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Determination and fractionation of metals in beer – a review

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Abstract

Major, minor and trace metals are important in beer fermentation since they supply the appropriate environment for yeast growth and influence yeast metabolism. A real concern is the content of Cu and Fe, which are involved in beer conditioning and ageing through reactions resulting in formation of reactive oxygen species. The reactive oxygen species readily oxidize organic compounds present in beer, changing the quality of foaming and the flavor stability of beer. In view of brewing technology and beer processing, knowledge regarding functions of metals and their speciation in brewing liquors and beer is of special significance. Metals in beer also have a certain nutritional importance but their actual effect related to beer consumption depends on the type of species they form with low and high molecular mass organic ligands which naturally occur in beer. This review covers the determination and fractionation of metals in beer using atomic spectrometry methods. Special attention is drawn to the role of metals in beer and brewing, possible metal associations, methods of beer preparation before analysis on the total metal content and approaches to metal partitioning in beer.

Keywords: *Beer, metals, determination, fractionation, atomic spectrometry*

Introduction

Sources of metals in beer

Beer is a product of a yeast alcoholic fermentation of extracts of malted cereals, usually barley malts, with or without a starchy material, and to which hops are added. All natural components used for brewing, including water, cereals, barleys and yeasts, are the main endogenous sources of metals in beer. For that reason, the mineral composition of beer reflects the composition of ingredients used for brewing and refers to processes involved in a beer production. The content of metals is variable and depends on quality of substrates taken, type of beer brewed and country of origin of beer (Hardwick 1995; Moll 1995; Goldammer 1999; Baxter and Hughes 2001; Briggs et al. 2004).

Metals in beer may also come from other substances added during brewing so as to control fermentation and maturation processes. Another exogenous source of metals in beer can be a contamination from brewery equipments, i.e. pipes, fluid lines, vessels and tanks, used for beer handling, including fermentation, conditioning, filtration, carbonating and packing. Also containers (kegs, casks, cans), in which a final quality product is stored and transported, can be a potential source of beer contamination. Indeed, moderately acidic brewing liquors and beer (pH about 4.2 on average) can to a large extent contribute to a metal ions ingestion, especially in case of Al cans, kegs and casks (Sharpe and Williams 1995; Vela et al. 1998; Wyrzykowska et al. 2001). Aluminum can liners deterioration and an interaction of beer with containers usually leads to an increase in concentrations of Al, Co, Cr, Cu, Fe or Ni, which readily migrate to the beer. In the case of Al (Vela et al. 1998), it can be presumed that the longer the length of beer storage, the higher concentrations of metals that are found in the beer. Additionally, the higher the temperature of storage, the more rapid is the rate of can

corrosion which takes place, regardless of its coating, and the higher the accumulation of metals in beer that occurs.

Metals in brewing

Besides the nutritional value of metals from beer with respect to their potential relationship and contribution to daily diary nutrition requirements, they are also very important and influential in the brewing process itself. When considering the growth and metabolism of yeasts, many metals are essential co-factors for numerous fermentative enzymes, being necessary components of transport systems fulfilling different charge-balancing or structural roles. Concentrations of Ca(II), Co(II), Cu(II), Fe(III), K(I), Mo(II), Mn(II), Mg(II), Ni(II) and Zn(II) ions required for the appropriate yeasts growth are reasonably small. At high concentrations, these metals are usually toxic or exert a salt stress on the yeasts as in case of Na(I) for example (Briggs et al. 2004).

With respect to improvements in yield and a fermentative capacity, as well as a maintaining consistency of a product quality, metal metabolism of yeasts is of special interest to brewers (Walker et al. 1996; Chandrasena et al. 1997). Normally, the metal ion composition of brewing liquors notably changes during brewing and the rate of this alteration depends on raw materials used for beer production and conditions of the brewing process. The required metal uptake by yeast cells normally relies on the concentration of particular ions in the growth media and, to a great degree, on their bioavailability. The latter factor depends on both solubility and properties of different ion-complexing ligands originated from raw materials. Free metal ions are the most bioavailable to yeast cells, but chelating and adsorbing constituents present in wort and

other brewing liquors regulate metal ions availability by formation of complexes of different stability. Certainly, such a reduction of bioavailability in the case of Ca(II), Mg(II) or Zn(II) ions has a detrimental effect on a yeast growth kinetics and a fermentation performance (Walker et al. 1996; Chandrasena et al. 1997). Information on the role of some specific major and minor metals in yeast metabolism and the fermentative and sensory performance of beer is given in Table I (Hardwick 1995; Moll 1995; Walker et al. 1996; Chandrasena et al. 1997; Goldammer 1999; Baxter and Hughes 2001; Briggs et al. 2004).

Nevertheless, it is evident that with reference to beer stability and quality not only the mineral composition of beer and brewing liquors should be controlled but also the speciation forms of the metals.

Beer ageing and haze formation

It is recognized that in the presence of Cu^{+} and Fe^{2+} ions, stable molecules of O_2 dissolved in beer capture electrons and form superoxide anions (O_2^{-}). Usually, resulting metal cations (Cu^{2+}, Fe^{3+}) can be reduced again to respective lower oxidation state forms by such pro-oxidant molecules as some polyphenols (Kaneda et al. 1992; Bamforth et al. 1993, Uchida and Ono 1996; Andersen and Skibstead 1998; Kaneda et al. 1999; Vanderhaegen et al. 2006). By further protonation, O_2^{-} anions form perhydroxyl radicals ($OOH^{\bullet}$) that are recognized to have a much higher oxidative activity than the other reactive oxygen containing species. In other reaction O_2^{-} anions can be reduced by Cu^{+} and/or Fe^{2+} cations to peroxide anions (O_2^{2-}), which can be then easily protonated to hydrogen peroxide (H_2O_2). Additionally, intensely reactive hydroxyl radicals ($OH^{\bullet}$) can be also produced from resulted H_2O_2 or O_2^{-} by metal ions

induced Fenton and Haber-Weiss reactions (Kaneda et al. 1999; Vanderhaegen et al. 2006).

The reactive oxygen containing species (O_2^-, $OOH^\bullet$, H_2O_2, $OH^\bullet$) are crucial to beer staling process because they willingly react with different classes of organic compounds present in beer, including polyphenols, isohumulones, alcohols, aminoacids, fatty acids, iso-α-acids, α-acids and β-acids, permanently changing a sensory beer profile and determining its flavor stability. Apparently, the rate and level of the reactive oxygen species formation is related to Cu and Fe amounts (Cu^+/Cu^{2+}, Fe^{2+}/Fe^{3+}), and thus, concentrations of Cu and Fe in beer are of special significance and should be minimized and/or controlled during the brewing process.

It is also documented that polyphenolic compounds contribute to beer ageing reactions. Some of polyphenols are pro-oxidants capable of transferring electrons to metal ions and reducing Cu^{2+} and Fe^{3+} to respective Cu^+ and Fe^{2+} forms, which indirectly stimulates the activation of O_2 in beer. In this process, polyphenols become radicals and can also react with other beer constituents leading to their deterioration or can decompose, producing various off-flavors. On the other hand, polyphenols act as anti-oxidants capturing Cu and Fe ions and forming their stable complexes. Apart from polyphenols, aminoacids, phytic acids and melanoidins in brewing liquors and beer have also this ability and sequestrate metal ions to promote the active oxygen radical species formation (Vanderhaegen et al. 2006).

Free metal ions are supposed to affect formation of a protein-polyphenol haze (chill haze) resulting from a cross-linkage of proteins by low molecular weight polyphenols (Siebert et al. 1996; McMorrough et al. 1999). In general, polyphenols readily polymerize but it is presumed that metal ions catalyze this process. Additionally,

when polyphenols subsequently interact with proteins after their polymerization, a "permanent haze" of particle size between 1 and 10 μm is formed in beer.

Finally, it should be noted that the polyphenol polymerization is strongly promoted by their oxidation (Kaneda et al. 1990; Kaneda et al. 1992; Bamforth 1999b). Indeed, when the rate of the reactive oxygen species formation, in reactions activated and catalyzed by Cu and Fe ions, is high, the haze readily appears because of polyphenols oxidation.

Determination of metals in beer

Total metal content

The major metals of beer are Ca (4–140 mg l^{-1}), K (18–1100 mg l^{-1}), Mg (24–270 mg l^{-1}) and Na (1–230 mg l^{-1}). To the groups of minor and trace metals belong Al (0.005–2.2 mg l^{-1}), Ba (0.01–0.07 mg l^{-1}), Cd (0.0002–0.7 mg l^{-1}), Co (<0.0008 mg l^{-1}), Cr (<0.02 mg l^{-1}), Cu (0.008–0.8 mg l^{-1}), Fe (0.02–1.6 mg l^{-1}), Hg (<0.3 mg l^{-1}), Mn (0.03–0.8 mg l^{-1}), Ni (<0.3 mg l^{-1}), Pb (0.0008–0.03 mg l^{-1}), Sr (0.1–0.7 mg l^{-1}) and Zn (0.001–1.5 mg l^{-1}). The concentrations of major as well as some minor and trace metals in beers coming from different countries are given respectively in Table II (Ca, K, Na, Mg) and Table III (Al, Ba, Cd, Cu, Fe, Mn, Pb, Sr, Zn).

<Tables II and III near here>

With respect to a nutritional value of metals to humans and according to relatively high contents of some of them in beer, it is accepted that a moderate and reasonable beer consumption can be to some extent be a valuable source of

recommended daily dietary metal intake (Bamforth 2002). However, it should be also noted that although the information on the total metal content in beer is advantageous for the estimation of the metal nutrient uptake source, metals bioavailability from beer to humans and their absorbability in the gastric system crucially depends on speciation forms in which they are present in beer (Sharpe and Williams 1995; Svendsen and Lund 2000; Pohl and Prusisz 2007). For instance, it is indicated that Al (where excessive intake is acknowledged to be associated with Alzheimer's disease) is predominantly present in beer as citrate and phosphate complexes and that only a small portion of the total Al in beer is transported through the gut wall. Consequently, beer plays rather a minor part in the uptake of Al from the diet (Sharpe and Williams 1995).

The determination of the total metal composition of beer, including major, minor and trace metals, is of particular interest to brewers and consumers. Depending on the concentration and type, metals may be essential or toxic to the human body and can also affect the brewing process and beer quality in view of flavor stability and haze formation (Bellido-Milla et al. 2000; Svendsen and Lund 2000; Wyrzykowska et al. 2001; Alcazar et al. 2002; Vinas et al. 2002; Bellido-Milla et al. 2004; Pohl and Prusisz 2004; Asfaw and Wibetoe 2005; Nascentes et al. 2005; Llobat-Estelles et al. 2006; Onate-Jaen et al. 2006; Pohl and Prusisz 2007).

Some metals may be harmful above a certain concentration (Al, Cd, Hg, Pb), some of them are regarded to influence the foam and flavor quality (Cu, Fe, Mn), others are essential, and have advantageous effect on human health and well-being (Fe, Zn). Accordingly, in regard to different health and/or disease implications of metals, their nutritional value and impact on beer quality and sensory properties, the total allowed

content claims of metals in brewing liquors and beer is regulated (Baxter and Hughes 2001; Briggs et al. 2004).

Measurement methods

Due to quantities of metals in beer, typically varying in the range from 10^{-3} (Cd, Co, Cu, Cr, Pb) through 10^{-2}–10^{0} (Al, Ba, Fe, Mn, Sr, Zn) up to 10^{1}–10^{2} mg l^{-1} (Ca, K, Mg, Na), methods of choice for their determinations are foremost atomic spectrometric techniques. These include flame atomic absorption spectrometry (F-AAS) (Fantozzi et al. 1998; Bellido-Milla et al. 2000; Bellido-Milla et al. 2004; Onate-Jaen et al. 2006; Pohl and Prusisz 2007, Pohl 2007b), electrothermal atomic absorption spectrometry (ET-AAS) (Wagner et al. 1991; Sharpe and Williams 1995; Wagner 1995; Svendsen and Lund 2000; Vinas et al. 2002; Asfaw and Wibetoe 2005; Llobat-Estelles et al. 2006), and inductively coupled plasma optical emission spectrometry (ICP-OES) (Matsushige and de Oliveira 1993; Vela et al. 1998; Alcazar et al. 2002; Bellido-Milla et al. 2004; Pohl and Prusisz 2004; Asfaw and Wibetoe 2005; Sedin 2005; Sedin 2006). Other spectrometric techniques proposed for measuring the total metal contents in beer are integrated atom trap flame atomic absorption spectrometry (IAT-F-AAS) (Matusiewicz and Kopras1997), thermospray flame furnace atomic absorption spectrometry (T-FF-AAS) (Nascentes et al. 2005), and inductively coupled plasma mass spectrometry (ICP-MS) (Wyrzykowska et al. 2001).

Sample treatment

Degassing. As an analytical sample, beer is a very complex matrix with a relatively high content of different organic compounds originated from brewery processing

(mostly carbohydrates and proteins) and saturated with CO_2. To take an exact sample volume for analysis, beer requires degassing at first. The simplest way to degas beer is to leave it loosely capped for at least 24 hours after an initial addition of concentrated HNO_3 (Sharpe and Williams 1995). Usually, immersing a respective beer aliquot in an ultrasonic bath for 5 to 20 min performs degassing equally well. Apart from a sonication, beer can be also degassed by exposing to microwaves in a pressurized closed vessel microwave oven, operated at a moderate power for several minutes (Bellido-Milla et al. 2000; Bellido-Milla et al. 2004), by a percolation of an inert gas through a sample aliquot for an hour (Svendsen and Lund 2000; Asfaw and Wibetoe 2005) or by passing a beer portion through a 0.45 μm pore size membrane filter or a dance filter paper (Wagner et al. 1991; Wagner 1995; Matusiewicz and Kopras 1997; Pohl and Prusisz, 2007, Pohl 2007b).

Wet digestion. The presence of organic constituents, even at low levels resulting from a dilution of beer samples with water, is recognized as a noticeable source of matrix effects in the transport of samples to an atomization cell and the atomization processes the occur therein (Nascentes et al. 2005). As a result, destruction of the organic matter and matrix dissolution is normally carried out before beer analysis using atomic spectrometry techniques mentioned above.

Several methods have been reported for a beer mineralization. In general, beer samples are treated at a high temperature with concentrated oxidizing reagents, i.e. mineral acids and hydrogen peroxide (H_2O_2), prior to the organic beer matrix destruction. Decomposition procedures in open vessel system are carried out using covered glass or Teflon beakers heated on an electric plate. Typically, concentrated

H_2SO_4 (Watson 1994; Bellido-Milla et al. 2004) or HNO_3 (Matsushige and de Oliveira 1993; Svendsen and Lund 2000; Alcazar et al. 2002; Pohl and Prusisz 2007; Pohl 2007b) with subsequent admixture of H_2O_2 are applied as digestion reagents. Mixtures of H_2SO_4 and HNO_3 (Pohl and Prusisz 2004) or HNO_3 with HCl (Vela et al. 1998) are also used for that purpose. Rather more rarely beer can be ashed in a muffle furnace (at 550 ^oC) and the resulting residue dissolved afterwards using HCl and water (Fantozzi et al. 1998).

Digestion protocols based on use of a closed vessel temperature programmable microwave oven are also often used. Usually, beer is treated with HNO_3 (Wyrzykowska et al. 2001) or a mixture of HNO_3 with H_2O_2, where in the latter case the reagents are added simultaneously (Asfaw and Wibetoe 2005) or sequentially (Bellido-Milla et al. 2000; Bellido-Milla et al. 2004; Llobat-Estelles et al. 2006; Onate-Jaen et al. 2006). Certainly, the use of microwave heating systems has several advantages over the traditional open vessel dissolution approach. It considerably diminishes the time of the digestion protocol and the consumption of reagents, in addition, reduces a risk of an analyte loss and/or a sample contamination (Bellido-Milla et al. 2004). In consequence, better analysis reproducibility is achieved.

An interesting beer digestion procedure has been described by Matusiewicz and Kopras (1997), in which beer samples, after addition of HNO_3 and H_2O_2, are exposed to UV radiation for 4 hours.

Unfortunately, regardless of the mineralization procedure used, there can be still some remaining organic matter in the samples that may have an effect on the analyte responses, even when microwave assisted digestion procedure is executed. Lately, it has been established that typical conditions referred to decomposition temperature and

pressure in closed pressurized microwave digestion system cannot be sufficient for a complete beer mineralization (Bellido-Milla et al. 2004). The residual organic matter left is presumed to be nitrobenzoic acids, when using HNO_3 for digestion, and phenolic acids, when applying both HNO_3 and H_2SO_4. Additionally, it has been also recognized that use of HNO_3 with admixture of H_2O_2 produces more residual organic matter as compared to H_2SO_4 with H_2O_2 mixture, likely due to a very high temperature of boiling point of the H_2SO_4-water eutectic mixture and strong oxidation properties of the acid itself. Considering these attenuations, a standard addition is normally recommended for reliable determinations of metals in beer. Due to the contribution of residual organic matter to decomposed beer samples, open vessel digestions are acknowledged to be more effective than those performed in closed pressurized microwave ovens, especially in reference to use of H_2SO_4 (Bellido-Milla et al. 2004) The choice of adequate preparation and treatment methods subjected before beer analysis should be considered individually with reference to available laboratory instrumentation and equipment.

Direct analysis. To simplify and improve speed of beer analysis, digestion can be avoided. In case of measurements with ICP-OES, a direct aspiration of analyzed beer samples is often preferred, however, lower signal-to-background ratios are usually achieved and a high supplied power is required for plasma operation (Pohl and Prusisz 2004; Asfaw and Wibetoe 2005; Sedin 2005, Sedin 2006). Undiluted beer samples are also analyzed using ET-AAS (Wagner et al. 1991; Wagner 1995).

More frequently, a reasonably dilution (from 1/1 to 1/10) of beer aliquots with water, especially when using F-AAS or ET-AAS for measurements, is necessary in order to minimize possible physical and/or chemical matrix interferences accompanying

atomization processes. By sample dilution, the formation of a solid deposit on a burner head, or the coverage of a graphite tube by a remaining ash layer is avoided (Sharpe and Williams 1995; Svendsen and Lund 2000; Nascentes et al. 2005).

Instead of sample dilution necessary for measurements performed using ET-AAS, Llobat-Estelles et al. (2006) have proposed a mathematical method of beer analysis based on an apparent content curves model enabling the evaluation of bias errors corresponded to any modification of a shape and a position of analyte absorbance profiles. Accordingly, the described method facilitates accurate and reliable measurements of metals in beer notwithstanding the interferences stemming from different organic constituents present in undiluted beer samples.

Another approach to beer analysis by ET-AAS without need for comparatively long sample dissolution or a respective sample dilution has been reported by Vinas et al. (2002). Only oxidizing agents (HNO_3 and H_2O_2) are added to beer samples maintaining a proper oxidizing environment during the atomization and preventing an accumulation of carbonaceous residues inside a graphite tube. This method has been proved to give very repeatable and reliable results.

Differentiation and classification of beer

Information on the total metal content of beer is recognized to be valuable for differentiation and classification of beer (Bellido-Milla et al. 2000; Wyrzykowska et al. 2001; Alcazar et al. 2002). This is because metals are very good descriptors reflecting the composition of natural raw products (water, cereals, hops and yeasts) used for brewing and indicating processes involved in beer manufacturing, as well as beer storage and ageing.

For the purpose of pattern recognition of beer, different chemometric techniques are usually applied to a data matrix referred to mineral content assessed through the analysis of a high number of different beer brands. Typically, principal component analysis (PCA) is used for visualization of the results, while linear discriminant analysis (LDA) and artificial neural networks (ANN) are supervised learning methods, helpful in finding adequate classification rules. Indeed, information on the mineral beer composition including total concentrations of a number of metals, including Ag, Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, Ga, Hg, K, Mg, Mn, Na, Ni, Pb, Rb, Sr, V, Zn, along with respective pattern recognition analysis is suitable for differentiation and classification of beer according to type, origin and container, or kind of raw materials used for brewing. Among other metals, Mg, Mn and K are established to be the most important variables for such classification (Alcazar et al. 2002).

Using PCA it has been indicated that there exists a certain degree of associations between some metals and they tend to group together in beer, i.e. Cs–Hg, Cd–Co, Cr–Cs, Fe–Zn or Mn–V (Wyrzykowska et al. 2001). These metal cluster correlations found possibly result from a supposition of two main sources of metal intake in beer, i.e. the endogenous source associated with raw materials used for brewing and the exogenous source originated from contaminations taking place during the production and storage of beer.

Analysis of variance between the groups (ANOVA) is also a valuable statistical method for beer characterization according to type, country of origin and container. It is suitable as well for controlling the quality of final products achieved with respect to the taste and flavor stability. Accordingly, applying ANOVA to 25 different beers, in which Al, Ca, Cu, Fe, Mg, Mn and Zn were determined, it has been found that the

concentrations of Cu and Fe in canned beers are commonly higher than those in bottled beers (Bellido-Milla et al. 2000). In addition, notable differences in content of Ca, Cu, Fe, Mg, Mn and Na are observed among lager, stout, wheat and ale beers, demonstrating that the metal intake is differentiated according to type of beer and distinctive technology of its production.

Fractionation of metals in beer

Metal associations in beer

Although studies devoted to characterization and quality control of beer related to the total mineral composition are obviously valuable, it should be highlighted that the effect of metals on beer stability and wholesomeness does not depend on their concentrations but foremost on type of metal associations preset in beer. Different low and high molecular mass organic compounds in beer can bind metals, while an existing equilibrium between complexed and non-complexed metal ions is responsible for beer quality and nutritional properties.

Indeed, beer contains various classes of natural compounds, including polyphenolics, proteins, amino acids or other organic species that have a high capacity of binding the metals through donor N, O and S atoms. Thus, metals in beer can be present as non-complexed cations and in the form of complexes of different stability, and therefore, of different bioavailability and toxicity to humans (Montanari et al. 1999; Gorinstein et al. 2000; Cortacero-Ramirez et al. 2003; Nardini and Ghiselli 2004; Khatib et al. 2006).

Due to a crucial role of Cu(I) and Fe(II) ions in the process of molecular O_2 activation and further oxidation of organic compounds, responsible for beer stability

and flavor, the elucidation of possible associations of these metals with different endogenous beer ligands is of special interest and significance in view of brewing technology and beer processing (Irwin et al. 1991; Mochaba et al. 1996; Andersen and Skibsted 1998; Montanari et al. 1999; Blanco et al. 2003; Morales et al. 2005). During the storage of beer, Cu and Fe can form very stable complexes with amino acids, polyphenols, and melanoids, being the products of Maillard reactions. This capture of Cu and Fe ions and association with organic compounds certainly decrease the rate of formation and activity of reactive oxygen species (Bamforth 1999a; Blanco et al. 2003; Vanderhaegen et al. 2006). Non-complexed Cu and Fe ions may have rather a negative effect on beer quality, contributing to changes in beer stability and chemical deterioration with ageing. On account of this special role of Cu and Fe, the content of these metals determines the type of beer ageing characteristics and final beer aroma and taste (Vanderhaegen et al. 2006).

Speciation of Mn or Zn has been less studied. The ability of Mn(II) ions to form complexes with organic compounds is considered to be rather low (Onate-Jaen et al. 2006). Although divalent Mn cations have not been reported to participate in radical generation and oxidative staling reactions, chemical properties of this metal and association with high molecular polyphenolic and flavonoid constituents tend to a conclusion that Mn effect on beer colloidal and flavor instability is possible (Pohl and Prusisz 2007; Pohl 2007b). Non-complexed Mn ions may enhance the action of Cu and Fe in catalyzing staling reactions in beer, however, a relatively low concentration of Mn as compared to Cu and Fe may mask this effect (Mochaba et al. 1997).

Obviously, better understanding and elucidation of metal functioning in processes related to flavor stability, changes during maturation, taste and odor of beer,

health implication, and nutritional value referred to beer consumption require the knowledge about speciation and/or fractionation forms of metals in beer. In this view, methods enabling classification of the existing metal groupings in beer and assessment of metal fractionation pattern are very essential.

Fractionation analysis and metal partitioning pattern

So far, experimental evidence of metal partitioning in beer has been occasionally reported in the literature. The charge of Cu, Fe and Mn species in bottled beer (Ringnes Pilsner, Norway) was determined using cation (CE) and anion exchange (AE) cartridges combined with off-line ET-AAS measurements (Svendsen and Lund 2000). When analyzing effluents obtained after passing untreated beer aliquots through separate cartridges, it was found that over 94% of the total Mn is present in the form of positively charged species, likely simple Mn(II) cations. Fe was determined to be completely present as negatively charged species, e.g. Fe bound to different classes of organic compounds. Positively (37%) and negatively (72%) charged species were found for Cu also supposing the existence of different metal complexes. The analysis of beer aliquots spiked with free Cu(II), Fe(III) and Mn(II) indicated that beer contains endogenous ligands that readily bind Cu(II) and Fe(III) ions, forming negatively charged metal complexes. Only added Mn(II) was totally recovered as positive ions.

In the cited work by Svendsen and Lund (2000), untreated beer was also treated with a size exclusion column so as to assess possible associations of Cu, Fe and Mn with ligands of different molecular mass ranges. The size exclusion chromatograms, constructed on the basis of metal quantities found in column effluents, proved that Mn in beer is present in non-complexed forms, presumably as Mn^{2+}. The retention volumes

of eluted Cu and Fe species suggested that these metals are likely to be negatively charged species bound to polyphenolic compounds, which molecular mass ranges from 4 to 12 kDa (Cu) and from 4 to 9 kDa (Fe).

A strong CE resin Dowex 50Wx4 was applied by Pohl and Prusisz (2004) to evaluate the cationic fraction for Al, Ba, Cu, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb Sr and Zn in canned beer (Warka Full Light, Poland). It was presumed that the cationic fraction is highly bioavailable to human organisms through gastrointestinal system and comprises free metal cations, stable cationic complexes of metals with small molecular mass, non-volatile acids, e.g. pyruvic acid, malic acid, lactic acid, citric acid, oxalic acid, and labile metal species, dissociating on a resin bed. The content of metals in the cationic fraction was determined by passing an untreated beer sample through the column, followed by resin acid digestion and analysis of a resulting digest. It was found that for Al, Co, Cr and Cu, the contribution of the cationic fraction to the total metal content is relatively low and varies from 6% (Cu) to 11% (Co). For Mn, Ni and Zn, the CF was established to range from 23 to 28%. Much higher donation of the cationic fraction (68–100%) was determined for Ca, Ba and Sr, probably because these metals are present in beer as simple metal cations. Contrary, Fe and Pb were found to be completely present in the form of neutral or negatively charged metal complexes.

Metal fractionation protocols based on solid phase column extraction have been recently proposed for Mn and Zn partitioning in Polish canned and bottled beers (Pohl and Prusisz 2007; Pohl 2007b). Distinct Mn and Zn species, i.e. the polyphenolic metal fraction (PF) and the CF, were retained respectively on a Amberlite XAD7 adsorbing resin (1st column) and a strong CE resin Dowex 50Wx4 (2nd column) connected in a series (Pohl and Prusisz 2007). A comparable tandem column assemblage, in which the

adsorbing resin was replaced by a weak AE resin Reillex 402 was also described (Pohl 2007b). Metal concentrations in the distinguished fractions were measured using F-AAS after a subsequent elution of the columns. Only in case of the resin Reillex 402, exposing a very strong adsorptive behaviour toward organic substances through hydrogen bonds with phenolic, hydroxyl and carboxyl groups of the dissolved organic matter, Mn and Zn amounts retained on the column were evaluated by analyzing a portion of the effluent incoming the Dowex 50Wx4 column. Additionally, the residual metal fraction (RF) was assessed by determining metal amounts in effluents obtained from passing beer aliquots through the column systems. The schemes of both tandem column assemblages are given in Figure 1.

<Figure 1 near here>

With the proposed procedures Mn and Zn are portioned among hydrophobic complexes of metals with high molecular mass polyphenolics and flavonoids (PF), sum of free metal cations and stable cationic metal complexes with low molecular weight species, including amino acids, organic acids being of primary origin (citric, malic) or secondary products of alcoholic fermentation (pyruvic, lactic, ketoglutaric, succinic, citramalic, fumaric) (cationic fraction), and residual metal species, possibly being anionic and/or neutral metal associations either with low molecular mass ligands (RF). The distribution of Mn and Zn among PF, cationic fraction and RF in some Polish bottled and canned beers is given in Figure 2.

<Figure 2 near here>

In comparison to the results previously reported by Svendsen and Lund (2000), it seems that possible chemical forms and associations of Mn are more differentiated. As reported before, it was found that the predominant class of Mn species is the cationic fraction with donation to the total Mn concentration changing from less than 50% (non-pasteurized and award-winning beer brands) to 100% (low-priced beers). There is however a considerable contribution of the RF (14–42%), Mn is also present in the form of complexes with polyphenolic substances, as the PF accounts for up to 37% of the total Mn content. The abundance of the CF for Zn is high (27–85%) and also dependent on beer grade. Zn was found to be bound to polyphenols, but the contribution of the PF is low, i.e. from 2% (low-priced beers) to 21–53% (non-pasteurized beers).

Bearing in mind possible metal associations with natural organic substances present in beer (Montanari et al. 1999; Cortacero-Ramirez et al. 2003; Khatib et al. 2006), the hydrophobic PF can be regarded as hardly bioavailable to humans while the sum of the cationic fraction and the RF can be assumed as highly absorbable.

The metal groupings distinguished through the fractionation protocols described above are operationally defined and relate to a sorption behavior of metal species toward adsorbents and ion exchangers applied in the fractionation schemes (Pohl 2007a). Certainly, with respect to the total content analysis, the described methods enable retrieving the information on the abundance of individual metal fractions in beer and can yield useful knowledge about metal bioavailability, beer safety, authenticity and nutrition.

Conclusions

In view of excellent beer quality assurance, knowledge concerning metal composition in beer and brewing liquors at various steps of beer production is very important to the brewers. Metal ions affect fermentation, maturation and storage of beer and imply its final flavor and colloidal stability. Considering the nutritious value of metals and the effect they have on beer wholesomeness, information on the total metal content in beer is also advantageous to the consumers. Depending on the concentration, various metals may be toxic to human body or essential for health with a profound effect on well-being. When using chemometric data analysis and interpretation methods, the determination of metals in beer is useful in characterization and authentication of beer according to type, origin or packaging.

However, actual bioavailability and absorbability of metals and their effect on beer stability and haze formation depend not only on the total concentrations but also on type of distinct physicochemical forms in which they are present in beer. In commonly applied procedures focused on the total metal content determination after previous beer digestion, any information on the occurrence of possible metal species can not be achieved. Usually, methods linking chromatographic separation techniques with sensitive spectrometric detection can be useful for that purpose and helpful for elucidation of metal functions in beer processing and implications on beer quality, flavor and taste stability. In addition, methods stimulating gut and intestinal conditions can be suitable for estimation of metal bioavailability and toxicity to human organisms.

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Captions for figures

Figure 1. Fractionation schemes for classification of Mn and Zn in beer. (a) Amberlite XAD7–Dowex 50Wx4 and (b) Reillex 402 – Dowex 50Wx4 tandem column systems.

Figure 2. Fractionation patterns of Mn and Zn in beer (adapted from Pohl and Prusisz 2007; Pohl 2007b).

Table I. Role of some major and minor metals in brewing and effect on beer flavor.

Metal and description of its role in brewing and effect on beer flavor
Calcium
–Mash and wort pH reduction through reactions with phosphates, phytates, peptides, proteins and other mash and wort constituents (important for yeast metabolism and fermentation action, and fermentation enzymes stabilization)
–Oxalates precipitation (important for governing yeast cells flocculation)
–Content above 100 mg l^{-1} in brewing liquors causes phosphates removal and inadequate yeast growth nutrition supply
–Minor effect on beer flavor (recommended concentration in beer: 20–150 mg l^{-1})
Copper
–Oxidation/reduction catalysis action in reactive oxygen containing species formation (responsible for aerobic beer ageing and flavor stability during storage)
–High concentration is toxic and mutagenic to yeasts, causes irreversible beer haze (recommended upper limit in brewing liquors and beer: 0.1 mg l^{-1})

Iron

–Oxidation/reduction catalysis action in reactive oxygen containing species formation (responsible for beer quality and flavor stability)

–High concentration conveys metallic and harsh beer taste and dark color due to associations with phenolic substances, results also in yeast activity hampering and haze production (recommended upper limit in wort and beer: 0.1 mg l^{-1})

Potassium

–Charge homeostasis maintenance (required for yeast growth)

–Participation in osmoregulation and regulation of divalent cations and phosphates uptake by yeast cells

–Concentration above 10 mg l^{-1} has different laxative effects and imparts salty beer taste

Magnesium

–Mash and wort pH reduction (similarly as in case of Ca)

–Cell division stimulation and enzymes co-factoring (important for yeast growth and yeast fermentation metabolism)

–Protection of yeast cells from disadvantageous effects stemming from ethanol, high temperature and osmotic pressure

–High concentration contributes to sour and bitter beer taste (recommended upper limit in beer: 30 mg l^{-1})

Manganese

–Oxidation/reduction catalysis action in reactive oxygen containing species formation (responsible for beer flavor and colloidal stability)

–Proteins solubilization and enzymatic action support (important for proper yeast growth)

–High concentration causes unpleasant beer taste (recommended concentration in beer: 0.05–0.2 mg l^{-1})

Sodium

–No specific chemical and metabolic influence

–High concentration contributes to sour and salty beer taste; level of 75–150 mg l^{-1} contributes to round smoothness and proper beer sweetness (recommended upper limit in beer: 150 mg l^{-1})

Zinc

–Micronutrient to yeast growth and metabolism, and protein synthesis

–Participation in ethanol production as enzymes co-factor

–High concentration is damaging to yeasts and affects fermentation, deficiency leads to impaired fermentation progress (recommended concentration in brewing liquors: 0.15–0.5 mg l^{-1})

Table II. Concentration of major metals in beer of different country of origin.

	Concentration, mg l^{-1}				
	British	Dutch	German	Spanish	Others
Calcium (Bellido-Milla et al. 2000; Alcazar et al. 2002; Briggs et al. 2004)					
	40.0–140	42.2–69.8	3.80–108	29.0–86.2	16.5–111
Potassium (Bellido-Milla et al. 2000; Alcazar et al. 2002; Briggs et al. 2004)					
	135–1100	124–648	46.7–833	22.9–496	17.5–442
Magnesium (Bellido-Milla et al. 2000; Alcazar et al. 2002; Briggs et al. 2004; Pohl and Prusisz 2004)					
	60.0–200	55.5–265	23.7–266	42.0–110	50.0–112
Sodium (Bellido-Milla et al. 2000; Alcazar et al. 2002; Briggs et al. 2004)					
	21.9–230	10.4–47.6	1.19–120	3.95–103	4.13–66.4

Table III. Concentration of minor and trace metals in beer of different country of origin.

	Concentration, mg l^{-1}						
	British	Dutch	German	Norwegian	Polish	Spanish	Others
Aluminum (Sharpe and Williams 1995; Vela et al. 1998; Alcazar et al. 2002; Vinas et al. 2002; Briggs et al. 2004; Pohl and Prusisz 2004)							
		0.08–0.18	0.05–1.24			0.05–2.2	0.005–6.50
Barium (Alcazar et al. 2002)							
		0.020–0.032	0.034–0.049			0.014–0.033	0.016–0.068
Cadmium (Briggs et al. 2004)							
			0.0002–0.020			0.031–0.40	0.095–0.68
Copper (Matusiewicz and Kopras 1997; Bellido-Milla et al. 2000; Svendsen and Lund 2000; Wyrzykowska et al. 2001; Alcazar et al. 2002; Vinas et al. 2002; Bellido-Milla et al. 2004; Briggs et al. 2004; Pohl and Prusisz 2004; Asfaw and Wibetoe 2005; Nascentes et al. 2005; Llobat-Estelles et al. 2006)							
	0.008–0.80	0.032–0.068	0.019–0.80	0.029–0.050	0.029–0.15	0.024–0.080	0.022–0.16
Iron (Matusiewicz and Kopras 1997; Bellido-Milla et al. 2000; Svendsen and Lund 2000; Wyrzykowska et al. 2001; Alcazar et al. 2002; Bellido-Milla et al. 2004; Briggs et al. 2004; Pohl and Prusisz 2004; Asfaw and Wibetoe 2005)							

0.067–0.50	0.064–0.43	0.040–1.55	0.036–0.093	0.015–0.53	0.096–0.92	0.041–1.06

Manganese (Matusiewicz and Kopras 1997; Bellido-Milla et al. 2000; Svendsen and Lund 2000; Wyrzykowska et al. 2001; Alcazar et al. 2002; Bellido-Milla et al. 2004; Briggs et al. 2004; Pohl and Prusisz 2004; Asfaw and Wibetoe 2005; Nascentes et al. 2005; Pohl and Prusisz 2007; Pohl 2007b)

0.033–0.18	0.047–0.30	0.040–0.51	0.046–0.78	0.053–0.47	0.031–0.25	0.032–0.36

Lead (Briggs et al. 2004; Nascentes et al. 2005)

		0.003–0.024			0.001–0.006	0.0008–0.033

Strontium (Alcazar et al. 2002)

	0.23–0.41	0.14–0.30			0.13–0.74	0.13–0.39

Zinc (Wagner et al. 1991; Matusiewicz and Kopras 1997; Bellido-Milla et al. 2000; Wyrzykowska et al. 2001; Alcazar et al. 2002; Briggs et al. 2004; Nascentes et al. 2005; Pohl and Prusisz 2007; Pohl 2007b)

	0.013–0.064	0.013–1.48		0.004–0.12	0.001–0.98	0.007–0.28

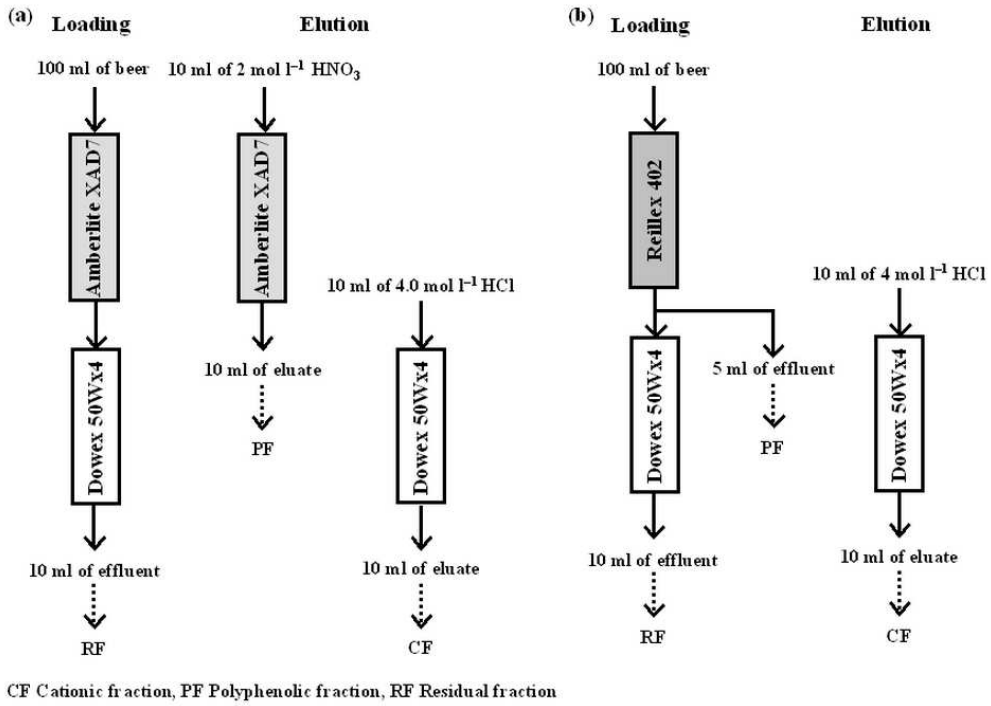

Figure 1. Fractionation schemes for classification of Mn and Zn in beer. (a) Amberlite XAD7–Dowex 50Wx4 and (b) Reillex 402 – Dowex 50Wx4 tandem column systems.
40x28mm (600 x 600 DPI)

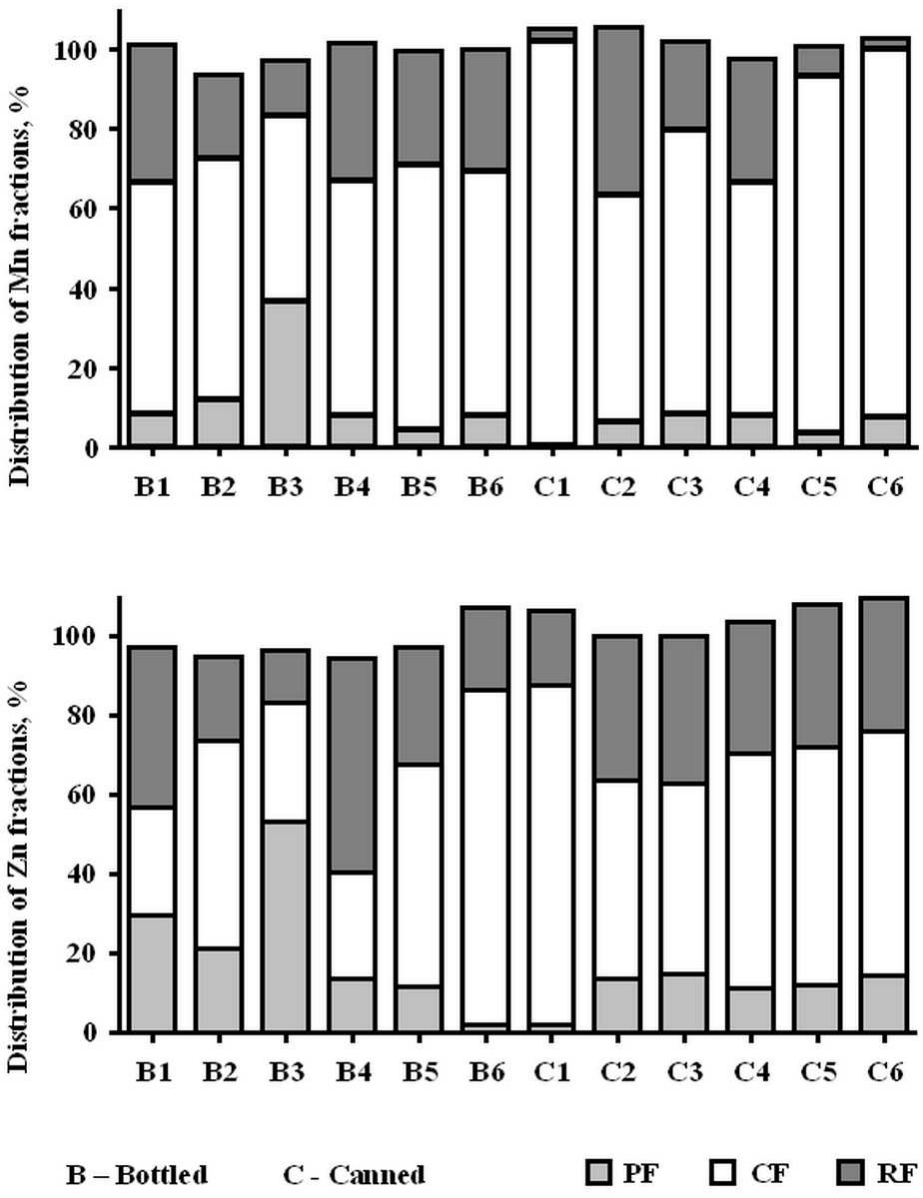

Figure 2. Fractionation patterns of Mn and Zn in beer (adapted from Pohl and Prusisz 2007; Pohl 2007b).
40x50mm (600 x 600 DPI)