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CHROMATOGRAPHY OF BEER

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The objectives of the review are the collection, concise description and evaluation of the various chromatographic techniques used for the separation and quantitative determination of macro- and microcomponents present in beers.

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Introduction

Beer is a popular beverage all over the world. It has been established many times that the moderate consumption of beer exerts favourable influences on human's health such as nutritional benefits, anti-mutagenic and anti-carcinogenic effects, reduction of cardiovascular diseases, hypolipidemic effect, stimulation of immune system, anti-osteoporosis effect and the reduction of the risk of dementia. It has been also found that the excessive consumption of beer may results in heath disorders, allergy induction, increase in the plasma concentration of uric acid, mutation and induction of cancer, increase of the risk of dementia, obesity and social misbehaviour.[1,2]

Gas chromatography (GC)

Gas chromatographic methods are generally applied for the separation and quantitative analysis of volatile and semi-volatile compounds. As beers contain volatile compounds of both natural and synthetic origin GC separation technologies found application in the investigation of the micro- and macro-components of beers. Headspace solid phase microextraction (HS-SPME) followed by gas chromatography mass spectrometric detection (GC-MS) was used for the study of the correlation between quantitative sensorial descriptors and chromatographic signals. Sensorial descriptors were estimated by conventional descriptive analyses (QDA). Genetic algorithm (GA) and ordered predictors selection (OPS) were applied as tools of selection of variables. The calculations revealed that the combination of sensorial descriptors and chromatographic signals can be successfully employed for the evaluation of foods and beverages.[3]

The maximum ethanol concentration in blood was measured after forced consumption of non-alcoholic beer. The investigations were carried out with headspace gas chromatography-flame ionization detection (HS-GC-FID). The limit of detection (LOD) was 0.0000 g L^{-1}, the maximum concentration of ethanol in blood was 0.0056 %. The investigations indicated that forensic implications cannot be expected from the consumption of non-alcoholic beer.[4] The aroma stability of beers under different storage conditions was investigated by HS-SPME/GC-MS and the influence of the extraction conditions on the efficacy of extraction was studied in detail. The data were evaluated by linear regression analysis. The calculations revealed that the sample volume (V) and extraction temperature (T) exert the highest impact on the extraction yield. The effect of storage temperature and the length of storage on the volatile profile was also determined. The measurements indicated that both the length and temperature of the storage influence the aroma profile of beers[5] GC-olfactometry, GC-MS, and GC combined with pulsed flame photometric detection were employed for the study of the occurrence of polyfunctional thiols in sorghum beer. Target compounds were selectively extracted with p-hydroxymercurybenzoic acid. It was established that the application of *Vernonia amygdalina* influences considerably the character of beer.[6] A novel dispersive liquid-liquid microextraction (DLLME) coupled with GC-MS were employed for the separation and quantitative determination of 18 biogenic amines in beers. It was found that the simultaneous extraction/derivatization of the amines results in a fast and simple extract enrichment. The extracting agent consisted of acetonitrile (dispersive solvent; 1.0 mL), toluene (extracting solvent; 325 µL), and isobutyl chloroformate (derivatizing agent; 25µL). The recoveries ranged from 72 to 113 %. The inter-day and intra-day precision was 13 and 14 %, respectively. LOD was always lower than 2.9 µg L^{-1}. It was established that the putrescine, tyramine, dimethylamine, cadaverine, pyrrolidine, and 1,3-diaminopropane were the dominant biogenic amines in beers.[7] The newest results in the theory and practice of the application of GC-olfactometry (GC-O) of alcoholic beverages have been previously discussed. The principles of GC-O methodology, sample preparation techniques, data collection procedures, GC instrumentation and other analytical conditions have been explained in detail. Examples for the analysis of alcoholic beverages such as wine, beer and spirits are presented.[8]

High performance liquid chromatography (HPLC)

Natural components

The allergy to beer has been extensively investigated. It has been established that in the majority of cases beer allergy was correlated with the hypersensitivity to the non-specific lipid transfer protein (LTP). The immun reactive LTP was separated by HPLC. The measurements indicated that allergy to beer considerably depends on the type and on the character of brewing process.[9] The cause of the rapid deterioration of flavour of beer has been intensively

investigated using HPLC technologies. It was established that this procedure may be due to the vulnerability of iso-alpha-acids to the light, the oxidation of iso-alpha acids. The stability of beers can be improved by adding phenolic compounds with antioxidant properties, addition of pure stereoisomers cis-iso-alpha acids or their reduced species, or use of riboflavin-binding proteins.[10] Real degree of fermentation (RDF) is an important characteristic of brewhouse performance. The relationship between RDF and the properties of malted barley starch has been intensively investigated. The molecular size distribution was determined with high performance size exclusion chromatography (HPSEC). Target compounds were detected with multi-angle laser light scattering. The data were analysed by various multivariate mathematical-statistical methods such as cluster analysis, analysis of variance, principal component analysis. Starch properties explained the 86% of the RDF variance suggesting that other malted barley constituents may influence the relationship.[11]

A simple and rapid analytical method was developed for the determination of 9 heterocyclic amines in beer and beer-like drinks. Measurements were carried out by employing hydrophilic interaction liquid chromatography-mass spectrometry. The recoveries of the measurements varied between 72-123 % for pilsner beer and 61-96 % for dark beer. As heterocyclic amines were not detected in the samples it was concluded that the health risk due to heterocyclic amines in beers and beer-like drink is extremely low (English abstract).[12] Dispersive liquid-liquid microextraction (DLLME) followed by LC with fluorometric detection has been employed for the analysis of thiamine (vitamin B-1) in foods. Derivatization was carried out by the oxidation of thiamine with ferricyanide at pH 13 to form fluorescent thiochrome. DLLME method was carried out by mixing 0.5 mL acetonitrile (dispersing agent) containing 90 μL of tetrachloroethane (extraction solvent). The mixture was added to 24 % of sodium chloride. Phase separation was achieved by centrifugation and the sedimented phase was analyzed by HPLC. LOD was 0.09 ng mL^{-1}, relative standard deviation was 3.2 %. It was established that the method can be applied for the analysis of thiamine monophosphate and thiamine pyrophosphate after acidic and enzymatic treatments. The procedure was successfully employed for the determination of thiamine in various foods and food products such as beer, brewer's yeast, honey, baby foods, infant formulas, fermented milk, cereals, and purees. Before HPLC analyses solid samples were hydrolysed with trichloroacetic acid. It was found that the results obtained by analyzing certified reference material were in excellent agreement with the certified value.[13]

A method using derivatization followed by RP-HPLC was developed for the determination of diacetyl an important flavour compound. Because of its importance as flavour compound and its impact on human health a considerable number of chromatographic method have been developed and applied for their determination in beers. A new method based on the derivatization of diacetyl and the subsequent separation of the derivatized product has been published. Diacetyl was derivatized with 4-nitro-o-phenylenediamine (NPDA) at 45^0C, for 20 min, at pH 3. The resulting 6-nitro-2,3-dimethylquinoxaline was separated on a RP18 column.

Target compound was detected at 257 nm. LOD was 0.0008 mg L^{-1}, the recoveries ranged 94-99.0 % The relative standard deviation range was 1.20-3.10 %. The concentration of diacetyl in the samples varied between 0.034 – 0.110 mg L^{-1}. Because of the excellent separation capacity, linearity and good repeatability the method was proposed for the determination of diacetyl in beer samples.[14]An automatic headspace in-tube extraction (ITEX) method was employed for the determination of acetaldehyde, ethyl acetate, diacetyl, and other volatile compounds in wine and beer. The optimization process established that the vial and sample sizes and the trapping material are most important parameters of the accuracy of the method. Small 2 mL vials containing a very small amount of sample (20 μL of 1:10 diluted sample) and a trap filled with 22 mg Bond Elut ENV was applied. The effective extraction required 100 x 0.5 mL pumping strokes at $60\ ^0$C. Determination coefficients were over 0.995, the repeatability was better than 7 %, and reproducibility was lower than 8.3%. It was stated that the method can be used for the investigation of volatile compounds in complicated matrices.[15] The influence of immobilized matrix on the fermentation process has been studied in detail and the results were compared with the results obtained with the results of traditional brewing. Cell microencapsulated technology was applied as a new brewing technique. Cell were microencapsulated in alginate. The investigated parameters included glucose concentration, cell multiplication, cell viability, specific gravity, pH, Brix, and ethanol. Fermentation processes were followed by both sensorial and instrumental methods (gas chromatography). No significant differences were found between the sensory characteristics of the beers brewed by the traditional and modern technologies. Although the profile of the headspace compounds were different it does not influenced the sensorial character of the beers.[16] It has been previosly established that the yeast (*Saccharomyces cerevisiae*) and lactic bacteria are able to accumulate and biotransform inorganic selenium into organo Se compounds. Selenium biotransformation was followed during brewing by using *S. cerevisiae* and *Saccharomyces uvarum* for Ale and Lager fermentations, respectively. Se-enriched beer was produced by adding sodium selenite (0.02, 1.0. 2.0. 10.0 and 20 μg mL^{-1}). The fermentation brew contained yeast, malt extract and water. Target compounds were separated by HPLC-ICP-MS and anion exchange HPLC. It was established that selenomethionin was the most frequent organo Se compound found during the investigations.[17] The concentration of biogenic amines (BA) and polyamines (PA) were determined in alcoholic and non-alcoholic beer in the Czech Republic. The measurements established that the concentration of histamine, phenylethylamine and tryptamine was very low in the samples. The amount of the PA spermine and spermidine was also low. However, the total amount of BA and PA exceeded the ''healthy'' level of 100 mg L^{-1}.[18] A new method was developed for the removal of volatile compounds from complicated accompanying matrices. Nitrogen gas stripping combined with high vacuum (NSHV) was employed for the removal of volatile components without the application of heat. It was established that the efficacy of the method is higher than that of the traditional rotary evaporation. The application of the new method was proposed for the removal of volatile compounds from beer and possibly other liquid samples as well.[19] The production of eight biogenic amines (BAs) (histamine, tyramine (TYR), tryptamine, putrescine, cadaverine (CAD), phenylethylamine, spermine and spermidine by 81 lactic acid bacteria (LAB) strains (*Lactobacillus, Lactococcus, Leuconostoc, Enterococcus,*

Pediococcus, Tetragenococcus and Bifidobacterium) was investigated. It was established that contaminating LAB can increase the concentration of CAD and TYR in beer.[20] The new extraction technique gas diffusion microextraction (GDME) has been employed for the determination of methylamine, dimethylamine and ethylamine in beers and other fermented beverages. Amines were extracted with GDME, and derivatised with phenyl isothiocyanate (PITC). Target compounds were analysed by HPLC using UV detection. LOD was between 12 – 46 μg L^{-1}, LOQ ranged from 39 to 153 μg L^{-1}. Because its simplicity GDME was proposed for the extraction of aliphatic amines in fermentated beverages.[21] The influence of the different beers on the mechanism of gastric acid secretion has been studied in detail. Organic acids and bitter compounds were separated by HPLC-DAD and UPLC-MS/MS. It was found that ethanol, and organic acids such as succinic acid, malic acid, and citric acid influence the gastric acid secretion. It was further established that the bitter acids such as alpha-, beta-, and iso-alpha acids play a considerable role in the regulation of gastric acid secretion.[22]

Environmental pollutants

A triple quadruple LC-MS/MS method was developed for the determination of the *Fusarium* mycotoxins deoxynivalenol (DON), 3-acetyl-DON (3-ADON) and the conjugated mycotoxin deoxynivalenol-3-glucoside (D3G). Before HPLC analysis the samples were degassed, the matrix compounds were precipitated and the dried rests were dissolved in the solvent. The relative standard deviation of the measurements were 4-16 %. Recoveries for DON, D3D and 3-ADON were 60-90, 39-69 and 96-124 %, respectively. It was established that beers contain in average 6.6 μg L^{-1} DON and D3G, while 3-ADON was not detected. The occurrance of mycotoxins together with endotoxins was investigated in indigenous banana beer. The measurements prove the presence of both mycotoxins and endotoxins causing health risk for consumers.[23] The influence of unmalted barley on the oxidative stability of wort and beer has also been investigated by GC-MS. The results indicated that the addition of barley increased the oxidative beer stability.[24] GC-MS method was applied for the determination of the effect of wood aging on beer flavor and the concentration of monophenols.The investigation indicated that the addition of oak chips modified considerably the flavor profile of the beer. The woody, vanilla-like, spicy, and smoky aroma increased. The concentration of monophenols as vanillin, acetovanillone, syringaldehyde, acetosyringone, guaiacol, ethylguaiacol, eugenol, thymol, and salicylaldehyde were also elevated. Both sensory effects and monophenol concentrations were correlated with the origin and toasting deuce of the oak chips applied.[25] The concentration of phenolic compounds and their antioxidant activity in barley and malt extract were investigated by liquid chromatographic technologies. Barley and malt were extracted with ethyl acetate and the component of the extracts were separated with RP-HPLC. Detection was carried out on two wavelengths one for the detection of phenolic compounds the other for the reduced form of the radical cation 2,2'-azinobis(3-ethylbenzo-thiazoline-6-sulfonic acid (ABTS). It was found that prodelphinidine 83 and procianidin B3 are responsible for the overwhelming majority of the antioxidant activity. It was further established that malting and brewing markedly decreases both the concentration and antioxidant activity of phenolic compounds.[26] The effect of mashing technology processes on the composition and quantity of phenolic compound was investigated by HPLC, and by other two electron spin resonance method. The measurements indicated that the concentration of polyphenols decreases during the mashing process.[27] HPLC/MS combined with clean upon immunoaffinity column was employed for the determination of aflatoxins B1, B2, G1 and G2 in brewing raw material and beer. LOD ranged 0.04 – 0.12 μg kg^{-1} in barley and malt, 0.08 – 0.58 μg kg^{-1} in hops, 0.04 – 0.12 μg kg^{-1} in brewers'yeast, and spent grains, and 1.5 – 4.7 ng L^{-1} in beer.LOQ varied from 0.13 – 0.39 μg kg^{-1} in barley and malt, 0.25 - 1.94 μg kg^{-1} in hop samples, 0.13 – 0.39 μg kg^{-1} in brewers yeast and spent grains, and 5.1 – 15.2 μg L^{-1} in beer. It was established that the aflatoxin levels did not exceed the maximum allowable limit set by the European Union[28]. The formaldehyde level in beers was also measured by HPLC. The target molecule was preconcentrated with the cloud point extraction method. Formaldehyde was complexed with 2,4-dinitrophenylhydrazine in an aqueous solution of the nonionic surfactant Triton X-114. The target compound was concentrated at the temperature of 60^0C. LOD was 0.7 ng mL^{-1}. The concentration of formaldehyde ranged 172-385 ng mL^{-1}. This new sensitive and rapid procedure was proposed for the determination of formaldehyde in beer.[29] The occurrence of Ochratoxin A (OTA) in various manufactured food products has been investigated. Sampled were prepurified on an immunoaffinity column (IAC). Separation and quantitative determination was carried out by HPLC-FD, and confirmed with LC-ESI-MS/MS. Recoveries were between 78.3 – 103.3 %. RSDs (relative standard deviations) was 2.1 – 4.3 %. It was concluded from the data that the consumption of the food products investigated present no risk to consumers[30]. The concentration of OTA was measured in various foods and food products such as composite samples of cereal-based baby foods, beer, breakfast cereals (corn and rice and wheat based), loaf bread, peanuts, and pistachios. Samples were pretreated by liquid-liquid extraction followed by separation on immunoaffinity column and HPLC measurements. The results indicated that the median estimated daily intake of OTA through the foodstuffs were below the latest provisional (PTDIs) tolerable daily intakes recommended by the Food Safety Authority (EFSA) in 2006 and the Joint FAO/WHO Expert Committee on Food Additives (JECFA).[31] Plasma ochratoxin A levels were determined in men and women in the Molise region Italy. The relationship between the concentration of ochratoxin A in plasma and some physicochemical parameters such as dietary habits, specific disease risk biomarkers (body mass index) (BMI), C-reactive protein (CRP), cardiovascular risk score were calculated. The amount of ochratoxin A in plasma was evaluated by HPLC. CRP was measured by a latex particle enhanced immunoturbidimetric assay. LOD was 25 ng L^{-1}, the mean ± standard deviation was 0.229 ± 0.238 ng mL^{-1}. The investigations revealed that cereals, wine, beer and jam/honey influence the level of OTA in plasma. It was further established that the positive correlation between OTA and CRP suggests a possible role of OTA in inflammation and in the genesis of cardiovascular diseases and cancer.[32] A novel method was developed for the separation and quantitative determination of chloroacetic, bromoacetic and iodoacetic acids in alcoholic beverages. Monohalogenic acids (m-HAAS) were extracted using static headspace extraction and were esterified to

increase their volatility. It was stated that the method is suitable for the detection of monohalogenated acids at the concentration of μg L^{-1}.[33] Formaldehyde was classified as carcinogenic to humans. It can cause leukemia and nasopharyngeal cancer. The exposure to formaldehyde in alcoholic beverages has been discussed in detail. It was concluded that formaldehyde in alcoholic beverages cause a negligible risk for consumers, the main risk factors are ethanol and acetaldehyde.[34] SPME using on-fibre derivatization was applied for the analysis of furfural in infant formulas, beers and vinegars. The poly(dimethylsiloxane)divinylbenzene (PDMS/DVB) fibre was employed and O-2,3,4,5,6-(pentafluorobenzyl)-hydroxylamine hydrochloride (PHBFA) was loaded onto the fibre. Food samples of 2 mL were placed in a glass vial. SPME was carried out at 80 C^0 for 20 min under magnetic stirring. SPME fibre was desorbed at the injection port of GC/MS. The method detection limits (MDLs) were lower than 5%. Recoveries were 100 ± 5 %, MDLs ranged from 3.09 to 14.05 μg L^{-1}.[35] HPLC combined with UV detection and triple quad MS was applied for the separation and quantitative determination of the Maillard reaction product 6-(2-formyl-1-pyrrolyl)-l-norleucine (formyline).This new pyrrole amino acid resulted from the reaction of free and protein-bonded lysine residues with the 1,2-dicarbonyl compounds such as 3-deoxypontosone, pentose sugars, and degradation products of disaccharides. Formyline and its structural analog pyrraline were detected in enzymatically hydrolyzed food matrices (milk and whey products, breakfast cereals, pasta, and bakery products). It was further established that formyline and pyrraline are constituents of beer proteins.[36] A GC/MS method was employed for the determination of ethyl carbamate (EC) in fermented foods and beverages. It was established that rice wines contained up to 515 ± μg kg^{-1} ethyl carbamate; rice cooking wine 206 ± 87 μg kg^{-1}; white spirits, wine and beer 72 ± μg kg^{-1}; Soy sauces and vinegars contained also EC in the concentration of 47 ± 27 μg kg^{-1}. EC was determined in normal sufu (63μg kg^{-1}) and in red sufu (182 μg).[37]

HOP

High speedcounter-current chromatography (HSCCC) and HPLC were applied for the separation of xanthohumol (XN) and related prenyl flavonoids from the hops (*Humulus lupulus L*). The solvent system employed for the analysis of XN consisted of n-hexane-ethyl acetate-methanol-water 5; 5; 4; 3. It was established that the method can be successfully used for the isolation of XN from hop extract. The purity of XN was over 95%, the yield of the extraction was 96.60%. UV, H1 NMR, and C13 NMR technologies were applied for the elucidation of the exact structure of XN.[38]

Electrically driven systems

Capillary zone electrophoresis (CZE) has been used for the analysis of compositional carbohydrates in polysaccharides and foods. The method made possible the simultaneous determination of thirteen reducing carbohydrate such as aldohexose, aldopentose, maltose and lactose. Analytes were derivatized with 1-phenyl-3-methyl-5-pyrazolone (PMP). The conditions of the CZE measurement were: background electrolite 175 mM borate buffer (pH 11.0), organic modifier: methanol; detection 245 nm. The quantitative recoveries of compositional carbohydrates in the samples were between 93.2 – 104.0 %, the RSD values varied between 2.9% - 4.9 %. The method was proposed for the quality control of reducing carbohydrates in food analysis. A thin-layer electrochemical flow cell coupled with capillary electrophoresis with contactless conductivity detection (EC-CE-C^4D) was employed for the derivatization and quantification of aliphatic alcohols (ethanol, 1-propanol, 1-butanol, and 1-pentanol). The simultaneous electrooxidation of the analytes were carried out on a platinum working electrode in acid medium. The analysis time was 2.5 min., the LOD was 5 x 10^{-5} mol L^{-1}. It was established that no significant differences can be found between the results obtained with the new method and those obtained by the traditional GC/MS procedure.[39]

The volatile compounds of hop were analysed by a headspace (HS)-trap method followed with GC-MS. The investigations revealed that steam distillation is not suitable for the preconcentration of thermolabile compounds, such as caryophyllene oxide. It was stated that the HS-trap procedure is fast and sensitive, requires small sample volume and minimal sample preparation steps.[40] Headspace solid-phase microextration followed with GC/MS was employed for the determination of the terpenoid (monoterpenes and sesquiterpenes) metabolic pattern of hop-essential oil. The parameters of the extraction (type of fiber coatings, extraction temperature, extraction time, ionic strength, and sample agitation) were optimized: analytes were extracted for 30 min at 40 ^{0}C using a 50/30 μm divinylbenzene/carboxene/polydimethyl siloxane coated fiber. The measurements established that hop essential oil contained 56.1 % monoterpenes (13 compounds); 39.9 % sesquiterpenes (10 compounds); 1.41 % oxygenated monoterpenes (3); 0.04 % hemiterpenes (1). The main metabolites were identified as monoterpene β-myrcene (53.0±1.1 %); and cyclic sesquiterpenes, α-humulene (16.6±0.8 %); β-caryophyllene (14.7±0.4 %). It was further proposed that hop essential oil can be applied as a powerful biosource of terpenoid metabolites.[41]

Abbreviations

ABTS	2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid)
BA	biogenic amines
BMI	body mass index
CAD	cadaverine
CRP	C-reactive protein
CZE	capillary zone electrophoresis
EC	ethyl carbamate
EFSA	European Food Safety Authority
DLLME	dispersive liquid-liquid microextraction
DON	deoxyvalenol
D3G	deoxyvalenol-3-glucoside
FID	flame ionization detector
FD	fluorescence detection
GA	genetic algorithm
GC	gas chromatography

GC-MS	gas chromatography with mass spectrometric detection
GC-O	gas chromatography-olfactometry
GDME	gas-diffusion microextraction
HPLC	high performance liquid chromatography
HPLC-DAD	high performance liquid chromatography diode array detection
HPSEC	molecular size distribution
HS	headspace
HSCCC	high-speed counter-current chromatography
HS-SPME	headspace solid phase microextraction
IAC	immunoaffinity column
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LAB	lactic acid bacteria
LC-MS/MS	quadruple LC-MS/MS
LOD	limit of detection
LOQ	limit of quantitation
LTP	non-specific lipid transfer protein
MDLs	method detection limits
NSHV	nitrogen gas stripping coupled with high vacuum
OTA A	ochratoxin A
OPS	ordered predictors selection
QDA	quantitative descriptive analysis
PMP	1-phenyl-3-methyl-5-pyrazolone
PA	polyamines
PDMS/DVB	poly(dimethylsiloxane)/divinylbenzene
PFBHA	O-2-3-4-5-6-(pentafluorobenzyl) hydroxylamine hydrochloride
RDF	real degree of fermentation
RSD	relative standard deviation
SPME	solid-phase microextraction
T	extraction temperature
TRY	tyramine
UPLC	ultra performance liquid chromatography
V	sample volume
XN	xanthohumol

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