Changes of the Metal Composition in German White Wines through the Winemaking Process. A Study of 63 Elements by Inductively Coupled Plasma–Mass Spectrometry

Maria del Mar Castiñeira Gómez,*[†,§] Rolf Brandt,[†]
Norbert Jakubowski,[†] and Jan T. Andersson[§]

Institute of Spectrochemistry and Applied Spectroscopy, Bunsen-Kirchhoff-Strasse 11,
D-44139 Dortmund, Germany, and Department of Inorganic and Analytical Chemistry,
University of Münster, Corrensstrasse 30, D-48149 Münster, Germany

Elemental patterns are often used for the classification or identification of the origin of wines. A prerequisite is that the concentration of the elements is not strongly influenced by the addition of different substances such as yeast and fining products during the winemaking process. Inductively coupled plasma−mass spectrometry (ICP-MS) has been used in this study to determine in total 63 elements (including some nonmetals and the rare earth elements) in five German white wines from five regions of origin. The whole winemaking process was studied, from the must to the ready wine. Microwave acid digestion was used for sample preparation, and indium was added as internal standard for a semiquantitative analysis. Two winemaking processes were compared: with the addition of clarifying agents (bentonites) before and after the fermentation. The concentration of only a few elements such as Li, B, Mg, Ca, Rb, Cs, and Pb seems to be constant throughout the whole winemaking process (changes of $<\pm50\%$) and are independent of the time of addition of the bentonites. When bentonites are added before fermentation, the concentration of other elements, such as V, Co, and Fe, remains constant. If bentonites are added after fermentation, the concentration of some other elements such as Sr, Zn, and Mn is nearly unaffected. These elements are therefore robust elements for origin studies in German white wines.

KEYWORDS: Wine; winemaking process; elements; ICP-MS

INTRODUCTION

In wine, mostly elements are present naturally and their concentration pattern has been already used for years for origin determinations. The elemental composition of a wine varies over a wide range and is influenced by environmental factors, such as climate, weather, and soil, as well as by cultivar and by processing and storage of the grapes, must, and wine. There are several sources contributing to the metal composition of a finished wine, and one speaks about primary and secondary metal contents. Primary metals enter the wine from the vineyard soil via the roots of the grapevine and the grapes. This is generally the characteristic and larger part of the total concentration. The secondary metals are introduced during the processing stages, from the grape harvest to the finished wine, and can be considered as impurities or contamination.

Anthropogenic contamination from mining, industries, and other human activities has decreased in recent years and is detectable in wines only sporadically. Nowadays the most important sources of contamination that can change the primary elemental content in wines lie in enological activities: (a) in agricultural practices during the vine-growing (use of fertilizers, mineral pesticides, etc.) and grape harvesting and (b) in the winemaking process, including must production, wine preparation (treatment of the wine with different substances for quality improvement), and wine storage. German wine regulations (*1*) force the producer to check for the correct concentrations of the elements Al, As, Pb, B, Cd, Cu, Zn, and Sn in the ready wine.

During recent years, only a few works have been published concerning the changes of metal concentrations during the winemaking process. Muranyi et al. (*2*) studied the effect of filtering on the elements Cd, Pb, Cu, Zn, Mn, Al, Fe, and Ca in Tokay wines. They also investigated the migration of major and minor elements (Na, K, Ca, Mg, P, Fe, Mn, and Zn) in an Eger grape−must−wine system (*3*). Kristl et al. studied the contents of Cu, Mn, Zn, Cd, Cr, and Pb at different stages of the winemaking process in Slovenian wines (*4*). Further research about this area has been published by H. R. Eschnauer (*5, 6*). Some papers can be found on the contamination with rare earth elements (REE) of wines when bentonites are used as fining agents (*7*).

* Corresponding author (e-mail castineira@isas-dortmund.de).
† Institute of Spectrochemistry and Applied Spectroscopy.
§ University of Münster.

The processes for making red and white wines differ in that red wines are obtained by the alcoholic fermentation of musts in the presence of the solid parts of the berry (skins and seeds), whereas white wines are exclusively produced by the fermentation of grape juice (*8*). At later stages different substances are added to improve the quality of the wine. Especially white wines usually require more enological care because clarity is an essential quality required by consumers. To achieve this, bentonites and other fining agents are added during the winemaking process. Bentonites (*8*) are hydrated aluminum silicates, mainly consisting of montmorillonites (e.g., Al_2O_3, $4SiO_2$, nH_2O), that are used for removing proteins in white wines and colloidal coloring matter in red wines. They contain exchangeable cations (Mg^{2+}, Ca^{2+}, Na^+, etc.) that vary according to their geographical origin and therefore are the agents with the potential biggest influence on the final wine concentration. German wine regulations require that the possible increase in concentration of the metals Ca, Mg, Fe, As, and Pb after the addition of bentonites for clarification (*9*) is checked by examining every new bentonite in use. Other fining agents are added jointly to intensify the yellow color and to eliminate undesirable odors or tastes. A single fining agent is not always sufficient to obtain good results, and a combination of several proteins and inorganic fining agents may be more effective.

The knowledge of how the winemaking process influences the elemental content during the production of white wines is of vital importance for origin studies in wines through elemental patterns. In the ideal case, the content should not change significantly or the changes should be reproducible to the same extent in all of the winemaking processes.

The aim of this work is to study the changes of as many elements as possible during the typical white winemaking process. Five German white wines from five wine regions were selected for the study, and two in-house and small-scale common white winemaking processes were selected and compared. The main difference is that in one of them the clarification takes place in the young wine after fermentation (process A) and in the other the clarification occurs in the nonfermented must (process B). Clarification essentially modifies must ferment ability. Clear must is known to ferment with more difficulty than cloudy must, and white wine quality is generally thought to be enhanced by a somewhat difficult and slow fermentation. Another difference between both processes is the time needed for the fermentation and aging steps (5 and 4 weeks, respectively, in process A, and 7 and 6 weeks, respectively, in process B).

A meaningful determination of metal concentration in different samples (must, wine) can be performed only if matrix influences are excluded. The must matrix is naturally different and more complex, due to the presence of depositions and colloidal suspensions of pectins or proteins, than the young or ready wine matrix (high ethanol content), and a sample preparation that destroys the organic components is necessary. Moreover, in this way, disorders on the plasma due to the presence of particles and high content of organic compounds are avoided.

Several analytical techniques have been used to carry out multielement analysis of wine: neutron activation analysis (*10*), transmission near-infrared spectrometry (*11*), total reflection X-ray fluorescence analysis (*12*, *13*), atomic emission spectrometry (*14−18*), atomic absorption spectrometry (*11*, *15−20*), inductively coupled plasma optical emission spectrometry (*18*, *21−24*), and inductively coupled plasma−mass spectrometry (ICP-MS) (*7*, *10*, *13*, *25−30*). Semiquantitative analysis,

Figure 1. Wine-growing regions in Germany: Ahr, Baden, Franken, Hessische Bergstrasse, Mittelrhein, Mosel-Saar-Ruwer, Nahe, Pfalz, Rheingau, Rheinhessen, Saale-Unstrut, Sachsen, Württemberg (www.germanwine.de).

as a screening method, has already been carried out for the elemental determination in wine by using ICP-MS (*7*, *10*, *13*, *29*). The accuracy of this fast approach by using internal standard allows element determination down to the nanograms per liter level to be carried out with an error range of 25−50%. This is perfectly acceptable for the present purposes and therefore a semiquantitative approach was chosen to obtain a first general overview of the winemaking process in a reasonable time. Due to the method's inherent error range of 50%, the chosen criteria to select an element as robust were (a) that its concentration did not change by >±50% when for the must and ready wine, and (b) no such change in concentration is observed between any steps of the winemaking process.

EXPERIMENTAL PROCEDURES

Material and Reagents. All chemicals used were purchased from Merck (Darmstadt, Germany): HNO_3 subboiled 14 M of p.a. quality, EtOH (99.8%) of p.a. quality, and indium from an ICP standard solution of 10 μg/mL in 5% HNO_3. The multielement standard solution 5 with 10 μg/mL of Be, Bi, Ce, Co, In, Mg, Ni, Pb, and U in 2% HNO_3 was from Cläritas, Spex, Germany.

The samples were filtered through a 0.45 μm pore size sterile acetate cellulose membrane (Minisart, Sartorius, Göttingen, Germany). Polypropylene (PP) tubes were used for storing the samples. They were carefully cleaned before use with 5% HNO_3 subb.

Wine Samples. Five sets of samples from the must to the ready white wine were analyzed. The samples were from the following German wine-growing regions (**Figure 1**): Rheingau (RG), Rheinhessen (RH), Baden (B), Pfalz (P), and Mittelrhein (MR). According to the German Wine Institut [data from the German Wine Institut of the wine production of Germany in 2001 (www.deutscheweine.de).], three of them are major producers (26.7% of total wine production comes from Rheinhessen, 22.4% from Pfalz, and 12.4% from Baden) and the
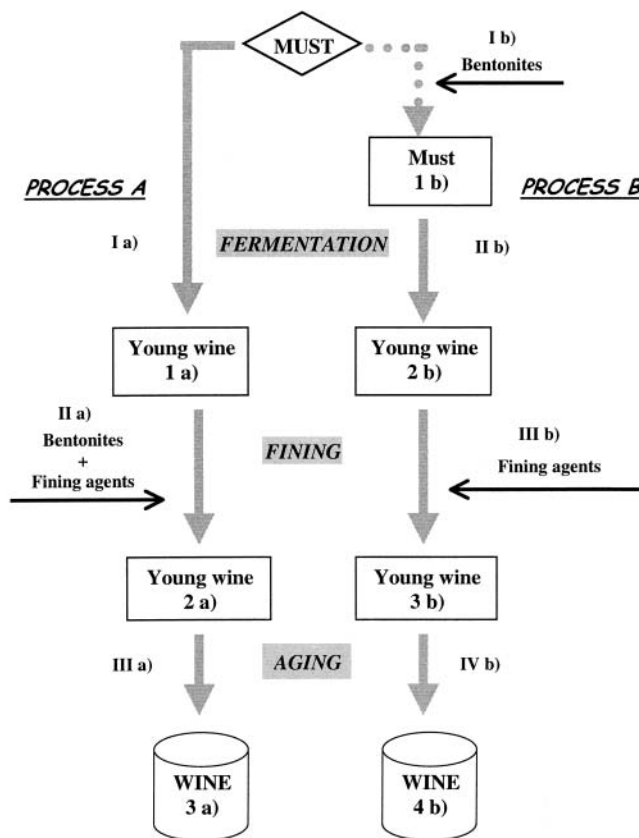
Changes of Metal Composition of German White Wines

J. Agric. Food Chem., Vol. 52, No. 10, 2004 **2955**

Figure 2. Alternative winemaking processes for German white wines with bentonite addition after fermentation (process A) and before fermentation (process B): (I a, II b) fermentation, 10 g/hL *O. freddo** (yeast), 100 mg/L Kadifit* ($K_2S_2O_5$), for sulfiting; (II a) fining, 50 g/hL Na Calit* (sodium–calcium bentonite), 50 mL og KS-Speedfloc*, 50 mL of Gelita-Klar* (gelatin); (III a, IV b) aging, 4 weeks; (I b) clarification, 150 g/hL Seporit* (calcium bentonite); (III b) fining, 50 mL of KS-Speedfloc* (silica sol), 50 mL of Gelita-Klar* (gelatin). *Products and wine samples supplied by Erbslöh Geisenheim Getränketechnologie GmbH & Co. KG (www.erbsloeh-geisenheim.de).

other two are minor producers (Rheingau represents only 3% of the total production and Mittelrhein, 0.4%). All of them were prepared from grapes harvested in 2001 and coming from different types of soil: the RG samples are from a loess soil, the RH samples from alluvial deposits/gravel, the Pfalz samples from sandier loess soil, the MR from schist soil, and finally the Baden samples from volcanic loess soil. All of the samples were obtained in microvinification conditions from the research department of Erbslöh Geisenheim Getränketechnologie GmbH & Co. KG (*31*). A scheme of the processes can be seen in **Figure 2**, where two typical winemaking processes are compared: In process A bentonites are added to the young wine after fermentation at the same time as the fining agents. In process B bentonites are added before fermentation, and fining agents are added separately at a later stage.

The vinification started with the must being split in two portions, one of which (sample a) followed process A and the other one (sample b) followed process B. All products added to the wine were supplied by the company Erbslöh Geisenheim Getränketechnologie GmbH & Co. KG (*31*). In process A the following products were used: (i) during the fermentation, the yeast (*Oenoform freddo*) was added followed 10 days later by SO_2 for sulfiting in the form of potassium metabisulfite, $K_2S_2O_5$ (the treatment with SO_2 suppresses oxidation processes and the growth of undesirable microorganisms); (ii) for fining or protein stabilization different products were used—a sodium–calcium bentonite low in iron (NaCalit), a silica solution with 30% highly active silicic acid (Klar-Sol Speedfloc), with flocculation properties, and a 20% liquid gelatin especially developed for beverage treatment (Gelita-Klar). In process B there is an additional step at the beginning consisting of the

addition of a calcium bentonite low in iron (Seporit) to the must. The RG must was extraclarified before any of the processes in the following way: 5 mL/hL Trenolin Super DF (a depsidase-free pectinase in the form of a liquid preparation for quick pectin decomposition), 30 mg/L SO_2 (for sulfiting), 150 g/hL Most-rein (a preparation of clay minerals of the montmorillonite group, gelatin, and traces of highly pure active carbon, which contains only very low metal levels; it is used for the elimination of pesticide residues and other undesirable substances as well as the prevention of off-taste and off-odor or fermentation disturbances), and 3 g/hL Blattgelatine (a liquid gelatin–caseinate derivative for the reduction of disturbing tannins in must).

The must samples were taken on October 8, 2001, and the samples from process A on November 14, 2001 (young wine after fermentation), on November 21, 2001 (young wine after clarification), and on Decebmer 17, 2001 (ready wine after process A). The samples from process B were taken on October 9, 2001 (must after clarification), on November 28, 2001 (young wine after fermentation), on December 6, 2001 (young wine after fining), and on January 14, 2002 (ready wine after process B). Thus, in process A the must spent 5 weeks in the fermentation process. Afterward, the young wine stood in contact with the bentonites and the fining agents for 1 week and then was stored for ~4 weeks before the sample of ready wine was taken. In process B the must was first in contact with the bentonites for 1 day only. Afterward, the clarified must was fermented for 7 weeks. The young wine was then fined during 1 week and finally stored for ~6 weeks before the final wine sample was taken. In this way the process of "a" wines took 10 weeks and that of "b" wines, 14 weeks.

The process started with 5 L of fresh must. Half of this amount was employed for each process. The volume of the samples taken at every step was 500 mL. They were first filtered twice, once using a paper filter (Schleicher & Schuell, 520 B$^1/_2$) and then a glass filter (Schleicher & Schuell, GF 8). The filters were previously washed with water, and then the first 200 mL of sample was discarded in order to avoid contamination. Afterward, they were stabilized with 40 mg/L sodium azide to stop fermentation and to avoid bacterial spoilage and stored in PP containers in the freezer at −18 °C until analysis.

Sample Preparation. To remove the appreciable amounts of deposits and colloidal suspensions present, the samples were filtered (membrane of cellulose acetate, 0.45 μm pore size) and then acid digested in a microwave oven (Multiwave, Perkin-Elmer, Überlingen, Germany). Two and a half milliliters of sample was mixed with 2.5 mL of 14 M HNO_3 subb. and subjected for 25 min to microwaves at 1000 W, resulting in a T_{max} of 170−190 °C and a P_{max} of 30 bar. The clear solution was transferred to 25 mL PP volumetric flasks, resulting in a 1:10 dilution; 0.100 mL of a 10 μg/mL indium standard solution in 5% HNO_3 was added as internal standard to give a concentration of 40 ng/mL. Every sample was prepared in triplicate. Six samples could be digested simultaneously. Six blanks were always digested first and then 30 must or wine samples in the following five runs. Thus, a total of 24 blanks and 120 samples were processed.

ICP-MS Analysis. ICP-MS measurements were carried out using a quadrupole mass spectrometer VG Plasma Quad PQ II Turbo Plus (VG Elemental, Winsford, Cheshire, U.K). For sample introduction a traditional quartz Meinhard nebulizer (type A, Meinhard, Santa Ana, CA) with a Scott type spray chamber made from quartz and cooled to 5 °C was used at a rate of 0.4 mL/min. Tuning was performed by optimizing the signal for $^{140}Ce^+$ and reducing the signals of $^{70}Ce^{2+}$ and $^{156}CeO^+$ to <3% of the $^{140}Ce^+$ signal. Data were evaluated in a semiquantitative approach based on a response curve using the 100 ng/mL multielement standard and 40 ng/mL of In as internal standard. The procedure used and operational parameters are described in detail elsewhere (*13*). The isotopes for the 63 elements under study, limits of detection (LOD; 3*s*, 24 blanks), and limits of quantification (LOQ; 10*s*, 24 blanks) are listed in **Table 1**. The values for arsenic cannot be totally trusted and are given justly as a guide because As forms volatile species and could be lost during the microwave digestion step.

RESULTS AND DISCUSSION

The samples were digested before ICP-MS measurements in order to (1) eliminate molecular interferences and plasma

2956 *J. Agric. Food Chem.*, Vol. 52, No. 10, 2004

Castiñeira et al.

Table 1. Limits of Detection (LOD = 3*s*, 24 Blanks) and Limits of Quantification (LOQ = 10*s*, 24 Blanks)

element	*m*/*z*	LOD	LOQ	element	*m*/*z*	LOD	LOQ
Li	7	0.3	1.0	Sb	121	0.4	1.2
Be	9	0.4	1.4	Te	126	1.8	6.0
B	10	15	49	Cs	133	0.1	0.2
Mg	25	25	84	Ba	138	1.2	4.1
Al	27	103	345	La	139	0.1	0.2
Ca	43	150	500	Ce	140	0.2	0.6
Sc	45	2.2	7.2	Pr	141	0.1	0.2
Ti	49	33	109	Nd	146	0.3	0.9
V	51	0.3	1.0	Sm	147	0.3	0.9
Cr	53	12	41	Eu	151	0.1	0.3
Mn	55	1.1	3.5	Gd	157	0.4	1.2
Fe	57	372	1240	Tb	159	0.05	0.15
Co	59	0.3	1.0	Dy	163	0.2	0.5
Ni	60	5.1	17.0	Ho	165	0.03	0.11
Cu	65	2.3	7.8	Er	166	0.2	0.6
Zn	66	8.9	29.7	Tm	169	0.1	0.2
Ga	69	0.2	0.7	Yb	174	0.1	0.5
Ge	73	1.1	3.6	Lu	175	0.1	0.2
As	75	1.1	3.8	Hf	178	0.3	0.9
Se	82	13	45	Ta	181	0.1	0.2
Rb	85	0.2	0.7	W	182	0.3	0.9
Sr	88	0.2	0.7	Re	185	0.2	0.7
Y	89	0.2	0.6	Os	190	0.4	1.4
Zr	90	1.8	5.9	Ir	193	0.1	0.4
Nb	93	0.1	0.2	Pt	195	0.2	0.8
Mo	98	0.4	1.2	Au	197	0.2	0.7
Ru	102	0.2	0.6	Tl	205	0.1	0.4
Rh	103	0.1	0.2	Pb	208	1.3	4.5
Pd	108	0.3	1.0	Bi	209	0.2	0.6
Ag	109	0.2	0.7	Th	232	0.2	0.6
Cd	111	0.5	1.6	U	238	0.1	0.4
Sn	120	1.0	3.4				

a Concentrations are given in ng/mL.

disorders caused by the organic content of the samples (high sugar content in must and high EtOH content of the samples), (2) avoid the presence of colloidal suspensions and particulates on the nebulization system, and (3) equalize matrix influences.

Element Content and LOD/LOQ. Of the 63 elements selected for measurements, 22 were detected below the LOQ in nearly all of the samples, namely, Ge, Se, Ru, Rh, Pd, Ag, Cd, Sn (except B 0 and B 1b samples), Sb, Te, Eu, Gd (except P 1b), Tb (except P 1b and RG 1b), Ta, W, Re, Os, Ir, Pt, Au, Tl, and Bi. Other metals such as Ni, Cr, Ta, Th, and U lie only slightly over the LOQ in one or two steps of the winemaking process. The same is true for most of the REEs except La, Ce, and Yb. Moreover, in process A, the REEs Sm, Tm, and Lu are below the LOQ throughout the whole winemaking process. Therefore, the results for only 31−37 elements for process A and 37−41 elements for process B are discussed. The contents of all the metals under study at different stages of the processes are shown in **Table 2A** (process A) and **Table 2B** (process B) for the Pfalz region as an example. In **Table 3** are summarized the elements whose concentration in wine is controlled by the German wine regulation (*1*), namely, Al, As, Pb, B, Cd, Cu, Zn, and Sn. In none of the final wines were found values above the legal limits. The concentration of those elements is quite high in the original must (especially Cu in RH and B samples with values >2 μg/mL). Cd and Sn lie below the LOQ during the whole winemaking process. Other metals were less clear-cut, showing increases or decreases in concentration to different degrees depending on the step of the winemaking process and the region of origin. Elements such as Al, As, and Pb increase in concentration during the winemaking process, whereas others, notably B, Cu, and Zn, decrease. The final concentration of these elements in the wine is much lower than the maximum values

allowed by the German wine regulations, so that any contamination introduced during the winemaking process should be of no concern.

Changes in Metal Concentrations from Must to Wine. A general trend is the lower concentrations of all the elements after the fermentation step followed by higher concentrations after the addition of bentonite and/or other fining agents. During the aging some elements also experience small changes in either direction. The magnitude of those changes is different and depends more on the element and its concentration than on the winemaking process or wine origin.

Some elements remain constant from the must to the final wine, independent of the winemaking process and the region:

• Li, B, and Rb decrease by no more than 50% in concentration. Boron decreases after fermentation. In process A, boron increases after bentonite and fining agents addition, but in process B the picture is less clear, an increase after bentonite addition being observed in only two of five cases. Fining without bentonites has the least effect on the boron concentration. During aging there are two trends—in process A boron decreases during this period and in process B boron slightly increases. The changes in boron concentration on going from must to wine correspond to a reduction of ∼10−40%.

• Mg and Ca experience a slight increase in concentration during the winemaking process. The changes after bentonite addition (suspected as the main source of contamination) are by <50% due mainly to their already high content in the original grape juice. The most important change in concentration throughout both processes seems to occur after aging, when there is an increase in concentration for almost all of the wines in both processes of ∼20%.

• The behavior of Cs depends on the wine and the region. On average, the concentration increases or decreases by no more than 50% of the initial content in must.

• Pb slightly increases during the winemaking process. In this experiment it did not present any change over ±50% in concentration when the initial must and the ready wine were compared; however, it did when young wine 1 and ready wine 3 in RH and RG wines in process A were compared and when wine 3b and ready wine 4b in P wine in process B were compared. The decrease following the fermentation is the most significant one and amounts to 10−20% in most cases (except in RH with a decrease of ∼60% in both processes). Pellerin et al. (*32, 33*) reported that in must and wine, most of the lead is complexed by a pectic polysaccharide rhamnogalacturonan(II) that is not degraded during vinification. Kristl et al. (*4*) suggested that only noncomplexed lead could be removed as precipitates of PbS in lees deposit after fermentation. Changes following the addition of bentonites and fining plus bentonites are minor increases (except for RH, being again ∼60%). After aging, Pb concentrations increased again. The largest observed increase of 56% was after aging in the case of the Pfalz wine in process B.

Unfortunately, in real winemaking processes, the secondary content of Pb in wine can be easily increased due to the corrosion of metals or alloys used in the wine cellar. Increases of ∼80% above the primary content have already been observed (*6*).

• Zn decreased during both winemaking processes. In process B, the MR and RH wines lost >50% of Zn. Zinc is present in the must samples in a concentration >2 μg/mL (except in B and RG). In general, the Zn concentration decreases by 30−50% after fermentation (except for RG in process A), increases (in 3 of 5 cases) after bentonite addition in process B, and

Changes of Metal Composition of German White Wines

J. Agric. Food Chem., Vol. 52, No. 10, 2004 **2957**

Table 2. Metal Concentrations[a] at Each Step of Winemaking Processes A and B in White Wines from the German Wine-Growing Region of Pfalz

A. Winemaking Process A

element	m/z	must	young wine 1a	young wine 2a	wine 3a	element	m/z	must	young wine 1a	young wine 2a	wine 3a
Li	7	16.4	11.9	13.6	14.6	Rb	85	217	138	142	160
Be	9	2.1	1.3	7.0	5.6	Sr	88	288	249	311	338
B*	10	7.0	4.6	5.2	4.6	Y	89	3.1	<0.5	3.7	4.1
Mg*	25	80.1	71.5	75.8	86.3	Zr	90	9.3	<1.7	60.1	66.3
Al*	27	1.5	1.6	4.1	3.7	Nb	93	0.4	<0.2	2.3	2.3
Ca*	43	125	117	129	139	Mo	98	3.7	1.6	3.2	2.2
Sc	45	33.2	55.9	56.2	64.8	Cs	133	0.9	0.7	0.6	0.7
Ti	49	96.2	103	120	117	Ba	138	340	109	149	139
V	51	4.3	2.6	5.1	6.2	La	139	1.3	0.2	0.9	1.4
Mn*	55	1.5	1.5	1.5	1.7	Ce	140	1.2	<0.3	4.3	4.1
Fe*	57	3.9	3.5	4.6	4.6	Nd	146	1.4	<0.9	1.4	<0.9
Co	59	3.4	3.0	5.7	5.8	Eu	151	<0.3	<0.3	<0.3	<0.3
Ni	62	<26	<26	50.0	<26	Ho	165	<0.1	<0.1	<0.1	0.13
Cu	65	451	44.8	36.3	37.0	Yb	174	0.5	<0.4	1.0	1.0
Zn*	66	2.2	1.6	2.0	1.7	Hf	178	<0.75	<0.75	1.3	1.8
Ga	69	26.7	15.1	18.5	14.4	Pb	208	16.1	12.9	21.1	29.3
As	75	<3.7	<3.7	6.7	6.7	U	238	<0.4	<0.4	0.6	0.7

B. Winemaking Process B

element	m/z	must	must 1b	young wine 2b	young wine 3b	wine 4b	element	m/z	must	must 1b	young wine 2b	young wine 3b	wine 4b
Li	7	16.4	17.6	15.0	14.6	15.6	Mo	98	3.7	5.0	2.2	2.1	2.3
Be	9	2.1	5.6	3.1	2.99	3.29	Cs	133	0.9	0.6	0.7	0.8	0.8
B*	10	7.0	6.2	4.0	4.3	5.0	Ba	138	340	200	79.4	91	98
Mg*	25	80.1	85.6	80.3	79.1	88.3	La	139	1.3	6.4	1.0	0.4	1.0
Al*	27	1.5	9.2	2.4	2.6	7.3	Ce	140	1.2	11.7	1.9	1.2	1.1
Ca*	43	125	148	128	123	144	Pr	141	0.2	1.1	0.3	<0.2	<0.2
Sc	45	33.2	36.6	48.0	54.9	57.8	Nd	146	1.4	4.8	1.0	<0.9	<0.9
Ti	49	96.2	90.1	91.2	111	100	Sm	147	<0.9	1.1	<0.9	<0.9	<0.9
V	51	4.3	5.0	3.6	3.7	4.4	Eu	151	<0.3	<0.3	<0.3	<0.3	<0.3
Mn*	55	1.5	1.8	1.8	1.8	2.0	Gd	157	<1.2	1.5	<1.2	<1.2	<1.2
Fe*	57	3.9	5.0	4.3	4.3	4.3	Tb	159	<0.14	0.2	<0.14	<0.14	<0.14
Co	59	3.4	4.2	3.7	3.3	3.6	Dy	163	<0.4	1.1	<0.4	<0.4	<0.4
Cu	65	451	381	28.7	17.2	17.9	Ho	165	<0.1	0.4	<0.1	<0.1	<0.1
Zn*	66	2.2	1.7	1.0	1.1	1.2	Er	166	<0.5	1.3	<0.5	<0.5	<0.5
Ga	69	26.7	17.5	10.0	11.1	9.2	Tm	169	<0.2	0.2	<0.2	<0.2	<0.2
As	75	<3.7	4.3	3.8	<3.7	5.1	Yb	174	0.52	1.7	1.0	0.7	0.9
Rb	85	217	204	130	142	171	Lu	175	<0.2	0.2	<0.2	<0.2	0.2
Sr	88	288	409	346	332	416	Hf	178	<0.75	1.5	<0.75	<0.75	<0.75
Y	89	3.1	11.9	3.4	2.7	3.5	Pb	208	16.1	21.0	15.8	14.7	22.9
Zr	90	9.3	50.3	9.6	10.8	14.3	Th	232	<0.4	3.6	<0.4	<0.4	<0.4
Nb	93	0.4	1.08	<0.2	<0.2	<0.2	U	238	<0.4	2.6	<0.4	<0.4	<0.4

[a] Concentrations are given in ng/mL unless denoted by an asterisk (∗), which are given in µg/mL.

Table 3. Maximum Allowed Concentrations of Some Metals in German Wine[a]

element	C_{max}, µg/mL	element	C_{max}, µg/mL
Al	8	B (as H_3BO_3)	80
Cu	2	Cd	0.01
As	0.1	Zn	5
Pb	0.25	Sn	1

[a] *Weinverordnung Anlage 7*, www.chemlin.de.

increases in 4 of 5 cases after the fining step. Following aging, Zn decreases in 8 of 10 cases and increases in the 2 cases of MR 4b and B 4b by 40 and 26%, respectively. Zinc was found to be present in wines at a level of 0.7−1.7 µg/mL. In general, the Zn concentration decreases during the winemaking process in all cases, for MR and RH to the extent of 50−60% depending on the process. In the case of the RG wine, the decrease is almost insignificant (<10% for both processes).

• V, Sr, Co, Mn, and Fe concentrations increase during both winemaking processes. This increase is below 50% for Sr and Mn in process A and for V, Co, and Fe in process B.

All of these 13 elements belong remarkably to the group of 17 elements that are already essential for plants or just needed for general confirmation (*34−36*); for instance, Li, V, and Sr are already known to be essential for some plants. The trace elements essential for plants are those that cannot be substituted by others in their specific biochemical roles and that have a direct influence on the organism so that it can neither grow nor complete some metabolic cycle.

Other elements show significant changes depending on both process and region:

• Cu and Ba decrease during the winemaking process, by a factor of ∼2 in the case of Ba and by at least 1 order of magnitude in the case of Cu. Their concentrations markedly decrease after the fermentation and slightly increase after the clarification with bentonites and addition of fining agents.

The copper content in musts ranged from 0.45 to 6.8 µg/mL, in wine after process A from 0.04 to 0.13 µg/mL, and in wine after process B from 0.02 to 0.14 µg/mL. Cu is the element that is most severely affected by the winemaking process and can decrease by a factor of 5−20 after fermentation depending on the wine and the winemaking process. After fermentation, the concentrations of Cu in young wines were quite similar in both processes. In the case of the RH samples for which the initial content of Cu was quite high (∼7 µg/mL), only ∼1% of the metal remained after fermentation in both winemaking
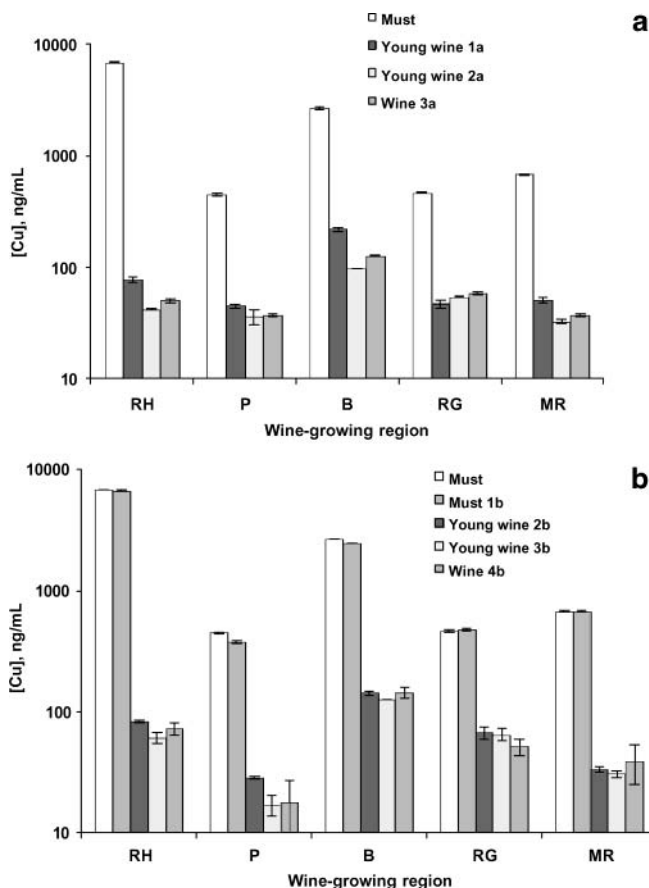
Figure 3. Copper concentration throughout winemaking processes A (**a**) and B (**b**). Results are shown at each step of the winemaking process and for the wine-growing regions Rheinhessen (RH), Pfalz (P), Baden (B), Rheingau (RG), and Mittelrhein (MR).

processes. The Cu concentration is not significantly affected by the addition of bentonites to the must in process B or when fining agents are added at a later stage (except in the RH and P wines). In process A, the Cu concentration diminishes in four of five cases after bentonites and fining agents. In the Pfalz wine the loss is 20% and in the other four wines almost 50%. After aging, Cu slightly increases in all cases (except RG 4b). On the whole the copper concentration significantly decreases from the must to the ready wine and is similar after both processes except for RH and P samples. In RH 4b, the Cu concentration is ∼50% higher than in RH 3a, and in P 4b it is ∼50% lower than in P 3a.

Kristl et al. (*4*) also observed a considerable Cu depletion from the must to the wine in white and red Slovenian wines. Trends for the copper concentration in both processes are shown in **Figure 3**.

• Be, Sc, Al, Zr, Y, and the REEs show higher concentrations in the wine than in the must, independent of winemaking processes. Only two cases (RG 4b and MR 3a) showed lower aluminum concentration than in the must. The case of zirconium is illustrated in **Figure 4**.

The difference in metal concentration between different musts is higher than between different wines in both processes. This means that during the winemaking process, the losses or gains of elements seem largely to counterbalance each other independently of the wine origin, and thus similar concentrations in the ready wines are found.

Those elements for which concentration does significantly vary during any of the winemaking process do not present the same changes in all five wines. These changes are not reproducible from wine to wine and therefore cannot be selected as robust elements for origin determination.

Winemaking Process Step by Step. *Must after Clarification with Bentonites.* In process B, the must was clarified with bentonites by addition of calcium bentonite for a single day. In this study, it has been observed that the concentration of the following elements increased by >50%: Be, Al, Zn, Ga, Y, Zr, Nb, La, Ce, Pr, Nd, Dy, Ho, Yb, Hf, Th, and U. Particular attention should be given to the elements Al, Zr, La, Ce, and Pr; their concentrations in some cases go up by a factor of 5−50 times after bentonite addition, depending on the must. Aluminum increases by a factor of 2−10 after bentonite addition. These changes were less pronounced for musts of high aluminum content such as MR and RH wines, leading to final concentrations in the ready wine similar to those in the must. Aluminum is one of the metals most strongly affected by the winemaking process. The fact that the addition of bentonites is a major contributor to the aluminum content has already been reported (*5*). Because the largest part of the metals extracted from bentonites is expected to be complexed by organic acids in must, a possible correlation between must acid content and pH and concentration of these elements in the must has been studied. The MR must, with a total or titratable acidity of 13.4 g/L and a pH of 2.9, and the B must, with an acidity of 6.6 g/L and pH of 3.53, should be the strongest and weakest metal extractors, respectively, but this was not observed. However, it can be seen that at least the elements Al, Y, Zr, Ce, and Pr are extracted to a higher degree from the bentonites by those musts with a lower original concentration on these elements.

In the same way, elements that in must were below the limits of quantification can appear after bentonite contact well above those limits, thus increasing in concentration by >1 order of magnitude. This is the case for Hf, some lanthanides or REEs such as Tb, Er, Tm, Lu, and some actinides such as Th and U. The effect of bentonite addition on the REE content of young wines has already been reported (*7*). Two bentonites of different origin were used for extraction experiments, performed both with a reference wine of low concentration of REEs and with a tartaric acid solution (1%). In both cases an increase of 1−2 orders of magnitude for all of the REEs was observed in the extracts.

Some elements showed an irregular behavior depending on the wine series: Zn decreased in concentration by between 50 and 100% for three of five cases (when the initial concentration in must is >2000 ng/mL) and for the remaining two cases Zn increased; Ba decreased by between 30 and 50% for three of five cases (when the must concentration is >300 ng/mL) and for the remaining two cases increased.

The elements Mg, Ca, Cu, Rb, and Cs seem to be altered by the bentonite addition only to a very small extent (<±20%). Although the bentonites do exchange Ca and Mg ions, the concentration in must is already quite high so it is not significantly altered.

The RG must was submitted to an extra clarification treatment before the fermentation. This clarification step consisted of the addition of small amounts of bentonite, enzymes, active carbon, SO$_2$, and gelatin. RG 0 is the RG must after this process. This treatment did not cause any significant changes in comparison with the other musts, nor were the concentrations appreciably different from those of the other wines after fermentation.

Young Wines after Fermentation and Sulfiting. As a general trend, the metal content in the young wines was lower than in the must. The concentration is reduced to different degrees
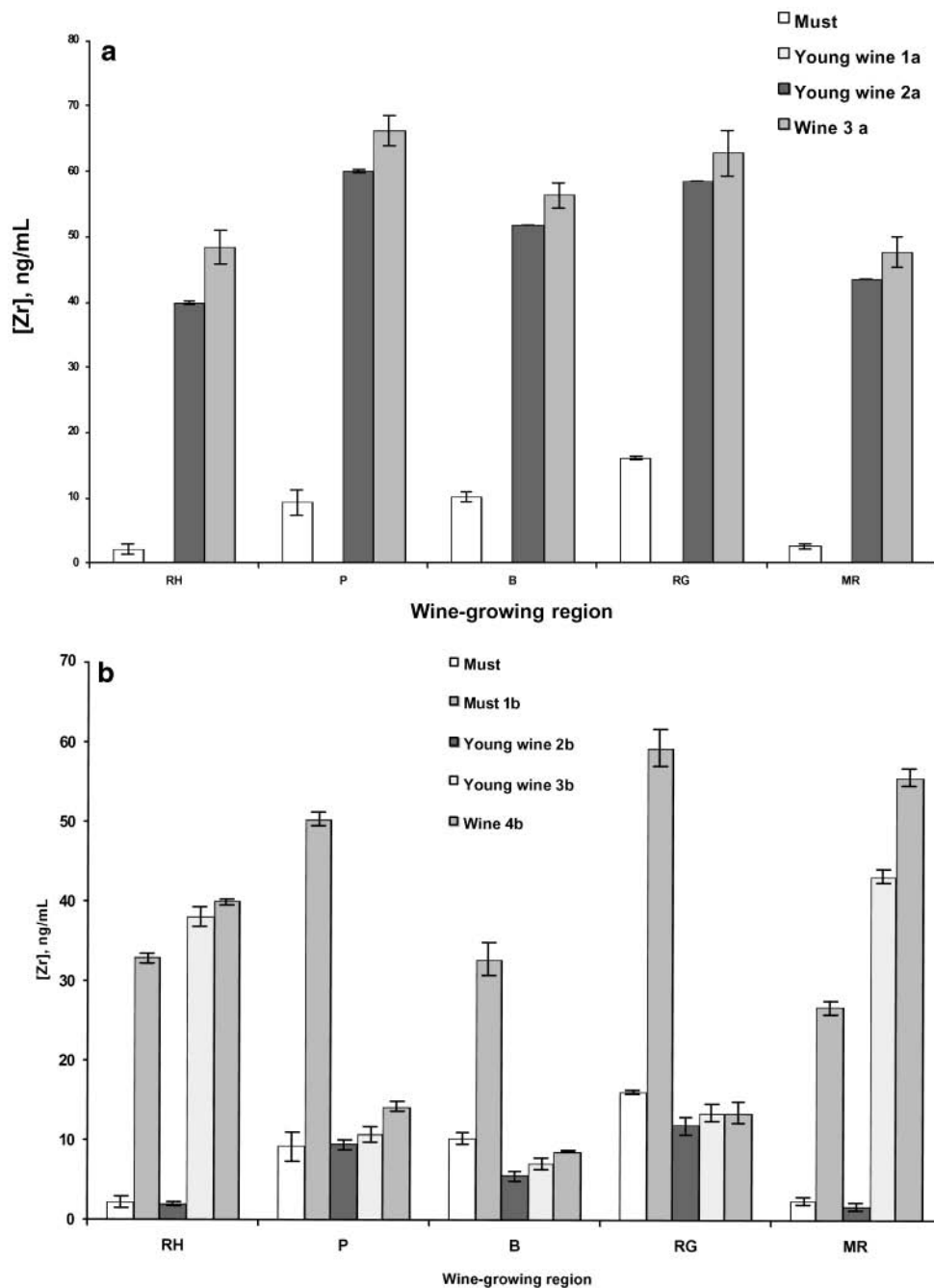
Changes of Metal Composition of German White Wines

J. Agric. Food Chem., Vol. 52, No. 10, 2004 **2959**

Figure 4. Zirconium concentration throughout winemaking processes A (**a**) and B (**b**). Results are shown at each step of the winemaking process and for the wine-growing regions Rheinhessen (RH), Pfalz (P), Baden (B), Rheingau (RG), and Mittelrhein (MR).

depending on the element and occurs at different stages. Some elements are lost to >90% in the fermentation process, namely, Cu, Y, Zr, Nb, Mo, Yb, La, Ce, Pr, Nd, Dy, Ho, Hf, Ta, Th, and U. However, Zr, Nb, Mo, and Yb are less affected in process B, with losses of 50−80%. Ba also decreases in concentration by a factor of 3−5 depending on the wine and on the process. Cu is, as already mentioned (see Changes in Metal Concentrations from Must to Wine), the element that is most severely affected by the winemaking process and can decrease by a factor of 5−20 after fermentation depending on the wine and the winemaking process.

This loss after the fermentation process has two explanations: either the yeast *Saccharomyces cerevisiae* is an effective bioaccumulator of metal ions, which are adsorbed by and eliminated with the yeast lees (*4*), or there is a sudden change in chemical conditions from the must (an aqueous medium) to

the young wine (an alcoholic medium) that leads to the precipitation of different compounds. For example, it is well-known that during the fermentation copper is easily converted to insoluble salts such as CuS and Cu_2S, the formation of which is also favored by the sulfiting agent $K_2S_2O_5$. This may also be the case for other toxic metals such as Cd, Zn, or Pb. In this study, the level of Cd remained below the LOQ, and for Zn and Pb, a reduction of 10−40% (depending on the process) was observed.

The concentration for some elements such as Mn and Fe goes up slightly in some cases. A possible explanation could be the presence of minerals such as CaO or Fe_2O_3 in dry yeast. Normally dry yeasts are supplied with small amounts of minerals that are necessary for their healthy development. The elements Sc and Ti, measured as the isotopes [45]Sc and [49]Ti, seem to increase significantly after fermentation, but this may be an

artifact due to strong interferences of C and S species (mono-isotopic ^{45}Sc has interferences from ^{12}C^{16}O$_2$^{1}H$^+$; and ^{49}Ti, having less interferences by S species, is nevertheless overlapped by ^{32}S^{17}O$^+$ and ^{32}S^{16}O^{1}H$^+$) because through the microwave digestion, the wine carbon content could not be totally eliminated.

No large differences are found in the young wines independent of whether the must was clarified with bentonites before the fermentation or not. The changes are the same in both processes, which is surprising because the concentration of some elements went up 5-fold after bentonite addition. No significant difference between young wines was found independent of the different times of fermentation (process A takes 5 weeks and process B 7 weeks). Also, this difference in contact time of the young wine with yeast and yeast lees during the fermentation does not seem to affect seriously the metal concentration of the young wine.

Young Wine after Fining. In process A fining is understood as the addition of bentonites, gelatin, and a silica solution at the same time. In process B, only gelatin and a silica solution are added at this point because bentonites were already previously utilized. In both processes fining took 7 days.

Aluminum increases by a factor of 2–10 after bentonite addition. In musts with high aluminum content such as MR and RH wines the concentration changed less pronouncedly, the final concentrations in the ready wine being similar to those in the must. Aluminum is one of the metals most strongly affected by the winemaking process. That the addition of bentonites is a major contributor to the aluminum content has already been reported (*7*).

When bentonites are added to the young wine together with the other two fining agents (gelatin and a silica solution), the elements Sc, Li, B, Mg, and Cs are not affected, but the rest of the metals increase their concentrations to different degrees. Especially the elements Zr, Nb, La, Ce, Pr, Nd, Ho, Yb, Hf, and U are enriched by almost 1 order of magnitude, an effect that can be ascribed to the bentonites as was found also for the addition of bentonites to the must.

In process B, most of the elements remain constant or suffer slight changes ($\pm$10%) in concentration. Cu, Zn, Ga, Ba, Yb, La, and Ce are lost to 20–50%.

Wine after Aging. After fining, the samples were filtered and stored in glass containers. In process A, the sample designed as ready wine, wine 3a, was taken after 4 weeks, and in process B the ready wine, wine 4b, was taken after 6 weeks. Slight increases or decreases in the concentrations of some elements are observed. Especially outstanding are increases of >50% for elements such as La, Ce, or Al in some of the process B wines.

Comparison between Ready Wines. There are only a few small differences in metal content between both ready wines, wine 3a and wine 4b. The only significant difference lies in some typical elements associated with the bentonite treatment. In the case of the wine 3a, higher concentrations for the elements Be (except RH and MR), Y, Zr, and Nb (in three of five cases and by a factors of 5 for Zr and 10 for Nb); La (except RG and MR), Ce (by a factor between 2 and 8), or Yb have been observed. Also, some elements such as Pr, Nd, Dy, Ho, Hf, Ta, and U lie over the LOQ in the wine that followed process A. Only aluminum is present in higher concentration among the "b" wines by a factor of 2–3 in comparison with the "a" wines, except for RH and MR wines, in which the concentrations are quite similar.

There are two possible explanations for this fact:
(i) In process A the young wine and not the must is the one

in contact with bentonites, and it could be that due to its different chemical composition (low sugar content, high EtOH content, and high acid content), it can more efficiently extract metals from the bentonites. To our knowledge there are no published studies on bentonite extraction with musts.

(ii) Additionally, in process B the bentonites are in contact with the must for only 24 h (in process A, for 7 days).

CONCLUSIONS

An interpretation of the results presented on these two winemaking processes should take into account that they were performed under ideal experimental conditions of microvinification in glass containers. Commercial operations differ in that (1) the large production scale in comparison with the amounts used here could lead to different results, (2) an additional source of metal contamination is present in the form of brass or stainless steel equipment in the wine cellars, and (3) enological agents (potential pollute agents) other than those used here can be additionally added.

Only a few elements such as Li, B, Mg, Ca, Rb, Cs, and Pb seem to stay nearly constant (changes in concentration of <50%) throughout the winemaking processes and are independent of the time of bentonite addition. In process A, when bentonites are added after the fermentation, additional elements such as Sr, Zn, or Mn remain nearly constant as well; in process B, when bentonites are added at the beginning of the winemaking process, some elements such as V (for P, RG, RH), Co (P, RG, B), or Fe also seem not to be altered by the winemaking process.

There are some elements that change significantly in all investigated wines and processes: the concentrations of Cu and Ba decrease by >1 order of magnitude after the fermentation process. The concentrations of Al, Y, and Zr also decrease by a factor of >10 after fermentation but increase again by 1–2 orders of magnitude after clarification (addition of bentonites) and/or fining. The concentrations of REEs decrease by >1 order of magnitude after fermentation but increase again by 1–2 orders of magnitude after clarification (addition of bentonites) and by almost 1 order of magnitude during aging.

The changes of these elements depend on the winemaking process and also on the wine. Because those variations occur to different extents and are not reproducible from wine to wine, they cannot be selected for origin studies.

In conclusion, it can be said that Li, B, Mg, Ca, Rb, Cs, and Pb are robust elements for studies of origin of German white wines. Their concentrations remain quite constant throughout the winemaking process. Important changes in concentration for some other elements such as V, Mn, Fe, Co, Zn, or Sr seem to be dependent only on the winemaking process and might possibly be used for origin studies as well. Moreover, to judge from the limited number of wines employed in this study, the concentrations of these elements are different from region to region so that they should be investigated as markers for the differentiation between regions.

The studies have been performed in wines only from the vintage 2001, so the study of the vintage-to-vintage variation in the concentration of these elements would be also of great interest before those elements are concluded to be good German regional markers.

All of these 13 elements will be used for provenance studies in German wines in future studies.

LITERATURE CITED

(1) German wine regulation, revised form, from May 14, 2002: Annex 7 to Article 13, paragraph 1, section 2.

(2) Murányi, Z.; Papp, L. "Enological" Metal Speciation Analysis. *Michrochem. J.* **1998**, *60*, 134−142.

(3) Murányi, Z.; Papp, L. Untersuchung der Migration von Makro- und Mikroelementen in einem Egerer (Ungarn) Trauben-Most-Wein-System. I. Die Migration von Na, K, Ca, Mg, P, Fe, Mn und Zn im Trauben-Most-Wein-System von Blaufränkisch, Zweigelt, Riesling und Királyleányka ("Königstöchterchen"). *Vitic. Enol. Sci.* **1996**, *51*, 25−28.

(4) Kristl, J.; Veber, M.; Slekovec, M. The contents of Cu, Mn, Zn, Cd, Cr and Pb at different stages of the winemaking process. *Acta Chim. Slov.* **2003**, *50*, 123−136.

(5) Eschnauer, H. R.; Scollary, G. R. Aluminium in Wein. *Vitic. Enol. Sci.* **1995**, *50*, 24−30.

(6) Eschnauer, H. R.; Scollary, G. R. Zur Önologie und Ökologie von Blei. *Vitic. Enol. Sci.* **1996**, *51*, 6−12.

(7) Jakubowski, N.; Brandt, R.; Stuewer, D.; Eschnauer, H. R.; Görtges, S. Analysis of wines by ICP-MS: Is the pattern of the rare earth elements a reliable fingerprint for the provenance? *Fresenius' J. Anal. Chem.* **1999**, *364*, 424−428.

(8) Ribereau-Gayón, P.; Glories, Y.; Maujean, A.; Dubourdieu, D. *Handbook of Enology; Vol. 1: The Microbiology of Wine and Vinifications; Vol. 2: Stabilization and Treatments*; Wiley: Chichester, U.K., 2000.

(9) German wine regulation, revised form, from May 14, 2002: Annex 5 to Article 12.

(10) Perez-Jordan, M. Y.; Soldevila, J.; Salvador, A.; Pastor, A.; de la Guardia, M. Inductively coupled plasma mass spectrometry analysis of wines. *J. Anal. At. Spectrom.* **1999**, *14*, 33−39.

(11) Sauvage, L.; Frank, D.; Stearne, J.; Millikan, M. B. Trace metal studies of selected white wines: an alternative approach. *Anal. Chim. Acta* **2002**, *458*, 223−230.

(12) Haswell, S. J.; Walmsley, A. D. Multivariate data visualisation methods based on multi-elemental analysis of wines and coffees using total reflection X-ray fluorescence analysis. *J. Anal. At. Spectrom.* **1998**, *13*, 131−134.

(13) Castiñeira, M. M.; Brandt, R.; von Bohlen, A.; Jakubowski, N. Development of a procedure for the multi-element determination of trace elements in wine by ICP-MS. *Fresenius' J. Anal. Chem.* **2001**, *370*, 553−558.

(14) Kwan, W.-O.; Kowalski, B. R.; Skogerboe, R. K. Pattern recognition analysis of elemental data. Wines of *Vitis vinifera* cv. Pinot noir from France and the United States. *J. Agric. Food Chem.* **1979**, *27*, 1321−1326.

(15) Maarse, H.; Slump, P.; Tas, A. C.; Schaefer, J. Classification of wines according to type and region based on their composition. *Z. Lebensm. Unters. Forsch.* **1987**, *184*, 198−203.

(16) Etievant, P.; Schlich, P.; Bouvier, J.-C.; Symonds, P.; Bertrand, A. Varietal and geographic classification of French red wines in terms of elements, amino acids and aromatic alcohols. *J. Sci. Food Agric.* **1988**, *45*, 25−41.

(17) Moret, I.; Scarponi, G.; Cescon, P. Chemometric characterization and classification of five Venetian white wines. *J. Agric. Food Chem.* **1994**, *42*, 1143−1153.

(18) 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.

(19) Latorre, M. J.; Garcia-Jares, C.; Medina, B.; Herrero, C. Pattern Recognition Analysis Applied to Classification of Wines from Galicia (Northwestern Spain) with Certified Brand of Origin. *J. Agric. Food Chem.* **1994**, *42*, 1451−1455.

(20) Pérez-Magariño, S.; Ortega-Heras, M.; González-San José, M. L. Multivariate classification of rosé wines from different Spanish protected designations of origin. *Anal. Chim. Acta* **2002**, *458*, 187−190.

(21) Thiel, G.; Danzer, K. Direct analysis of mineral components in wine by inductively coupled plasma optical emission spectrometry (ICP-OES). *Fresenius' J. Anal. Chem.* **1997**, *357*, 553−557.

(22) Danzer, K.; De la Calle Garcia, D.; Thiel, G.; Reichenbächer, M. Classification of wine samples according to origin and grape varieties on the basis of inorganic trace analysis. *Ann. Lab.* **1999**, *31*, 26−33.

(23) Brescia, M. A.; Caldarola, V.; De Giglio, A.; Benedetti, D.; Fanizzi, F. P.; Sacco, A. Characterization of the geographical origin of Italian red wines based on traditional and nuclear magnetic resonance spectrometric determinations. *Anal. Chim. Acta* **2002**, *458*, 177−186.

(24) Brescia, M. A.; Košir, I. J.; Caldarola, V.; Kidrič, J.; Sacco, A. Chemometric classification of Apulian and Slovenian wines using ^{1}H NMR and ICP-OES with HPICE data. *J. Agric. Food Chem.* **2003**, *51*, 21−26.

(25) Stroh, A.; Brückner, P.; Völlkopf, A. Multielement analysis of wine samples using ICP-MS. *At. Spectrosc.* **1994**, *15*, 100−106.

(26) Augagneur, S.; Medina, B. Determination of rare earth elements in wine by inductively coupled plasma mass spectrometry using a microconcentric nebulizer. *J. Anal. At. Spectrom.* **1996**, *11*, 713−721.

(27) Baxter, M. J.; Crews, H. M.; Dennis, M. J.; Goodall, I.; Anderson, D. The determination of the authenticity of wine from its trace element composition. *Food Chem.* **1997**, *60*, 443−450.

(28) Barbaste, M.; Medina, B.; Sarabia, L.; Ortiz, M. C.; Pérez-Trujillo, J. P. Analysis and comparison of SIMCA models for denominations of origin of wines from the Canary Islands (Spain) builds by means of their trace and ultratrace metals content. *Anal. Chim. Acta* **2002**, *472*, 161−174.

(29) Almeida, C. M.; Vasconcelos, M. T. S. D.; Barbaste, M.; Medina, B. ICP-MS multi-element analysis of wine samples—a comparative study of the methodologies used in two laboratories. *Anal. Bioanal. Chem.* **2002**, *374*, 314−322.

(30) Taylor, V. F.; Longerich, H. P.; Greenough, J. D. Multielement analysis of Canadian wines by inductively coupled plasma mass spectrometry (ICP-MS) and multivariate statistics. *J. Agric. Food Chem.* **2003**, *51*, 856−860.

(31) www.erbsloeh-geisenheim.de.

(32) Pellerin, P.; Doco, T.; Vidal, S.; Williams, P.; Brillouet, J.-M.; O'Neill, M. A. Structural characterization of red wine rhamnogalacturonan II. *Carbohydr. Res.* **1996**, *290*, 183−197.

(33) Pellerin, P.; O'Neill, M. A. The interaction of the pectic polysaccharide rhamnogalacturonan II with heavy metals and lanthanides in wines and fruit juices. *Analusis* **1998**, *26*, M32−M36.

(34) Adriano, D. M. *Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability and Risks of Metals*, 2nd ed.; Springer-Verlag: Berlin, Germany, 2001.

(35) Kabata-Pendias, A.; Pendias, H. *Trace Elements in Soils and Plants*, 3rd ed.; CRC Press: Boca Raton, FL, 2000.

(36) Greenland, D. J.; Hayes, M. H. B. *The Chemistry of Soil Processes*; Wiley: Chichester, U.K., 1981.