	93

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Table 1. continued

Parameter	Method of analysis	Source
Low-molecular weight organic acids (including carboxylic acids) analysis	Co-electroosmotic capillary zone	96
	Electrophoresis	97
Determination of volatile profile compounds	Headspace extraction followed by GC-MS	23
Volatile sulphur compounds	GC with flame photometric detector or Sievers' chemiluminescent detector	23
	Headspace single-drop microextraction (HS-SDME), direct single-drop microextraction (D-SDME) or headspace solid-phase microextraction followed by GC with detection flame photometric	99
	Dynamic headspace sampling followed by capillary GC coupled to a flame photometric detector or sulphur chemiluminescent detector	100, 101
Determination of volatile and semi-volatile sulphur compounds	Headspace solid-phase microextraction and GC with pulsed flame photometric detection	93
Quantification of sulphur dioxide	Measurement of NADH (after enzymatic oxidation of sulphur dioxide to sulfate and hydrogen peroxide) by spectrophotometry (340 nm)	23, 102
Assessment of vicinal diketones	GLC with an electron capture detector	23
	GC with both packed and capillary columns	23, 104
	GC-headspace with electron capture detection	23
	Colorimetric analysis	23
Measurement of non-volatile flavor compounds	Automated Dumas combustion method (isolation of N_2 from other combusted products and its measurement in a thermal conductivity cell)	104 - 110
Inorganic salts and nucleotides (nucleic acids) analysis	HPLC methodology	23
Amount of dissolved oxygen in headspace (which affects flavor stability via oxidation)	Oxygen electrodes	23
Measurement of radical forms of oxygen	Electron spin resonance technology (ESR)	22
	Chemiluminescence measurement	22

isocratic HPLC procedure with amperometric electrochemical detection system. The technique gave good specificity and sensitivity and could therefore be used for routine monitoring of the above compounds in beer. Prior to the study, simultaneous determination of hydroxycinnamic acids and volatile phenols was not easily possible. These compounds were separately determined by using HPLC[33,36,44,45,48] or GC[49-54] analysis. Pusecker *et al.*[55] used a HPLC system coupled to nuclear magnetic resonance spectroscopy (HPLC-NMR) to determine bitter acids in hop and beer. NMR spectroscopic measurements afforded full structural information on hop bitter acids constituents of various hop products. In addition, as an alternative/complementary technique to the HPLC system for the food analysis, capillary zone electrophoresis (CZE) has gained some attention. The main advantages of CZE are high separation efficiency, improved selectivity, low operational costs and speed of analysis[56]. A principle known as co-electroosmosis capillary electrophoresis has been used successfully for the analysis of phenolic compounds[57,58]. Total amounts of polyphenols in beer and wine were analyzed by using spectrophotometric and fluorimetric procedures[39,59,60].

2. *Volatile Organic Compounds*

Membrane inlet mass spectrometry (MIMS) is a specific and sensitive method for the analysis of volatile organic compounds (VOCs) in a water or gas sample using a thin membrane, which is installed between the sample and the ion source of a mass spectrometer[61]. Organic compounds dissolve in a membrane, permeate through it and finally evaporate into the mass spectrometer[62]. The function of MIMS is similar to that of the purge-and-trap gas chromatography-mass spectrometry (P&T-GC-MS) and also to that of static headspace gas chromatography (HSGC) in the determination of VOCs in aqueous samples[63-65]. Compared with P&T-GC-MS and HSGC methods, methods based on MIMS have lower detection limits, shorter analysis times and also higher capability for continuous on-line monitoring[66-68].

MIMS has been utilized in the on-line monitoring of beer fermentation processes and also for the continuous analysis of aroma compounds (using automatic MIMS)[62]. Several other methods including liquid-liquid extraction[69], simultaneous extraction and distillation[70], SPE[71] and supercritical fluid extraction[72] have also been employed for the analysis of volatile compounds in beer. Most of the methods result in extracts with flavor compounds highly representative of the liquid matrix and not of the headspace. The most widely used headspace sampling techniques for volatile compounds include static headspace analysis, dynamic headspace analysis and the purge and trap technique[73]. Headspace solid-phase microextraction (HS-SPME) is a simple, fast, sensitive and solvent-free extraction technique that, at the same time, results in the concentration of the extracted materials [74-77]. Pinho *et al.*[78] reported a simple and sensitive method for the analysis of beer volatile compounds using headspace SPME with GC-MS analysis.

There are various extraction or enrichment techniques such as liquid-liquid extraction[79,80], low-pressure or steam distillation[81-83], low pressure distillation followed by purge and trap (P&T) with Tenax TA[84] and solid-phase extraction[85,86] that are practiced for the analysis of stale flavor carbonyl compounds in beer. SPME was applied for the analysis of underivatized E-2-nonenal and E,E-2,4-decadienal (stale-representating agents) in beer[87,88]. In 1999, a new extraction technique known as stir-bar sorptive extraction (SBSE) using stir bars coated with 50 - 300 μL of polydimethylsiloxane (PDMS) was developed by Baltussen *et al.*[89]. Ochiai *et al.*[88] applied SBSE with in-situ derivatization combined with GC-MS to determine sub-ng/mL levels of stale flavor carbonyl compounds including E-2-octenal, E-2-nonenal, E,Z-2,6-nonadienal and E,E-2,4-decadienal in beer. After extraction, the analytes were thermally desorbed in a thermal desorption system (used to function as an autosampler) followed by GC-MS analysis. Santos *et al.*[82,83] proposed a routine method for the determination of E-2-nonenal in beer by HPLC with UV detection, for the evaluation of beer aging (detection limit of 0.1 μg/L).

β-damascenone, which is a terpenic ketone, is a main flavor in many alcoholic beverages (including beer). It is also a key odor in a variety of fruits and vegetables[83]. Guido *et al.*[70] proposed a method for the simultaneous determination of E-2-octenal and β-damascenone in beer by reversed-phase liquid chromatography using UV detection. Their method included a steam distillation step followed by extraction/concentration using Sep-Pak Plus C_{18} RP cartridges and determination by HPLC using UV detection at 226 nm[70]. Onate-Jaen *et al.*[90] presented several spectrophotometric methods to differentiate among beers and evaluate their aging (Section 1.3). Evaluation of beer aging can be directly correlated with the adverse changes in beer flavor profile and also with the relevant off flavors[90].

Using GC-MS, Vanderhaegen *et al.*[91] analyzed 15 known volatile compounds from aging beers to monitor the development of typical aging flavors produced during beer storage due to the Maillard reaction, formation of linear aldehydes, ester formation, ester degradation, acetal formation, etherification and degradation of hop bitter compounds. Carboxylic acids[92], sulfur compounds[93] and bitter acids [55,94,95] in beer can be qualitatively and/or quantitatively determined by HPLC using a DAD or an MS detector. Electrophoresis has also been used for the analysis of bitter acids[29]. Co-electroosmotic capillary zone electrophoresis was applied for the analysis of low-molecular-weight organic acids in beer[96-98]. Volatile sulfur compounds can be analyzed by GC using either a flame photometric or a Sievers' chemiluminescent detector, both of which are specific for sulfur[23].

Xiao *et al.*[99] compared three different extraction methods including headspace single-drop microextraction, direct single-drop microextraction and SPME to analyze volatile sulfur compounds in beer. The determination was carried out by using a GC instrument equipped with a flame photometric detector (FPD). The current method of choice for the determination of sulfur compounds in beer industry includes a dynamic headspace sampling procedure, followed by capillary GC coupled to FPD or sulfur chemiluminescence detection[100,101]. Adsorption losses, the introduction of artifacts and signal quenching were some disadvantages attributed to the dynamic headspace coupled to FPD[101]. To overcome these issues, Hill and Smith[93] developed a simple and sensitive method using HS-SPME and GC with pulsed FPD for the analysis of trace levels of volatile and semi-volatile sulfur compounds in beer. Sulfur dioxide (SO_2) is commonly analyzed using an enzymatic method, where it is oxidized to sulphate by the enzyme sulphite oxidase. Hydrogen peroxide, which is also produced here, is reduced by the enzyme NADH-peroxidase. Then, NADH is measured by its absorption at 340 nm[23,102]. Dimethylsulphide was determined using headspace GC with a capillary column and flame ionization detector (FID)[103]. Vicinal diketones were assessed using GC with both packed and capillary columns[103,104], by GC-headspace (without a need for distillation) with electron capture detection[23], or by colorimetric methods[23]. Diacetyl (acetoin) was analyzed by Tian *et al.*[105] using headspace GC analysis. This method was described as a sensitive quantitative analysis and the results demonstrated that it could be used successfully to analyze the concentration of acetoin in beer.

Kohonen Neural Network maps were used by da Silva *et al.*[106] for the exploratory analysis of Brazilian Pilsner beers. The input data consisted of the peak areas of the volatile profile compounds from the samples obtained from headspace SPME coupled to gas chromatography. The chromatographic peaks were identified as originating from compounds such as alcohols, esters, organic acids, phenolic compounds and ketones that are typically found in the headspace of such samples. Analysis of the Kohonen maps showed that the 20 different brands of beers could be grouped into six sets, with three of these sets having only one sample according to the composition of their volatile fractions. The volatile species associated with the similarities and differences among each sample group were tentatively identified

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by mass spectrometry and their contributions to the grouping were discussed.

3. *Miscellaneous Compounds*

Non-volatile flavor compounds can be measured according to the automated 'Dumas' combustion method, where the sample is combusted in the presence of oxygen at about 1000°C to give oxides of nitrogen, which are catalytically reduced to free nitrogen. Other products of combustion such as carbon dioxide and water are removed by selective absorption and the remaining nitrogen is measured in a thermal conductivity cell[107-110]. Inorganic salts and nucleotides (nucleic acids) can be measured by HPLC[23]. The amount of dissolved oxygen in the headspace of beer packages that involves in the off flavor production through oxidative reactions can be determined by using oxygen electrodes[23]. Radical forms of oxygen, which are good indicators of flavor instability, can be detected by using electron spin resonance technology and also by chemiluminescence measurements, either directly or after the reaction of beer with the radical scavengers[22].

Rudnitskaya *et al.*[111] used an electronic tongue multisensor system as an analytical tool for the rapid assessment of taste and flavor of beer. The beer samples were distinguished using both sensory panel and ET data based on PCA. The ET was capable of predicting 20 sensory attributes of beer including bitter, sweet, sour, fruity, caramel, artificial and burnt tastes, as well as the taste intensity and body of beer.

(II) *Safety Aspects*

Presence of some detrimental chemical compounds (having chemical or microbial origin) in beer beyond their standard dose should be avoided in order to inhibit the corresponding chemical intoxication diseases, such as allergy-related disorders, certain cancers, neurodegenerative disorders, encephalopathies, some cases of osteomalacia and estrogenic-associated disorders[112-114]. Toxic amines, mycotoxins, nitrates, aluminum, formaldehyde and radical forms of oxygen in beer are among the compounds that might occur at levels higher than those expected, if appropriate hygienic precautions are not considered[111-118]. Several published articles on the analysis of chemical hygienic aspects (chemical safety) of beer are listed in Table 2. The most important methods to analyze these compounds are discussed below:

1. *Mycotoxins, Aluminum, Arsenic and Phthalates*

Mycotoxins in beer, such as zearalenone, have been analyzed by thin-layer chromatography (TLC)[122], HPLC[123,124], GC[125], and enzyme-linked immunosorbent assay (ELISA)[126]. Once developed, LC-based methods are less time-consuming to operate and sometimes might not need major sample preparation and/or derivatization steps. In addition, chromatographic techniques can allow the simultaneous analysis of several mycotoxins in a single run[123-125]. HPLC has been used for zearalenone analysis in the low μg/kg range with fluorescence detection[127]. Recently, SPE with immunoaffinity materials has become popular in mycotoxin analysis as a selective and time-saving, one-step sample clean-up tool[127,128]. However, multi-toxin analysis is not feasible with these columns since they are highly specific for only one target mycotoxin[129]. MS is applied as a highly sensitive and selective detector in this regard. This detector comes with many advantages including easy sample preparation, its universal applicability to a wide variety of different analytes and its suitability for multi-analyte detection (if used online with a chromatographic technique such as GC and HPLC). Single-ion monitoring (SIM) and multi-reaction monitoring (MRM) with tandem mass spectrometry (MS-MS) can provide specific and exact determination of compounds over a wide linear range[129]. Schothorst and Jekel[130] developed a method for the determination of trichothecenes in beer by capillary GC equipped with FID.

Reinsch *et al.*[131] described a method for the determination of ochratoxin A in beer. It was based on a combined anion exchange/reversed phase clean-up and liquid chromatography with tandem mass spectrometry. This method was compared with a modified standard method and validated on the basis of spiked beer samples. Due to its good reproducibility, repeatability and robustness, this method is a promising alternative to LC-FD (fluorescence detection) techniques. Also, Medina *et al.*[132] reported a method for the determination of mycotoxin in beer using Immunoaffinity column as a clean-up procedure. The limits of detection and quantification of the proposed method were 0.0008 and 0.0025 ng/mL, respectively, while the reference values for them were 0.0025 and 0.0075 ng/mL, respectively, in the AOAC method. In this method, emphasis was put on the clean-up step, assaying zinc acetate as a precipitating agent for dyes and other components of beer. Further clean-up was performed using SPE-silica cartridges after liquid-liquid extraction with ethyl acetate. The advantage of the proposed method was that the use of high-cost immunoaffinity columns was avoided for sample clean-up while the good performance of the reference method was met[132].

Aluminum in beer can be measured using several techniques, such as ion-selective electrode[23], atomic absorption spectroscopy and ion chromatography[23]. Bellido-Milla *et al.*[116] analyzed various trace metals including aluminum using flame atomic spectrophotometry. Husa´kova´ *et al.*[133] described a method for the direct and accurate determination of arsenic in beer (> 8.4 μg/L) by electrothermal atomic absorption spectrometry equipped with several improvement approaches such as deuterium background correction and a palladium modifier, resulting in a 40% increase in sensitivity in peak-height measurements.

Ye *et al.*[134] introduced a simple, low-cost, sensitive and selective method for the determination of trace levels of phthalate acid esters (around μg/L) in beer based on a SPME-GC analysis using a novel sol-gel calixarene-containing fiber, resulting in much lower matrix interference from the beer samples and also much lower limit of detection. Phthalate acid esters, which can easily migrate from plastic

Table 2. Several published articles on the analysis of chemical hygienic aspects of beer.

Parameter	Method of analysis	Source
Aluminium content	Specific electrodes (conductometry)	23
	Atomic absorption spectroscopy	23
	Ion chromatography	23
	Flame atomic spectrometry	119
Arsenic content	Electrothermal atomic absorption spectrometry with deuterium background correction (D2-ET-AAS)	133
Quantification of methylamines/amines	Positive chemical ionization with GC-MS	120
	HPLC by *o*-phthaldialdehyde (OPA) derivatization	121
Mycotoxins analysis	Thin-layer chromatography (TLC)	122
	HPLC	123, 124, 127
	GC	125
	Enzyme-linked immunosorbent assay (ELISA)	126
	Single-ion monitoring (SIM) and multi-reaction monitoring (MRM) with tandem instruments (MS-MS)	129
	Capillary GC with flame ionization detection	130
	Combined anion exchange/reversed phase clean-up and liquid chromatography with tandem mass spectrometry	131
	Immunoaffinity column (IAC) as a clean-up procedure	132
Phthalate acid esters	Solid-phase microextraction (SPME) followed by gas chromatography using a novel sol-gel calixarene-contained fiber	134
Formaldehyde	Chromatogram	135 - 138
	Electrometry	139 - 142
	Fluorimetry	143 - 147
	Spectrophotometry	148 - 151
	Flow injection catalytic spectrophotometric methods	154
	Air-deriving flow injection (FI) device with merging zone technique	155
Nitrate analysis	Ion chromatography	23
N-nitrosodimethylamine	SPME	154
Biogenic amine	Sensitive capillary electrophoretic methods	155
	Reversed phase-high performance liquid chromatography (RP-HPLC) with diode array detection	156
Measurement of radical forms of oxygen	Electron spin resonance technology (ESR)	22
	Chemiluminescence measurement	22

materials into the environment and even into the foods in contact with them, are the most common plasticizers.

2. *Formaldehyde, Nitrate, Nitrosamines and Biogenic Amines*

Formaldehyde can be analysed using GC or HPLC[135-138] as well as electrometric[139-142], fluorimetric[143-147], spectrophotometric[148-151] and flow injection catalytic spectrophotometric[152] techniques. However, none of these methods is suitable for the routine analysis of formaldehyde in beer. Although chromatographic techniques provide adequate sensitivity, they are slow and cannot be easily adopted for routine analysis. On the other hand, fluorimetric methods are always subjected to interferences from some carbonyl compounds in beer. Yue *et al.*[153] proposed a sensitive automated procedure for the rapid determination of formaldehyde in beer. The method was based on the catalytic action of formaldehyde in the redox reaction between Victoria Blue B and potassium bromate in a phosphoric acid medium. Both the sensitivity and speed of analysis were adequate and small amounts of reagents and sample were needed. An air-deriving flame ionization device with merging zones technique was applied in this procedure and provided excellent precision too.

Nitrate content in beer can be analyzed by ion chromatography[23]. Amounts of *N*-nitrosodimethylamine (NDMA) and *N*-nitrosodiethylamine in beer have been determined using GC-MS with positive-ion chemical ionization[120]. Pe´rez *et al.*[154] proposed SPME for the extraction of *N*-nitrosodimethylamine, a trace and highly potential active carcinogen, from beer using headspace sampling and GC-MS analysis. Polydimethylsiloxane/divinylbenzene

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fibers were used to evaluate the influence of equilibrium time, ionic strength, extraction time and temperature by means of a factorial design. The method was validated based on the linearity, reproducibility, limit of detection and limit of quantification. The method was applied to the quantitative analysis of NDMA combining the standard addition method with an internal standard method.

Using laser induced-fluorescence, Cortacero-Ramı´rez *et al.*[155] developed a sensitive capillary electrophoretic method for the simultaneous determination of 10 biogenic amines normally present in beer samples. Amines in the samples were first derivatized and then filtered and finally separated with an uncoated capillary tubing in the presence of 50 mM of sodium borate and 20% acetone at pH 9.3 in a 30 kV electric field. It was possible to analyze biogenic amines in brewing-process samples and in beer samples in less than 30 min, achieving a detection limit as low as 0.3 μg/L for ethylamine and 11.9 μg/L for 1,6-hexanodiamine. Tang *et al.*[156] employed a method involving the pre-column derivatization of the amines with 4-chloro-3,5-dinitrobenzotrifluoride and subsequent analysis by reversed-phase HPLC with diode array detection. Detection limits of biogenic amines were 0.056 - 0.87 μmol/L at a signal-to-noise ratio of 3. The proposed method was applied to the quantitative determination of spermine, phenethylamine, spermidine, histamine, tyramine, tryptamine and putrescine in beer with recoveries of 91.9 - 103.1% and relative standard deviation of 2.86 - 5.63%. Putrescine, histamine and tyramine were detected in all samples. Spermidine was detected in 89% of the beers. Spermine, tryptamine and phenylethylamine were found, respectively, in 78%, 61% and 44% of the beers examined.

(III) *Nutritional Aspects*

Beer comprises numerous health benefits (nutritional and medicinal) and epidemiological, experimental and clinical investigations have revealed that light-to-moderate consumption of beer brings a relatively wide spectrum of health benefits to humans. However, its excessive consumption (especially those with higher amounts of alcohol) results in adverse effects. Beer contains large levels of vitamins (especially B complex) and minerals such as selenium[6]. The medicinal effects of beer (anti-carcinogenic, cardioprotective, immunomodulation, anti-osteoporosis, anti-stomach ulcer, radioprotective and anti-microbial/anti-viral effects, retardation of dimentia and aging, prevention of diabetes, tension reduction (relaxation), ease of bowel movement in the elderly, facilitating renal excretion of aluminum as well as estrogenic properties in women), have been attributed to certain components such as ethanol, phenolics, proteins and peptides, folic acid, dietary fibers, glycine betaine in beer and also to its relatively lower pH[2,6]. The main methods to assess the nutritional compounds of beer are mentioned below:

1. *Carbohydrates and Proteins*

Fermentable carbohydrates (as caloric substances) of beer can be analyzed using HPLC technique[23]. Fructose, fructosans and pentosans have been quantified using colorimetric methods[23]. Chromatographic carbohydrate profile can be determined for quality control purposes[112]. Electrophoresis[29] and two-dimensional *J*-resolved nuclear magnetic resonance spectroscopy[157] have been used for the determination of carbohydrates in beer.

Total protein content is routinely measured by the Kjeldal method. However, some advanced instrumental methods have been used to analyze different types of beer proteins. Evans and Sheehan[158] showed that measurement of beer proteins by the "Bradford Coomassie Blue Dye Binding Assay" (a colorimetric method), which only measures proteins with MW > 5,000 amu, correlated well with Rudin head retention values. There have been some efforts to determine the protein profile of beer using simple assessment of ultraviolet absorbance by beer wort. However, other ultraviolet-absorbing materials such as bitter acids of hop interfere with the above absorbance[22]. ELISA has been used for measuring the levels of some protein fractions in malt[159]. Size exclusion chromatography (gel filtration) is applied for the analysis of polypeptides in beer[160]. Bamforth *et al.*[161] explored a method for assessing hydrophobic polypeptides in beer by measuring fluorescence based on interaction of the proteins with 1-anilino-8-naphthalenesulfonate. Gorinstein *et al.*[59] studied the changes in the protein and amino acid contents of beer using combined fluorimetry, ion-exchange chromatography, gel-electrophoretic separation and Fourier transform infrared (FT-IR) spectroscopy.

Khatib *et al.*[157] determined the amino acids in beer qualitatively and quantitatively using two-dimensional *J*-resolved nuclear magnetic resonance (NMR) spectroscopy. HPLC as well as electrophoresis has been reported as a suitable method for the determination of both amino acids and peptides in beers[29,162]. Kutlan and Molnar-Perl[121] proposed a new HPLC method for the simultaneous quantification of amino acids and amines by *o*-phthaldialdehyde derivatization of these compounds. Thorsten and Bruckner[163] quantified enantiomeric amino acids (*L*-amino acids and *D*-amino acids) by using GC complemented by an HPLC analysis.

2. *Ethanol*

Alcohol (ethanol) content can be determined using several analytical methods including the catalytic combustion using a "Servochem Automatic Beer Analyzer" (SCABA). The injected beer is divided into two streams. One stream enters a Paar U-tube densitometer and the other one passes down a column as a falling film where the alcohol is removed as a vapor with a counter current air flow and passed over an alcohol sensor. After calibration with known standards, the onboard computer will display the percentage of alcohol[164]. Another method is based on calculating the refractive index of the media[23]. GC-FID and direct injection onto a suitable column are both considered as precise methods for alcohol analysis[165]. Two-dimensional *J*-resolved nuclear magnetic resonance (NMR) spectroscopy has also been used for alcohol analysis in beer[157]. Alcohol content has been also analyzed using infrared[166] or near-infrared (NIR)[167,168]

spectroscopy or by combination of these two methods[169]. Liario *et al.*[168] determined different quality parameters (including alcohol content) in beer using attenuated total reflectance-Fourier transform infrared spectroscopy. For alcohol-free beers with alcohol content of less than 0.008%, an enzymatic method is proposed based on the "Boehringer test kit"[23]. The alcohol is oxidized first to ethanal and then to ethanoic acid with nicotamide adenine dinucleotide (NAD^+) and the reduction of the cofactor is measured spectrophotometrically at 340 nm[23].

Among other devices, biosensors have also been used for the analysis of ethanol. Biosensors are easy to operate. They have short response times, better sensitivity and higher selectivity levels[170]. Some biosensors based on alcohol dehydrogenase[171,172], alcohol oxidase[173,174], alcohol oxidase-peroxidase coupled to enzyme system[175,176], microbial[177,178] and plant tissue material[179] have been developed for ethanol determination. Immobilized catalase enzyme biosensor was able to determine ethanol concentration as low as 18 mM within a response time of 30 - 90 s[180]. Also, a hydrogen peroxide sensor based on antagonism of peroxide reaction to tyrosinase reaction using common substrates was reported[181]. Akyilmaz and Dinckaya[170] developed an amperiometric biosensor based on catalase enzyme for the determination of ethanol at 0.05 - 1.0 mM concentration, a detection limit of 0.05 mM and a response time of 3 min. GC is being applied as one of the most common methods for ethanol determination in beverages[182,183]. However, it is still relatively expensive and demands skilled operators and often a sample pretreatment.

A microplate based on dielectric thin membrane in combination with a tiny capillary was applied to simultaneously determine several parameters such as superficial tension, latent heat of evaporation, boiling point and heat capacity of alcohol-water mixture. The application of liophilic fluorescent reagent and fluorescein octadecyl ester (chromoionophore XI) in fiber-optic sensor for the determination of aliphatic alcohols (in the range of 10 - 60% v/v) has been reported[184]. An application of lipophilic trifluoroacetophenone derivatives in optical alcohol sensors was reported by Simon's group[185,186]. Synthesis of a variety of chromogenic alcohol-sensitive reactants has also been reported[187,188].

Several studies have applied potentiometric polymeric membrane electrodes for indirect detection of various alcohols[189-191]. Such response from these electrodes is mainly due to the effect of alcohols on the activity of the electrode's primary ion. In these sensors, a mechanism similar to that in the human gustatory system is used for artificial tasting purposes. Electronic tongues were also reported for the analysis of alcohol content in beverages[192,193]. Flow-through electronic tongues based on miniaturized solid-state potentiometric sensors were also utilized for the recognition of beers[194].

Lvova *et al.*[195] developed an analytical instrument allowing rapid and on-line control of ethanol content in beverages in a wide range of concentration. In this research, the potentiometric responses of porphyrin-based solvent polymeric membranes towards several aliphatic monoatomic alcohols in single-, two- and four-component solutions were evaluated. Sensitivity of membranes in single-component alcohol solutions decreased in the following order: ethanol > methanol > butanol. Boujtita *et al.*[196] developed a disposable amperometric biosensor for ethanol analysis. It comprised a screen-printed carbon electrode doped with 5% cobalt phthalocyanine and coated with alcohol oxidized; a permselective membrane on the surface acts as a barrier for interfering elements. The measurement of ethanol was based on the signal produced by hydrogen peroxide, a product of the enzymatic reaction. MIMS (as mentioned in Section I) is another technique for monitoring ethanol concentration during the brewing fermentation[197].

3. *Phenolic Compounds and Riboflavin*

The methods for the analysis of phenolic compounds and different ions in beer were mentioned in Section I. Several methods have been developed to evaluate antioxidant capacity of food based on the evaluation of the free radical scavenging capacity. The most commonly used methods are based on molecular absorption spectrophotometry (MAS) using a UV-VIS instrument. This is due to the simplicity of the operation and also its relatively lower cost. They are considered as indirect methods since free radicals from an aromatic organic compound are measured[198-200]. Electrochemical techniques[201] and also techniques based on the determination of the lag time by electron spin resonance (ESR) have as well been reported[202,203].

Riboflavin content in beer was determined by capillary electrophoresis/blue light emitting diode (LED)-induced fluorescence detection combined with a dynamic pH conjunction technique. LEDs have been developed since the 1960s and constitute an exceptionally stable light. They are considered as an ultra-high intensity sources. LEDs became commercially available in mid-1990s at a variety of wavelengths in the visible spectrum[204]. A dynamic pH conjunction method represents one of the on-line sample concentration techniques[205,206]. The principal mechanism of this takes advantage of velocity-difference-induced focusing, in which an analyte migrates differentially within two distinct segments of the background electrolyte resulting in the compression of the analyte into a narrow zone prior to reaching the detector[205]. The traditional chromatographic methods, such as HPLC, GC and supercritical fluid chromatography (SFC) require complicated procedures to prepare and pre-concentrate (by liquid-liquid or SPE) the analytes[204]. HPLC[207] and electrophoresis[29] have been used for the analysis of vitamins in beer. Sikorska *et al.*[208] used fluorescence spectroscopy for monitoring changes occurring in beer during storage under different conditions. Table 3 shows several publications on the analysis of nutritional components of beer.

II. *Microbiological Analysis*

Only a narrow range of microorganisms can grow in

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beer. Lactic acid bacteria are the main spoilers[209]. Spoilage microorganisms can deteriorate the qualitative profile of beer. These undesirable effects cost millions of dollars in economic losses annually. In this category, some advanced methods of qualitative determination (detection and identification) or quantitative determination (enumeration) of microorganisms (not their chemical or physical impacts in food) are mentioned.

The conventional plate count procedure for qualitative or quantitative microbiological analysis suffers from both specificity and sensitivity aspects. In addition, it takes a relatively long time before results are available[210]. Therefore, newer rapid and reliable methods of detection are of great interest for the food industry. Different methods including immunological assays[211-215], polymerase chain reaction (PCR) [209,216-218], fluorescence or chemiluminescence[219-222], and quartz crystal microbalance[223] have been developed that are applied for identification purposes. Although hybridization and PCR techniques are very specific and suitable for screening purposes, they still fail to produce accurate results when enumeration of viable microorganisms is needed[210]. Among these methods, the most promising ones are those that are based on immunoassay technology since they present higher sensitivity and specificity in considerably shorter times. Monoclonal antibodies have the advantage of ensuring reproducibility and a permanent reagent supply[210]. They have been applied for the specific detection and identification of lactic acid bacteria [213,214,224-226]. They have been employed in the conventional and modified ELISA methods[213,226,227], immunoblotting[224,225] and filter epifluorescence antibody[228] techniques. However, these methods do not provide sufficient accuracy and sensitivity for the analysis[210]. On the other hand, luminescence assays are highly sensitive. Therefore, a combination of immunoassay technology with luminescence detection (detection of light produced by a chemiluminescence reaction coupled to an antigen-antibody interaction) may provide a specific and sensitive detection system with high potential for quantifying viable bacteria[210]. Juvonen *et al.*[229] developed and evaluated group-specific PCR methods to detect and differentiate strictly anaerobic beer-spoilage bacteria. A group-specific primer pair targeting a 342-bp variable region of the 16S rRNA gene was designed and evaluated in end-point PCR with gel electrophoresis and in real-time PCR with SYBR Green I dye. The PCR methods developed allow the detection of all the nine beer-spoilage Pectinatus, Megasphaera, Selenomonas and Zymophilus species in a single reaction and their differentiation sub-group level, and reduce the analysis time for testing of their presence in beer samples by 1 - 2 days. The methods can be applied for routine quality

Table 3. Several publications on the analysis of the nutritional and medicinal aspects of beer.

Parameter	Method of analysis	Source
Fermentable carbohydrates analysis	HPLC	23, 112
	Colorimetric methods	23
	Electrophoresis	29
Protein content determination	Hydrophobic interaction chromatography	161
	Two-dimensional *J*-resolved nuclear magnetic resonance (NMR) spectroscopy	157
		162
	HPLC	29
	Electrophoresis	121
	HPLC by *o*-phthaldialdehyde (OPA) derivatization of amino acids	23
	Dialysis	23
	Electrophoresis	22
	Ultraviolet absorbance	86
	Enzyme-linked immunosorbant assay (ELISA)	160
	Size exclusion chromatography (gel filtration)	33
	Measuring fluorescence	
Alcohol (ethanol) analysis	Catalytic combustion using a "Servochem Automatic Beer Analysier" (SCABA)	164
	Infrared or near-infrared (NIR) spectroscopy or combination of two methods	167, 168
	Refractive index analysis procedure	23
	GC with flame ionization detector	165
	Two-dimensional *J*-resolved nuclear magnetic resonance spectroscopy	157
	Attenuated total reflectance-Fourier transform infrared spectroscopy	168
	Spectrophotometry (340 nm) (measuring reduction of co-factor; an enzymatic procedure)	170
	Biosensors (including amperometric ones)	171 - 181, 196

Table 3. continued

Parameter	Method of analysis	Source
	Gas-liquid chromatography (GLC/GC)	182, 183
	Determination of alcohol by analysing several physical parameters (e. g. supercritical tension, latent heat of evaporation, boiling point and heat capacity of alcohol-water mixture) using microplate based on dielectric thin membrane in combination with a tiny capillary	195
	Potentiometric polymeric membrane electrode	189 - 191
	Electronic tongues (array of cross-sensitive sensors coupled with adequate data treatments)	192, 194, 195
	Potentiometric responses of porphyrin-based solvent polymeric membranes toward different alcohols (on-line monitoring of alcohol)	195
	Fibre-optic sensors comprising lipophilic fluorescent reagents (for aliphatic alcohols)	184 - 188
	Membrane inlet mass spectrometry (MIMS) (continuous monitoring of ethanol)	197
Polyphenols analysis	(Table 1, flavor)	(Table 1, flavour)
Ions analysis	Electrodes (conductometry)	23
	Atomic absorption spectroscopy	23
	Ion chromatography	23
	Flame atomic spectrometry	119
	Integrated-atom-trap system mounted on a standard atomic absorption air-acetylene flame burner	199
	Flow injection (FI) system with in-valve column and bed injection	8
Determination of antioxidant capacity	Molecular absorption spectrophotometry UV-VIS (MAS) (based on the pre-formation of free radicals)	90, 198, 200
	Electrochemical techniques and procedure based on the determination of lag time by electron spin resonance (ESR)	201 - 203
Riboflavin	Capillary electrophoresis/blue light emitting diode (LED)-induced fluorescence detection combined with a dynamic pH junction technique	204 - 206
	HPLC with liquid-liquid or solid-phase extraction	206, 207
	Electrophoresis	29
	Fluorescence spectroscopy	202

control in brewery and for studying occurrence, diversity and numbers of the strictly anaerobic beer spoilers in the brewing process.

Television cameras have been used as imaging devices for rapid and sensitive immunochemiluminescence detection and viable enumeration of *Escherichia coli*[215,230] and a beer spoilage strain of *Lactobacillus brevis*[231]. March *et al.*[210] managed to detect and count viable beer-spoilage lactic acid bacteria using a monoclonal chemiluminescnece enzyme immunoassay and a cooled digital charge-coupled device (CCD) camera. The microorganisms in beer can be pre-concentrated (cells capture) using a sterile membrane filter with pore-sizes of 0.22 or 0.45 μm. With direct epifluorescent filter technique, the cells trapped on the filter are stained with a fluorescent dye such as acidine orange. Viable cells strains can be observed as orange and dead cell strains as green spots[22]. Commercially, the amount of contaminating micro-organisms in beer has been recommended to be assessed: (1) in a hemocytometer, (2) electronically in a Coulter particle counter or (3) using Abmeter, by optical procedure using NIR spectroscopy[22]. Hemocytometer is a counting chamber loaded onto a microscope slide. Wild yeasts have been quantitatively determined by GC analysis[112]. The growth kinetics of yeast cells during the fermentation process can be monitored by the assessment of optical density using a spectrophotometer at suitable wavelengths. Table 4 lists several published articles on the microbiological analysis of beer.

Haakensen *et al.*[232] designed a method to assess the ability of Lactobacillus and Pediococcus to spoil beer; that is, to evaluate the beer-spoilage potential of mentioned bacterial isolates. In searching for a method to differentiate between beer-spoilage bacteria and bacteria that cannot grow in beer, Haakensen *et al.*[232] explored the ability of lactobacilli and pediococci isolates to grow in the presence of varying concentrations of hopcompounds and ethanol in broth medium *versus* agar medium. The best method for differentiating between bacteria that can grow in beer and bacteria that do not pose a threat as beer-spoilage organisms was found to be a hop-gradient agar plate containing ethanol. This hop-gradient agar plate technique provides a rapid and

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Table 4. Several published articles on the microbiological analysis of beer

Parameter	Method of analysis	Source
Detection, identification and enumeration of bacteria and yeasts	Immunological methods/immunoassay (e.g., monoclonal antibodies, immunoblotting and epifluorescence antibody)	211 - 215, 224 - 229
	Polymerase chain reaction (PCR)	209, 216 - 218
	Fluorescence and chemiluminescence assays	219 - 222
	Quartz crystal microbalance	223
	Combination of immunoassay technology with luminescence detection	210
	Immunochemiluminescence detection with television camera	215, 230, 231
	Monoclonal chemiluminescence enzyme immunoassay with a cooled digital charge-coupled device (CCD) camera (viable count of spoilage lactic acid bacteria)	210
	Hemocytometer	22
	Coulter particle counter (electronic procedure)	22
	Abmeter (optical procedure using near-infrared, NIR, spectroscopy)	22
	GC (wild yeasts)	13

simple solution to the dilemma of assessing the ability of *Lactobacillus* and *Pediococcus* isolates to grow in beer, and provides new insights into the different strategies used by these bacteria to survive under the stringent conditions of beer.

Asano *et al.*[233] evaluated a microcolony method for the detection and identification of beer-spoilage lactic acid bacteria (LAB). In this approach, bacterial cells were trapped on a polycarbonate membrane filter and cultured on ABD medium, a medium that allows highly specific detection of beer-spoilage LAB strains. After short-time incubation, viable cells forming microcolonies were stained with carboxyfluorescein diacetate and counted with μFinder Inspection System. All of the slowly growing beer-spoilage LAB strains were detected within 3 days of incubation. The specificity of this method was found to be exceptionally high and even discriminated intra-species differences in beer-spoilage ability of LAB strains upon detection. The results indicated that this method allows rapid and specific detection of beer-spoilage LAB strains with inexpensive CFDA staining. For further confirmation of species status of detected strains, subsequent treatment with species-specific fluorescence in-situ hybridization (FISH) probes was shown effective for identifying the CFDA-detected microcolonies to the species level. In addition, no false-positive results arising from noise signals were recognized for CFDA staining and FISH methods. Taken together, the developed microcolony method was demonstrated as a rapid and highly specific countermeasure against beer spoilage LAB, and compared favorably with the conventional culture methods.

CONCLUSIONS

Flavor, chemical hygiene (chemical safety), nutritional and medicinal as well as microbiological attributes are among the key characteristics of beer. In this article, major instrumental methods of analysis relevant to chemical and microbiological characteristics of beer were discussed. These methods have been successfully applied for beer analysis. Ease of operation combined with a quick result is the most important characteristic one should look for when trying to select among several methods. Furthermore, adequate sensitivity (that is, a low detection limit) and selectivity as well as good accuracy and precision levels are expected in a typical method applied for beer analysis.

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