

## Evaluation of the Total Content and the Operationally Defined Species of Copper in Beers and Wines

P. POHL\*<sup>†</sup> AND I. SERGIEL<sup>‡</sup>

<sup>†</sup>Department of Analytical Chemistry, Faculty of Chemistry, Wrocław University of Technology, Smoluchowskiego 23, 50-372 Wrocław, Poland, and <sup>‡</sup>Department of Biotechnology, Faculty of Biological Sciences, University of Zielona Góra, Prof. Z. Szafrana 1, 65-516 Zielona Góra, Poland

A two-column solid phase extraction was used to determine the operationally defined speciation of Cu in lager beers and red wines. Applying a nonionic macroreticular adsorbing resin Amberlite XAD-16 and a gel type strong cation exchange resin Dowex 50Wx8–200, three different groupings of the chemical forms of Cu, including the hydrophobic, the cationic, and the residual species fractions, were separated and determined. The total content of Cu in the analyzed samples and its concentrations in the distinguished fractions were measured using flame atomic absorption spectrometry without any special preparation of the sample solutions. It was found that the residual species (72–82% of the total content), being polar and noncationic forms of Cu, are the most abundant fraction of Cu in the analyzed beers. In the case of wines, the fraction of the hydrophobic species was established to have the highest share in the total Cu content, that is, 27–77%. This fraction was presumed to contain relatively strong complexes of Cu with various flavonoids and other polyphenols.

**KEYWORDS:** Cu; wine; beer; fractionation analysis; SPE; FAAS

### INTRODUCTION

The investigation of the Cu species in alcoholic beverages has a certain chemical interest and food safety importance (1). In most respects, the Cu ions, similar to the Fe ions, are assumed to be responsible for the activation of O<sub>2</sub> and the initiation of beer and wine oxidative spoilage and staling (2–6). In the presence of the simple Cu(I) ions, the excessive amounts of O<sub>2</sub> may be reduced to the superoxide anions (O<sub>2</sub><sup>•−</sup>). This reaction can likely be stimulated by the presence of the certain phenolics that may initially reduce the Cu(II) ions to the respective Cu(I) ions. In the subsequent reactions, the resulting O<sub>2</sub><sup>•−</sup> anions can give rise to the perhydroxyl radicals (OOH<sup>•</sup>), the peroxide anions (O<sub>2</sub><sup>2−</sup>), and finally hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). The latter compound seems to be the main source of the hydroxyl radicals (OH<sup>•</sup>), possibly generated in the successive Cu-induced reactions (2–6). All the aforementioned oxygen species, that is, O<sub>2</sub><sup>•−</sup>, HOO<sup>•</sup>, H<sub>2</sub>O<sub>2</sub>, and HO<sup>•</sup>, are recognized to readily react with various organic constituents of beer and wine, including ethanol, carboxylic, and amino acids, in addition to different polyphenolic species. As a result, a variety of the carbonyl oxidation products such as aldehydes, quinones, and different radical intermediates tend to appear, leading to the flavor staling and stability degradation of beer and wine (2, 3, 7).

Because the rate of staling is supposed to be influenced not only by the total Cu concentration but mainly the type of species in which this metal is present, that is, the labile forms or the complexing ions with different organic compounds (8–10), the

determination of the ultimate Cu forms in beers and wines seem to be of special importance (11–16). It has lately been acknowledged that all stable organically bound forms of Cu probably decrease the generation and the oxidative activity of the reactive oxygen species responsible for the spoilage of finished beers and wines (2). Unfortunately, a number of research works that have been published so far primarily focuses on the determination of the total content of Cu rather than the identification and quantification of the ultimate Cu species present in beers and wines (17–31). The experimental evidence on the speciation of Cu in both beverages is rather uncommon, especially in the case of beer. The operationally defined speciation of Cu in wine has been more often explored using various electroanalytical techniques, that is, differential pulse anodic stripping voltammetry (32, 33), potentiometry with ion-selective electrodes (32), and stripping potentiometry (13–15). These techniques enabled to assess in white and red wines the labile Cu species, comprising free metal ions and those dissociated from the unstable Cu complexes with the organic compounds, and the simple Cu ions. Other methods for the fractionation of Cu in wine were the use of ultrafiltration membranes with different molecular cut-offs (34) and a two-step column solid phase extraction (SPE) with different ionic and nonionic polymer sorbents (16).

The present study was conducted with the intention to establish an analytical procedure relevant for the fast and simple determination of the total content of Cu and its fractionation forms, particularly the labile Cu species and the extent of the organic complexation of Cu, in beers and wines. For this reason, a two-column SPE fractionation scheme, in which a nonionic macroreticular adsorbing resin Amberlite XAD-16 (first column)

\*To whom correspondence should be addressed. Tel.: +48-71-320-3445. Fax: +48-71-320-2494. E-mail: pawel.pohl@pwr.wroc.pl.

connected with a gel type strong cation exchange resin Dowex 50Wx8-200 (second column), was proposed. The suitability of this approach to the chemical fractionation of Cu was examined in detail. Carrying out the fractionation analysis, three operationally defined groupings of the Cu species, differing in hydrophobicity and charge, were discriminated and determined. The results concerning the distribution of the Cu fractionations in the analyzed beers and wines were discussed in reference to the previously published works. The possible connections between the classified fractions and the chemical compounds that likely bind Cu were established.

## MATERIALS AND METHODS

**Instrumentation.** A Perkin-Elmer model 1100 flame atomic absorption spectrometer (FAAS) was applied for the determination of Cu concentrations. The measuring instrument was operated according to the specific instructions given by the manufacturer. Additionally, to achieve best performance and sensitivity, the position of a concentric nebulizer and a burner were adjusted with the flame running. The air-acetylene stoichiometric combustion flame was operated at 1.5 (fuel) and 8.0 (oxidant) L min<sup>-1</sup>. An absorption line of Cu at 324.8 nm and a spectral band at 0.7 nm were selected for the measurements. A hollow cathode lamp (HCL) for Cu was operated at 15 mA. The readouts were averaged for three readings using a hold mode with an integration time of 1 s. The concentrations of Cu in the solutions were measured using six external standard solutions at the level of 0.02, 0.05, 0.10, 0.20, 0.50, and 1.0 mg L<sup>-1</sup> (the calibration curve method) and the method of two standard additions. The LOD and LOQ of 0.005 and 0.015 mg L<sup>-1</sup>, respectively, were assessed for Cu under the selected operating conditions.

The Supelco glass columns (10 mm ID) with the coarse frits and Teflon stopcocks were used for SPE. A 4-channel MasterFlex L/S peristaltic pump (Cole-Parmer, U.S.A.) was employed to maintain and control the flow rates of the solutions passed through the SPE columns.

**Chemicals and Solutions.** All reagents used were of analytical grade (POCH, Poland). Deionized water was used throughout. A Merck (Germany) stock standard solution of 1000 mg L<sup>-1</sup> of Cu(II) was used to prepare the 10% (v/v) aqueous-ethanolic working standard solutions (50 mL) containing 0.2 mg L<sup>-1</sup> of Cu. In a corresponding way, the working standard solutions of Cu with tartaric (500 mg L<sup>-1</sup>) and tannic (500 mg L<sup>-1</sup>) acids added to complex this metal were also prepared. All these solutions were adjusted to pH 3.5, 4.0, 4.5, and 5.0 using 2 mL of the respective 0.10 mol L<sup>-1</sup> standard acetic acid–sodium acetate buffering solutions and applied for characterizing the sorption properties of the polymeric sorbents.

**Resin Preparation.** A Sigma-Aldrich nonionic macroreticular adsorbing resin Amberlite XAD-16 (matrix: polystyrene, apolar; particle size: 20–60 mesh; surface area: 800 m<sup>2</sup> g<sup>-1</sup>; mean pore size: 100 Å; pore volume: 1.82 mL g<sup>-1</sup>; dipole moment: 0.3 D) and a Sigma-Aldrich gel-type strong acidic cation exchanger Dowex 50Wx8-200 (matrix: styrene-divinylbenzene; cross-linkage: 8%; matrix active group: sulfonic acid; particle size: 100–200 mesh; sorption capacity: 1.7 meq mL<sup>-1</sup>) were taken as the sorptive phases for SPE. Prior to use, the adsorbing resin was dried in an oven for 4 h at 110 °C. The 1.0 g portions of the resulting dried adsorbent were wetted with methanol and then with water and poured later on into the columns as water slurries. The resin beds of Amberlite XAD-16 were washed with 10 mL of a 1.0 mol L<sup>-1</sup> HCl solution and subsequently rinsed with 20 mL of water to remove the excess of HCl. The 1.0 g portions of the cation exchange resin were wetted with water and then poured into the SPE columns. The resin beds formed were flushed with 10 mL of a 1.0 mol L<sup>-1</sup> HCl solution and next washed with 20 mL of water. Finally, 10 mL of a 1.0 mol L<sup>-1</sup> NaOH solution were passed through the columns, while the excess of NaOH was removed washing the resin beds with 20 mL of water. Water and the conditioning solutions were passed through the columns at a flow rate of 2.0 mL min<sup>-1</sup>.

**SPE Column Operation.** The sorption behavior of the simple ions of Cu(II) and the complexing ions of Cu with tartaric and tannic acids, representing the low and high molecular weight organic compounds, respectively, toward the Amberlite XAD-16 and Dowex 50Wx8-200 SPE columns were thoroughly examined. The working standard solutions were

passed through the SPE columns filled with the resins (1.0 g) at the flow rate of 1.0 mL min<sup>-1</sup>. After passing about 40 mL of each solution through the column, the respective column effluent (5.0 mL) was collected to measure the concentration of Cu not retained by the resin ( $C_{\text{eff}}$ ). The percentage efficiency of Cu retention was evaluated relating the content of Cu retained by the resin to its original content in the working standard solution ( $C_{\text{ws}}$ ), that is,  $100\% \times (C_{\text{ws}} - C_{\text{eff}})/C_{\text{ws}}$ . In case of the Dowex 50Wx8-200 resin, 0.5, 1.0, and 2.0 mol L<sup>-1</sup> solutions of HCl and HNO<sub>3</sub> were additionally tested for a complete elution of Cu from the resin. For this purpose, the working standard solutions of the simple Cu ions (pH 4.5) were passed through the SPE columns at the flow rate of 1.0 mL min<sup>-1</sup>, and then 10 mL of the selected solutions were used to recover the fraction of Cu retained. The relevant eluate portions (10 mL) were collected and analyzed for the amount of Cu desorbed from the resin ( $C_{\text{el}}$ ). The percentage efficiencies of Cu recovery were determined relating the content of Cu desorbed to its original content in the working standard solutions, that is,  $100\% \times C_{\text{el}}/C_{\text{ws}}$ . All the retention and recovery efficiencies given are average values for three independent replicates ( $n = 3$ ) and consider the respective column blanks. The figures behind are the standard deviations.

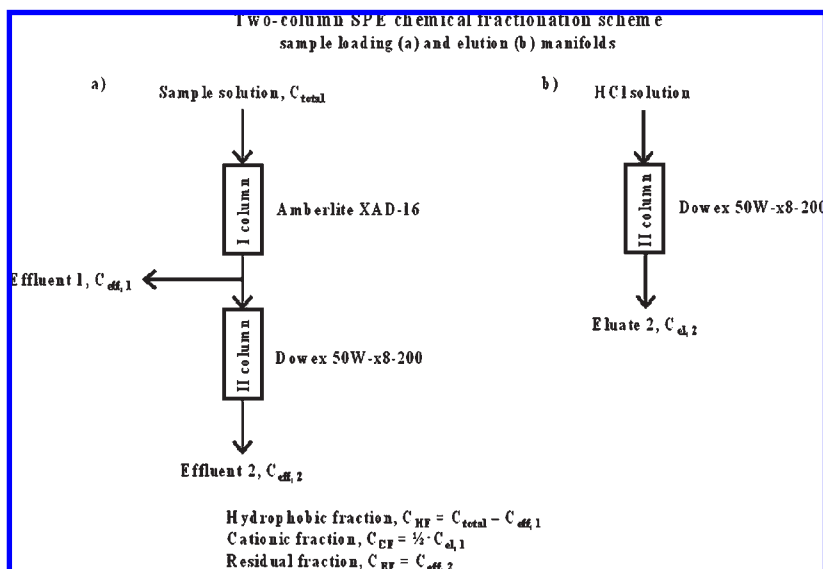
### Direct Analysis, Wet Digestion, and Fractionation Procedures.

Four Polish canned pale lager beers (B1, B2, B3, B4), containing from 4.5 to 6.2% (v/v) of ethanol, and four Bulgarian bottled semidry and dry red wines (W1 Cabernet Sauvignon, W2 Melnik, W3 Merlot, W4 Pinot Noir), with 12–14% (v/v) of ethanol contained, were investigated in this contribution. After opening, the analyzed beers and wines were filtered through the 0.45 μm Nylon 66 membrane filters (Supelco).

For the direct analysis, beers were measured undiluted, the wine samples were diluted four times with water. The recovery test was studied as follows: 10 mL portions of beer and wine samples were spiked with a 100 mg L<sup>-1</sup> standard solution of Cu(II) at such volumes that the added Cu amounts approximately doubled the final concentrations of Cu in the sample solutions. Then the direct analysis of the samples was carried out.

To determine the total content of Cu, portions of the resulting beer and wine filtrates (25 mL) were sampled and digested in concentrated HNO<sub>3</sub> with added 30% (m/v) H<sub>2</sub>O<sub>2</sub>. The samples were transferred into the 100 mL glass beakers, covered with the watch glasses and allowed to boil, but without spattering, to evaporate ethanol and reduce the sample volumes to approximately 1 mL. Afterward, concentrated HNO<sub>3</sub> (5.0 mL for beer and 10 mL for wine) was added and the sample aliquots were again evaporated to about 1 mL and let down to cool. In the next turn, 30% (m/v) H<sub>2</sub>O<sub>2</sub> (5.0 mL of beer and 10 mL for wine) was added and the heating was prolonged until the sample volume was reduced to near dryness. The residues left were dissolved and diluted with water to 10 or 25 mL, respectively, in the case of beer and wine samples. Each sample was analyzed in triplicate ( $n = 3$ ) along with the respective reagent blanks.

For the study of the Cu partitioning in beers and wines, 25 mL portions of filtered beers and wines were passed at the flow rate of 1.0 mL min<sup>-1</sup> through the SPE column filled with the nonionic Amberlite XAD-16 adsorbing resin. The resulting effluent was instantly directed to pass through the second SPE column filled with the Dowex 50Wx8-200 cation exchanger. After passing 20 mL of the sample through both SPE columns, a 5.0 mL portion of the effluent from the first SPE column was collected prior to the analysis for the content of Cu not retained by the adsorbent ( $C_{\text{eff}, 1}$ ). Another 5.0 mL portion of the effluent from the second SPE column was sampled to evaluate the content of Cu not retained by both linked columns ( $C_{\text{eff}, 2}$ ). Afterward, the columns were split out and the cationic Cu forms were eluted from the second SPE column using 10 mL of a 2.0 mol L<sup>-1</sup> HCl solution at the flow rate of 1.0 mL min<sup>-1</sup>. The respective 10 mL eluates were collected and analyzed for the content of Cu retained by the cation exchange resin ( $C_{\text{el}, 2}$ ). Subjecting the analyzed beer and wine samples to this fractionation procedure, three different species groupings for Cu were distinguished and determined (see a schematic manifold of the fractionation procedure in **Figure 1**). It included the organically bound, hydrophobic Cu species (retained on the first SPE column,  $C_{\text{HF}}$ ), the cationic Cu species (retained on the second SPE column,  $C_{\text{CF}}$ ), and the residual Cu species (not retained by both SPE columns,  $C_{\text{RF}}$ ). The percentage contributions of the discriminated classes of the Cu species were assessed relating the content of Cu in the separate fractions to its total content determined in the examined beer and wine samples. Each time the



**Figure 1.** Schematic manifold applied for the SPE fractionation procedure.

fresh resin portions were taken for SPE and the performing of the fractionation analysis.

## RESULTS AND DISCUSSION

**Adsorbent Characterization.** The nonionic macroreticular Amberlite XAD-16 adsorbing resin was used in the present contribution to differentiate the fraction of the hydrophobic species of Cu, mostly associated with phenolic substances present in beers and wines that have been found to have a relatively high affinity for the complexation of the divalent transition metal ions (11, 16). Previously, the Amberlite XAD-16 resin was used for the separation of the organically bound species fraction of some transition metals from their simple ions present in the lake water samples (35). Nevertheless, this resin has very little been explored and applied for the purpose of the speciation and fractionation studies of metals as compared with other Amberlite XAD resins, including XAD-2, XAD-7, and XAD-8 (16, 36–38). One difficulty, however, is that the Amberlite XAD resins, previously used to distinguish the organically bound metal species, were also found to retain considerable amounts of the simple metal ions. This unspecific retention could be possible due to the presence of some polar impurities that were assumed to act as cation exchange active sites (16, 36, 37, 39).

Here, at the outset, the Amberlite XAD-16 resin was also examined on its eventual cation exchange behavior and sorption of the simple Cu(II) ions. Analyzing the effluents collected after passing the working standard solutions of the Cu(II) ions through the columns, it was found that the studied adsorbent does not retain the simple Cu(II) ions at pH values ranged from 3.5 to 5.0. The concentrations of Cu determined in the effluents were on average  $100 \pm 1\%$  of its original concentration in the working standard solutions. This reveals that the Amberlite XAD-16 resin does not contain any polar impurities that could have a certain cation exchange capacity. It also suggests that an ultimate sorption of the metal ions on the nonionic adsorbing Amberlite-type resins reported in previous works could rather result, depending on pH, from a formation of hydrolyzed or complexed forms of metals, as was suggested before (35). In this way, no additional pretreatment with the solutions of Bi(III) or In(III) salts formerly reported (36, 37, 39) was necessary for the Amberlite XAD-16 resin used in the present contribution.

Additionally, it was found that Amberlite XAD-16 does not either retain Cu when passing the working standard solutions

(pH 3.5–5.0) with added tartaric acid. Again, the content of Cu determined in the respective effluents was on average  $99.8 \pm 1.3\%$  of its initial concentration in the working standard solutions.

The behavior of the Amberlite XAD-16 resin toward the organically bound Cu species was investigated on the example of the complexes of Cu with tannic acid. Apparently, it was established that the relative retention efficiencies for Cu at the studied pH are relatively low and this could possibly be attributed to low efficiencies of the formation of the respective complexes of Cu with tannic acid under the experimental conditions examined. Recently, it has been indicated that indeed the stability of the species of Cu bound by the high molecular weight phenolic substances critically depends on the experimental conditions (40). The efficiency of Cu retention for the Amberlite XAD-16 resin found here was changed from  $10.2 \pm 2.1\%$  (pH 3.5) to  $35.4 \pm 2.5\%$  (pH 5.0), indicating that Cu was present to a high degree as the uncomplexed forms. Additionally, there was hardly any difference noted (lower than 2%) between the retention efficiencies for Cu obtained when using two different ethanol concentrations, that is, 5.0 and 10% (v/v), in the working standard solutions. All these results achieved are consistent with the earlier findings (41) because they expose that for the more than 100 times higher concentrations of tannic acid as compared to the Cu(II) ions the contribution of the stable Cu-tannic acid complexes is much lower than the respective contribution of the simple Cu(II) ions or the labile Cu species.

Finally, the effect of the mass of the Amberlite XAD-16 resin in the range 0.6 to 2.0 g and the flow rate of the solutions passed through the SPE columns in the interval 0.5 to  $4.0 \text{ mL min}^{-1}$  was investigated on the retention of Cu from the working standard solutions containing tannic acid (pH 4.5). In the course of these experiments it was found that the retention efficiency for Cu remains unchanged regardless of the mass of the resin beds. However, when applying the sample flow rates higher than  $1.0 \text{ mL min}^{-1}$  it was found that the amount of Cu retained by the resin decreases. Hence, the resin mass of 1.0 g and the flow rate of  $1.0 \text{ mL min}^{-1}$  were selected for further experiments. The selection of a suitable eluent enabling a complete recovery of Cu retained on the Amberlite XAD-16 resin in the form of the complexes with tannic acid was abandoned since it was intended to use a nonelution approach to evaluating the contribution of the hydrophobic Cu species fraction.



**Cation Exchanger Characterization.** The Dowex 50W resins were used before for the operationally defined speciation of Cu, Fe, Mn, and Zn in beers and wines in different fractionation approaches (16, 38, 42, 43). In reference to the experience gained in these works on the retention of the complexes of Fe, Mn, and Zn with polyphenolic substances it was decided that the Dowex 50Wx8-200 resin used in the fractionation procedure proposed here should follow the Amberlite XAD-16 adsorbing resin. At the same time, it was verified that indeed this strong cation exchange resin completely retains Cu from the solutions containing tannic acid. The retention efficiencies found for Cu were ranged from  $100 \pm 1\%$  (pH 3.5) to  $98.7 \pm 1.6\%$  (pH 5.0). This suggests that a direct treatment of beer and wine samples with the Dowex 50W type strong cation exchangers could lead to an overestimation of the cationic fraction of Cu.

When passing the working standard solutions containing Cu and tartaric acid through the cation exchanger, the largest part of Cu was found in the respective column effluents, likely due to the presence of the neutral and negatively charged tartaric complexes of Cu, that is,  $\text{CuL}$  or  $\text{CuL}_2^{2-}$ , formed under the studied conditions. Accordingly, at pH 3.5 the sum of these complexes accounted for  $68.2 \pm 2.3\%$  of the total Cu content, while at pH 5.0 it was  $97.3 \pm 2.9\%$ . For both ethanol concentrations examined, that is, 5.0 and 10% (v/v), the differences between the results were indistinguishable. These findings were confirmed calculating the distribution of the Cu(II)–tartaric acid complexes at given pH values using a Hyperquad Simulation and Speciation (HySS) program, version HySS2006. Accordingly, it was assessed that under the studied conditions more than 60% (pH 3.5) to almost 100% (pH 5.0) of Cu(II) is present in the solution in the form of mentioned neutral and anionic complexes, unable to be retained by the cation exchange resin.

The studied resin was also found to quantitatively retain Cu from the working standard solutions of its simple divalent ions. The retention efficiencies determined for Cu were varied from  $99.2 \pm 1.1\%$  (pH 4.0) to  $101 \pm 1\%$  (pH 3.5). In addition, it was found that among different HCl and  $\text{HNO}_3$  solutions, 10 mL of 2.0 mol  $\text{L}^{-1}$  HCl solutions produces the complete recovery of Cu ( $100 \pm 1\%$ ) from the investigated cation exchanger.

**Total Cu Content Through Direct Analysis.** The presence of ethanol at the level of 4–15% (v/v) and a multitude of different organic substances and minerals makes beer and wine very complex matrices for the analysis by the atomic spectrometry methods. The measurements with FAAS can be accompanied by different physicochemical interferences, and therefore, the selection of a suitable sample pretreatment procedure and a calibration strategy is critical for obtaining the dependable and accurate results of the analysis. Considering the effort and time spent for routine analyses, the preparation procedures should however be uncomplicated and free from the risk of contamination by the impurities or losing the analytes. These criteria are fulfilled when the sample treatment is avoided and the beer and wine samples are directly analyzed. However, this kind of measurements of the total metal concentrations using FAAS is really infrequently reported in the literature (16, 19, 26).

Pursuing the interest in assessing the information on the total content of Cu in beers and wines in a fast and uncomplicated way, the suitability of the analysis of the undigested beer and wine samples was investigated and verified in the present contribution. Following the earlier reports (16, 19, 26), the method of two standard additions was applied for the determination of Cu in the analyzed samples to eliminate the possible matrix effects.

The total concentrations of Cu in the analyzed beers and wines were found to be respectively within the ranges 0.078–0.098 mg  $\text{L}^{-1}$  and 0.175–0.571 mg  $\text{L}^{-1}$  (see Table 1).

**Table 1.** Total Concentration of Cu (Mean Value  $\pm$  Standard Deviation) in the Analyzed Beers (B) and Wines (W)

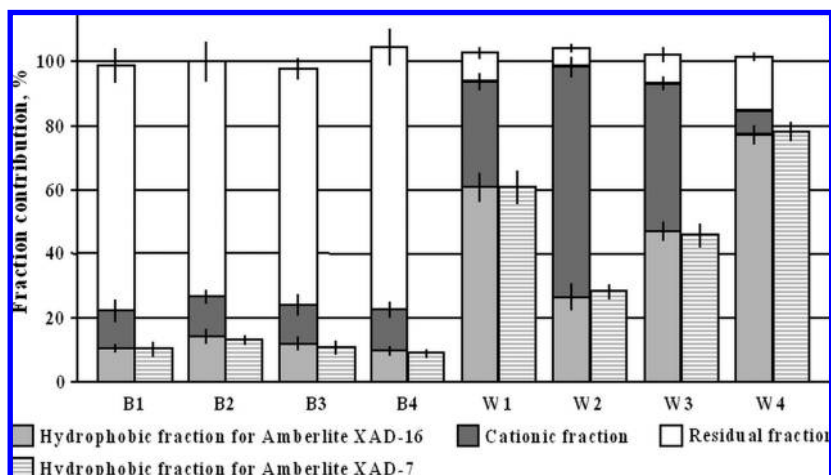
sample	total concentration (mg $\text{L}^{-1}$ )		total dissolved fraction <sup>c</sup> (mg $\text{L}^{-1}$ )
	direct analysis <sup>a</sup>	acid digestion <sup>b</sup>	
B1	0.086 $\pm$ 0.003	0.084 $\pm$ 0.006	<sup>d</sup>
B2	0.098 $\pm$ 0.008	0.104 $\pm$ 0.010	<sup>d</sup>
B3	0.092 $\pm$ 0.003	0.093 $\pm$ 0.004	<sup>d</sup>
B4	0.072 $\pm$ 0.004	0.081 $\pm$ 0.006	<sup>d</sup>
W1	0.368 $\pm$ 0.023	0.372 $\pm$ 0.048	0.353 $\pm$ 0.025
W2	0.238 $\pm$ 0.010	0.229 $\pm$ 0.018	0.243 $\pm$ 0.011
W3	0.175 $\pm$ 0.005	0.178 $\pm$ 0.007	0.173 $\pm$ 0.004
W4	0.571 $\pm$ 0.022	0.585 $\pm$ 0.045	0.561 $\pm$ 0.017

<sup>a</sup> Beers: measured in undiluted and degassed samples,  $n = 3$ ; wines: measured in 4-fold water diluted samples,  $n = 3$ . <sup>b</sup> Measured in digested beer and wine samples,  $n = 3$ . <sup>c</sup> Measured in 4-fold water diluted wine filtrates (0.45  $\mu\text{m}$ ),  $n = 3$ . <sup>d</sup> Not analyzed.

To verify if the proposed method of analysis provides reliable results, they were compared to those obtained after the wet digestion of beer and wine samples in the mixture of concentrated  $\text{HNO}_3$  with added 30%  $\text{H}_2\text{O}_2$  and using the external standard solutions for the calibration. Applying the paired *t*-test for the results achieved it was found that the respective Cu concentrations determined with both methods are comparable. The differences between the results were found to be statistically insignificant at the 0.05 significance level. In addition, the recoveries of added Cu were investigated for the analyzed beers and wines. It was established that they were within 98.5–100% in the case of beer samples and within 99.1–101% in the case of wine samples. This proves that the proposed procedure of the direct beer and wine analysis is reliable and supplies the dependable results, but in a much shorter time and without the use of the chemical reagents as compared to the sample decomposition and the analysis of the resulted digests.

It was reported before that the determination of Cu by FAAS in the samples containing 10% of ethanol produces about a 20–25% decrease in the sensitivity (26, 44). However, at the ethanol levels lower than 5.0%, the deterioration of the absorbance signals for Cu was recognized to be much less detrimental or did not appear at all (26). Hence, the influence of beer and wine matrices on the response from Cu during the direct measurements of the certain beer and wine samples by FAAS was checked here. Fortunately, the concentrations of Cu assessed under the use of two standard additions closely corresponded to those achieved using the method of the calibration curve with the external aqueous standard solutions. It was found that the total Cu concentrations obtained with these two calibration strategies do not statistically differ ( $p = 0.05$ ) from each other. These findings support formerly described observations (26, 44) that low amounts of ethanol in the analyzed samples do not affect the atomization process and the atomic cloud formation in the air-acetylene flame.

**Fractionation of Cu in Beers and Wines.** The experimental evidence of different chemical forms of Cu in beer can be regarded as unsatisfactory. So far, the charge of the Cu species has been established treating a degassed beer sample with the separate cation and anion exchange SPE cartridges (11). Determining the Cu content in the effluents collected, the information on the 72 and 34% shares of the nonanionic (the positively charged species, mostly the metal cations) and the noncationic (the anionic metal species associated with large molecular mass organic compounds) Cu species, respectively, was obtained in the cited work. In the case of wine, the operationally defined chemical speciation has been carried out using mainly the electrochemical stripping techniques and therefore, the information about the sum of the



**Figure 2.** Distribution of the Cu species fractions in the analyzed beers and wines. The results are average ( $n=3$ ) percentage contributions with respect to the total Cu concentrations; the pooled relative standard deviations are denoted.

free Cu(II) ions with the labile bound Cu species was retrieved. This fraction was reported to contribute 13–91% to the total Cu content (13, 14, 32). The operationally defined speciation of Cu in wine based on the use of the SPE columns with different ionic and nonionic solid sorptive phases has also been reported (16).

When the fractionation procedure described in the present work was used, three different fractions were distinguished, including the hydrophobic, the cationic, and the residual Cu species. The proposed procedure is simple to operate and does not produce high working costs. Furthermore, the total concentrations of Cu in the analyzed samples of beers and wines and the content of Cu in the separated fractions are determined without any sample pretreatment. As a result, the possible disturbance of the equilibrium between the Cu species and contamination of the samples are minimized. The percentage distribution of distinct fractions of the Cu species in analyzed beers and wines are given in **Figure 2**.

In the case of beers, it was established that the most abundant class of the Cu species discriminated with the two-column SPE fractionation procedure is the residual species fraction. This fraction was presumed to contain the Cu species that were not retained by the adsorbing resin, neither by the cation exchanger, and, therefore, it was attributed to the presence of nonhydrophobic and noncationic Cu forms, that is, any neutral and negatively charged species. The contribution of this fraction in the studied beers was found to be 74–82% of the total Cu content. These results are in a good agreement with the findings of Svendsen and Lund (11), who determined that the share of the noncationic species of Cu, found upon the analysis of the cation exchange cartridge effluent, was 72% of its total concentration in the analyzed beer.

The contributions of two other species fractions of Cu determined in the analyzed beers were much lower as compared to the residual species fraction. The hydrophobic species fraction, likely containing the polyphenolic bound Cu forms, was evaluated to account for 10–14% of the total Cu content. The donation of the cationic species fraction, presumably including the free Cu ions, the stable cationic complexes of Cu and the labile complexes of that metal, was established to change from 12 to 13% as related to the total Cu. It can be seen that the sum of the contributions of these latter fractions, being within the range 22–27%, well corresponds to the 34% contribution of the cationic fraction reported before by Svendsen and Lund (11), who assessed it measuring the content of the nonanionic Cu species in the effluent of the anion exchange cartridge. This suggests that the cationic Cu

species fraction, formerly evaluated using two separate cartridges with the ion exchangers, could be overestimated due to the retention of the organically bound Cu species.

In the case of the analyzed red wines, a higher variability in the results for the hydrophobic and the cationic species fractions can be observed as compared to the fraction distribution assessed for beers. A higher degree of variability in the content of different organic substances capable of complexing the Cu ions might be responsible for that. Indeed, the hydrophobic species fraction of Cu was found to change from 27 to 77% in reference to its total concentration. This fraction was presumed to be attributed to the strong complexes of Cu with various anthocyanins, flavonoids (flavanols, flavonols) and other polyphenols (i.e., tannins) that have been reported to be contained in red wines in relatively high amounts (45–49). Other large organic molecules, including peptides, proteins, and polysaccharides, could also contribute to this fraction because these compounds were reported to usually exist in the complexes with polyphenols (16, 49).

As can be seen from **Figure 2**, the range of the distribution of the cationic species fraction of Cu assessed for the analyzed wines is also extensive and accounts for 8–72% of its total concentration. Similarly, a great variability in the contribution of the free Cu(II) ions with the labile Cu species was formerly reported for wines analyzed with the electrochemical stripping techniques (13, 14, 32). This class of the species was supposed to contain the simple Cu ions and the labile complexes of Cu with amino and carboxylic acids, which were formerly recognized to be rather weak ligands of Cu at the typical enological conditions (45–49). Due to its nature, this fraction can be regarded as the most easily absorbable and with the highest propensity to undergo the spoilage and staling reactions in wine as was suggested before (13, 15, 16).

Finally, the less abundant class of the Cu species in the analyzed wines was found to be the residual fraction (6–17% of the total Cu content), which could possibly be attributed to the presence of stable anionic and/or neutral complexes of Cu. The contribution of this fraction well corresponds to the 5–11% contribution previously reported for the negatively charged species of Cu separated in red wines as well (16).

The information assessed through the fractionation analysis presented here could have a special value for brewers and wine-makers as well as common consumers of beers and wines. Apparently, the knowledge on the respective classes of the chemical forms of Cu, especially the hydrophobic and the cationic

species, can be useful for explaining the conditions of the Cu induced oxidative spoilage of beer and wine, resulting in the undesirable alteration of their quality and stability.

**Reliability of Results.** In general, the quality control of the results of the operationally defined speciation analysis is difficult. To verify the results obtained for the hydrophobic Cu species fraction, the samples of studied beers and wines were treated with another macroreticular adsorbing resin Amberlite XAD-7. The results achieved and related to the donations of this species fraction to the total Cu concentrations in the analyzed samples are given in **Figure 2**. It can be seen that the percentage contributions of the hydrophobic Cu species fractions obtained with both adsorbing resins are comparable, which proves the consistency and reliability of the fractionation approach proposed. The differences between the fraction contributions were found to change within  $-1.7$  to  $+1.1\%$ . In addition, the sums of the fraction contributions assessed with the fractionation procedure described are from  $97.8 \pm 5.1\%$  (sample B3) to  $104 \pm 6\%$  (sample B4). This suggests that Cu, present in the form of different physicochemical species varying in hydrophobicity and charge, was quantitatively recovered from the analyzed alcoholic beverages. The precision of the results achieved using the described partitioning procedure is also satisfactory. The pooled relative standard deviations found for the fractions classified were in the ranges  $1.2$ – $4.7\%$ ,  $0.5$ – $3.6\%$ , and  $1.3$ – $6.3\%$ , respectively, for the hydrophobic, the cationic, and the residual species fractions.

#### LITERATURE CITED

- Ibanez, J. G.; Carreon-Alvarez, A.; Barcena-Soto, M.; Casillas, N. Metals in alcoholic beverages: a review of sources, effects, concentrations, removal, speciation and analysis. *J. Food Comp. Anal.* **2008**, *21*, 672–683.
- Vanderhaegen, B.; Neven, H.; Verachtert, H.; Derdelinckx, G. The chemistry of beer aging—a critical review. *Food Chem.* **2006**, *95*, 357–381.
- Bamforth, C. W. Enzymic and non-enzymic oxidation in the brewhouse: a theoretical consideration. *J. Inst. Brew.* **1999**, *105*, 237–202.
- Danilewicz, J. C. Review of reaction mechanisms of oxygen and proposed intermediate reduction products in wine: central role of iron and copper. *Am. J. Enol. Vitic.* **2003**, *54*, 73–85.
- Li, H.; Guo, A.; Wang, H. Mechanisms of oxidative browning of wine. *Food Chem.* **2008**, *108*, 1–13.
- Waterhouse, A. L.; Laurie, V. F. Oxidation of wine phenolics: a critical evaluation and hypotheses. *Am. J. Enol. Vitic.* **2006**, *57*, 306–313.
- Elias, R. J.; Andersen, M. L.; Skibsted, L. H.; Waterhouse, A. L. Identification of free radical intermediates in oxidized wine using electron paramagnetic resonance spin trapping. *J. Agric. Food Chem.* **2009**, *57*, 4359–4365.
- Benítez, P.; Castro, R.; Barroso, C. G. Removal of iron, copper and manganese from white wines through ion exchange techniques: effects on their organoleptic characteristics and susceptibility to browning. *Anal. Chim. Acta* **2002**, *458*, 197–202.
- Clark, A. C.; Scollary, G. R. Copper(II)-mediated oxidation of (+)-catechin in a model white wine system. *Aust. J. Grape Wine Res.* **2002**, *8*, 186–195.
- Cacho, J.; Castells, J. E.; Esteban, A.; Laguna, B.; Sagrista, N. Iron, copper, and manganese influence on wine oxidation. *Am. J. Enol. Vitic.* **1995**, *46*, 380–384.
- Svendsen, R.; Lund, W. Speciation of Cu, Fe, and Mn in beer using ion exchange separation and size-exclusion chromatography in combination with electrothermal atomic absorption spectrometry. *Analyst* **2000**, *125*, 1933–1937.
- Alberti, G.; Pesavento, M.; Biesuz, R. A chelating resin as a probe for the copper(II) distribution in grape wines. *React. Funct. Polym.* **2007**, *67*, 1083–1093.
- Clark, A. C.; Scollary, G. R. Medium exchange stripping potentiometry for the measurement of labile copper in white wine. *Electroanalysis* **2006**, *18*, 1793–1799.
- Sanchez Misiego, A.; Garcia-Monco Carra, R. M.; Ambel Carracedo, M. P.; Guerra Sanchez-Simon, M. T. Electroanalytical determination and fractionation of copper in wine. *J. Agric. Food Chem.* **2004**, *52*, 5316–5321.
- Green, A. M.; Clark, A. C.; Scollary, G. R. Determination of free and total copper and lead in wine by stripping potentiometry. *Fresenius' J. Anal. Chem.* **1997**, *358*, 711–717.
- Karadjova, I.; Izgi, B.; Gucer, S. Fractionation and speciation of Cu, Zn, and Fe in wine samples by atomic absorption spectrometry. *Spectrochim. Acta, Part B* **2000**, *57*, 581–590.
- Llobat-Estelles, M.; Mauri-Aucejo, A. R.; Marin-Saez, R. Detection of bias errors in ETAAS. Determination of copper in beer and wine samples. *Talanta* **2006**, *68*, 1640–1647.
- Asfaw, A.; Wibetoe, G. Direct analysis of beer by ICP-AES: a very simple method for the determination of Cu, Mn, and Fe. *Microchim. Acta* **2005**, *152*, 61–68.
- Nascentes, C. C.; Kamogawa, M. Y.; Fernandes, K. G.; Arruda, M. A. Z.; Nogueira, A. R. A.; Nobrega, J. A. Direct determination of Cu, Mn, Pb, and Zn in beer by thermospray flame furnace atomic absorption spectrometry. *Spectrochim. Acta, Part B* **2005**, *60*, 749–753.
- Bellido-Milla, D.; Onate-Jaen, A.; Palacios-Santander, J. M.; Palacios-Tajero, D.; Hernandez-Artiga, M. P. Beer digestion for metal determination by atomic spectrometry and residual organic matter. *Microchim. Acta* **2004**, *144*, 183–190.
- Vinas, P.; Aguinaga, N.; Lopez-Garcia, I.; Hernandez-Cordoba, M. Determination of cadmium, aluminium, and copper in beer and products used in its manufacture by electrothermal atomic absorption spectrometry. *J. AOAC Int.* **2003**, *85*, 736–743.
- Wyrzykowska, B.; Szymczyk, K.; Ichichashi, H.; Falandysz, J.; Skwarzec, B.; Yamasaki, S. Application of ICP sector field MS and principal component analysis for studying interdependences among 23 trace elements in Polish beers. *J. Agric. Food Chem.* **2001**, *49*, 3425–3431.
- Bellido-Milla, D.; Moreno-Perez, J. M.; Hernandez-Artiga, M. P. Differentiation and classification of beers with flame atomic spectrometry and molecular absorption spectrometry and sample preparation assisted by microwaves. *Spectrochim. Acta, Part B* **2000**, *55*, 855–864.
- Matusiewicz, H.; Kopras, M. Methods for improving the sensitivity in atom trapping flame atomic absorption spectrometry: analytical scheme for the direct determination of trace elements in beer. *J. Anal. At. Spectrom.* **1997**, *12*, 1287–1291.
- Ajtony, Z.; Szoboszlai, N.; Susko, E. K.; Mezei, P.; Gyorgy, K. Direct sample introduction of wines in graphite furnace atomic absorption spectrometry for the simultaneous determination of arsenic, cadmium, copper, and lead content. *Talanta* **2008**, *76*, 627–634.
- Schiavo, D.; Neira, J. Y.; Nobrega, J. A. Direct determination of Cd, Cu, and Pb in wines and grape juices by thermospray flame furnace atomic absorption spectrometry. *Talanta* **2008**, *76*, 1113–1118.
- Alvarez, M.; Moreno, I. M.; Josa, A. M.; Camean, A. M.; Gonzalez, A. G. Study of mineral profile of Montilla-Moriles “fino” wines using inductively coupled plasma atomic emission spectrometry methods. *J. Food Comp. Anal.* **2007**, *20*, 391–395.
- Coetzee, P. P.; Steffens, F. E.; Eiselen, R. J.; Augustyn, O. P.; Balcaen, L.; Vanhaecke, F. Multi-element analysis of South African wines by ICP-MS and their classification according to geographical origin. *J. Agric. Food Chem.* **2005**, *53*, 5060–5066.
- del Mar Castineira Gmez, M.; Feldmann, I.; Jakubowski, N.; Andersson, J. T. Classification of German white wines with certified brand of origin by multielement quantitation and pattern recognition techniques. *J. Agric. Food Chem.* **2004**, *52*, 2962–2974.
- Diaz, C.; Conde, J. E.; Estevez, D.; Perez Olivero, S. J.; Perez Trujillo, J. P. Application of multivariate analysis and artificial neural networks for the differentiation of red wines from the Canary



- Islands according to the Island of Origin. *J. Agric. Food Chem.* **2003**, *51*, 4303–4307.
- (31) Aceto, M.; Abollino, O.; Bruzzoniti, M. C.; Mentasti, E.; Sarzanini, C.; Malandrino, M. Determination of metals in wine with atomic spectroscopy (flame-AAS, GF-AAS, and ICP-AES); a review. *Food Addit. Contam.* **2002**, *19*, 126–133.
- (32) Wiese, C.; Schwedt, G. Strategy for copper speciation in white wine by differential puls anodic stripping voltammetry, potentiometry with an ion-selective electrode and kinetic photometric detection. *Fresenius' J. Anal. Chem.* **1997**, *358*, 718–722.
- (33) Arcos, M. T.; Ancin, M. C.; Echeverria, J. C.; Gonzalez, A.; Garrido, J. J. Study of lability of heavy metals in wines with different degrees of aging through differential pulse anodic stripping voltammetry. *J. Agric. Food Chem.* **1993**, *41*, 2333–2339.
- (34) McKinnon, A.; Scollary, G. R. Size fractionation of metals in wine using ultrafiltration. *Talanta* **1997**, *44*, 1649–1658.
- (35) Tokalioglu, S.; Kartal, S.; Elci, L. Speciation and determination of heavy metals in lake waters by atomic absorption spectrometry after sorption on Amberlite XAD-16 resin. *Anal. Sci.* **2000**, *16*, 1169–1174.
- (36) Hiraide, M.; Hiramatsu, S.; Kawaguchi, H. Evaluation of humic complexes of trace metals in river water by adsorption on indium-treated XAD-2 resin and DEAE-Sephadex A-25 anion exchanger. *Fresenius' J. Anal. Chem.* **1994**, *348*, 758–761.
- (37) Erdemoglu, S. B.; Pyrzynska, K.; Gucer, S. Speciation of aluminum in tea infusion by ion-exchange resins and flame AAS detection. *Anal. Chim. Acta* **2000**, *411*, 81–89.
- (38) Pohl, P.; Prusisz, B. Fractionation analysis of manganese and zinc in beers by means of two sorbent column system and flame atomic absorption spectrometry. *Talanta* **2007**, *71*, 1616–1623.
- (39) Hiraide, M.; Arima, Y.; Mizuike, A. Separation and determination of traces of heavy metals complexed with humic substances in river waters by sorption on indium-treated Amberlite XAD-2 resin. *Anal. Chim. Acta* **1987**, *200*, 171–179.
- (40) Borges, F.; Guimaraes, C.; Lima, J. L. F. C.; Pinto, I.; Reis, S. Potentiometric studies on the complexation of copper(II) by phenolic acids as discrete ligand models of humic substances. *Talanta* **2005**, *66*, 670–673.
- (41) Kraal, P.; Jansen, B.; Nierop, K. G. J.; Verstraten, J. M. Copper complexation by tannic acid in aqueous solution. *Chemosphere* **2006**, *65*, 2193–2198.
- (42) Pohl, P. Manganese and zinc operational fractionation in beer by means of tandem ion exchange column assemblage and flame atomic absorption spectrometry. *Microchim. Acta* **2007**, *159*, 325–332.
- (43) Pohl, P.; Prusisz, B. Application of tandem column solid phase extraction and flame atomic absorption spectrometry for the determination of inorganic and organically bound forms of iron in wine. *Talanta* **2008**, *77*, 1732–1738.
- (44) Espinoza, M.; Olea Azar, C.; Massiff, G.; Villa, A. Development of a reference material for copper and iron in wine. *J. Food Comp. Anal.* **2008**, *21*, 684–688.
- (45) Vasconcelos, M. T.; Azenha, M.; de Freitas, V. Role of polyphenols in copper complexation in red wines. *J. Agric. Food Chem.* **1999**, *47*, 2791–2796.
- (46) Azenha, M. A. G. O.; Vasconcelos, M. T. S. D. Pb and Cu speciation and bioavailability in port wine. *J. Agric. Food Chem.* **2000**, *48*, 5740–5749.
- (47) Esparza, I.; Salinas, I.; Santamaria, C.; Garcia-Mina, J. M.; Fernandez, J. M. Electrochemical and theoretical complexation studies for Zn and Cu with individual polyphenols. *Anal. Chim. Acta* **2005**, *543*, 267–274.
- (48) Vestergaard, M.; Kerman, K.; Tamiya, E. An electrochemical approach for detecting copper-chelating properties of flavonoids using disposable pencil graphite electrodes: possible implications in copper-mediated illnesses. *Anal. Chim. Acta* **2005**, *538*, 273–281.
- (49) Esparza, I.; Santamaria, C.; Garcia-Mina, J. M.; Fernandez, J. M. Complexing capacity profiles of naturally occurring ligands in Tempranillo wines for Cu and Zn. An electroanalytical approach for cupric casse. *Anal. Chim. Acta* **2007**, *599*, 67–75.

---

Received June 11, 2009. Revised manuscript received September 2, 2009. Accepted September 07, 2009.