A Chemometric Approach to the Comparison of Different Sample Treatments for Metals Determination by Atomic Absorption Spectroscopy in Aceto Balsamico Tradizionale di Modena

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A comparison of different digestion procedures has been carried out for the analysis of metal concentration in samples of vinegars and Aceto Balsamico Tradizionale of Modena (ABTM) coming from an unique barrel set. In particular, classical wet, dry ashing, and closed vessel microwave digestion procedure have been utilized and compared for each investigated species. In a few cases, direct metal determination on ABTM (without treatment procedure) is proposed as possible alternative to sample manipulation. Flame atomic absorption spectrometry was used for the quantification of iron and zinc, while graphite furnace atomic absorption spectrometry was used for all the other elements (i.e., chromium, manganese, cobalt, nickel, copper, cadmium, and lead). The comparison among the different sample treatments was carried out by the use of statistical and chemometric tools. In particular, principal component analysis and ANOVA approaches were used to discriminate between the diverse analytical methods. Furthermore, for all the dissolving techniques, the analytical metal recovery was always evaluated by the application of the recovery function on the same sample matrix. In general, the recoveries were fairly good, ranging from 90 to 103%, except for Cd and Pb with dry ashing, which showed recovery values close to 55% and 67%, respectively. As regards the metals concentration of the investigated samples, the experimental data reveal for some species the presence of concentration slightly over the legal limit fixed for wine and wine vinegar.

KEYWORDS: Metals determination; principal component analysis; ANOVA; microwave; GFAAS; FAAS; ABTM; vinegar

INTRODUCTION

Trace elements are an important quality aspect for foods in general and are receiving increasing scrutiny by all scientists involved with human health and/or nutritional aspects (*1−3*). In fact, toxicologists are concerned with elements that are suspected to be detrimental to human health, while nutritionists are interested in the elements known to be necessary for maintenance of optimum health (*4, 5*). In any case, for both these aspects, people need accurate and precise data to give the best interpretation of the physiological interaction.

Furthermore, the presence of metals characterized by toxic properties in aged food products is always a depreciable event, because in many cases, they could undergo concentration phenomena. Determination of metals in food products represents a quite intriguing work and an attitude of "useful paranoia" toward contamination or accuracy, and precision is a prerequisite for good trace metal data.

Mineralization is an important step when analyzing heavy elements in food products. In fact, mineralization procedures are specific for the method used to determine an element in a particular food matrix, or on the contrary, depend directly on the product (*6, 7*).

Sample digestion represents probably the most important and critical step and is, sometimes, the source of uncertainty and contamination (*8*). There are several possibilities for sample treatment: (i) dry ashing with a muffle furnace and (ii) wet digestion with concentrated acids, bases, or oxidizing agents at ambient pressure or in closed vessels. Some steps of these procedures may lead to contamination errors (i.e., due to the atmospheric pollution of the muffle), loss of analyte (due to formation of volatile compounds, etc.), or to an incomplete mineralization of the organic matrix. For these reasons, the

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Table 1. Operating Parameters for Atomic Absorption Spectrometry

parameter	setting								
metal	Cr[b]	Mn[b]	Co[b]	Ni[b]	Cu[b]	Pb[b]	Cd[b]	Zn[a]	Fe[a]
lamp curr./mA	4	12	20	12	4	7	12	12	25
measur. mode	working curve std add	working curve std add	working curve std add	working curve std add	working curve std add	working curve std add	working curve std add	working curve	working curve
wavelength, nm	357.9	279.5	240.7	232.0	324.8	283.7	228.8	213.9	248.3
signal mode	BKG corr	BKG corr	BKG corr	BKG corr	BKG corr	BKG corr	BKG corr	BKG corr	BKG corr
integration, s	6	5	5	5	5	5	6	5	5
side	platform	platform	platform	platform	platform	platform	platform	flame	flame
slit/nm	0.7	0.2	0.2	0.2	0.7	0.7	0.7	0.7	0.7
flame/furnace	furnace	furnace	furnace	furnace	furnace	furnace	furnace	air/acetylene	air/acetylene
matrix modifier	$50\mu g Mg(NO_3)_2$	$50\mu g Mg(NO_3)_2$	$50\mu g Mg(NO_3)_2$	$50\mu g Mg(NO_3)_2$		$50\mu g PO_4$, 15 μg Pd	$15\mu g$ Pd$(NO_3)_2$		
concd unit	ng g^{-1}	μg g^{-1}	ng g^{-1}	ng g^{-1}	μg g^{-1}	μg g^{-1}	ng g^{-1}	μg g^{-1}	μg g^{-1}

[a] Philips PU9000model. [b] Perkin-Elmer 1100B model.

digestion procedure is probably the limiting factor to the efficiency of recovery.

In addition to the mentioned limitations, these methods are also characterized by an excessive sample manipulation that is very time-consuming. The introduction of microwave digestion systems for laboratory applications has offered enormous advantage in mineralization (*9, 10*) and extraction work (*11, 12*). Since then, many successful studies have been published, dealing with metal determination and/or organic analysis by atomic absorption spectroscopy, ICP-OES, ICP-MS, or gas chromatography in different matrixes. Actually, microwave heating can offer several advantages if compared with traditional wet or dry ashing methods (*13−16*). Microwave technique is rapid, efficient, safe, and reproducible in terms of repeatability of the mineralizing process (amount and energy intensity applied to samples).

The main aim of the present work is the comparison between conventional ashing techniques and microwave digestion for Aceto Balsamico Tradizionale of Modena (ABTM) matrix, in terms of quantity of metal recovered, recovery factor, and time of analysis.

In particular, the concentration of several essential (Co, Cu, Fe, Mn, and Zn) and nonessential (Cd, Ni, Cr, and Pb) elements in vinegar products coming from a unique ABTM casks set has been evaluated by flame atomic absorption spectroscopy (FAAS) or graphite furnace atomic absorption (GFAAS). We were also interested in following the variation of the metals concentration in the vinegar matrix, to gain information about the possibility of concentrating phenomena during the aging period relative to the investigated barrels set.

Because of the particular physical and chemical properties of the vinegars such as high density, high viscosity, high sugar, and organic acid concentration, different matrix effects might be expected, depending on the sample aging period (*6, 17−18*).

Statistical tools such as ANOVA, principal component analysis (PCA), and regression techniques were utilized to compare data coming from the different sample treatments.

For the sake of clarity, it is now necessary to give a few details about the nomenclature utilized throughout the paper. In particular, we use the term ABTM to indicate the marketable ABTM made from cooked must and aged at least 12 years (S1 sample), while the term vinegar is used to indicate any traditional ABTM products aged less than 12 years and therefore not marketable (S2 to S10 samples). In this context, the term sample is used in its broadest meaning, indicating the vinegar matrix as well as the ABTM ones.

EXPERIMENTAL PROCEDURES

Apparatus. All glassware was washed with nitric acid (HNO₃ 50%, v/v) and rinsed with deionized water.

(*a*) *Atomic Absorption Spectrometer.* A Philips Pye Unicam Pu9000, dual beam model with a single-slotted burner head was used to determine Fe and Zn. The instrument is equipped with a Deuterium lamp for the background correction. Flame: air-acetylene with oxidizing (lean blue) flame was used to determine Fe and Zn.

(*b*) *Atomic Absorption Spectrometer.* A Perkin-Elmer 1100B atomic absorption spectrophotometer with a deuterium background correction equipped with an HGA 700 furnace and an AS 70 auto sampler was used to determine Cr, Mn, Co, Ni, Cu, Cd, and Pb.

All the metals were determined using hollow cathode lamps (HCL), and the instrumental conditions are reported in **Table 1**.

(*c*) *Microwave Apparatus.* Commercial oven, model MDS-2000 (CEM Corp.) equipped with Teflon-coated oven cavity, removable 12 position sample carousel, exhaust fan, hose to permit venting of fumes into fume hood, and Teflon ACV type vessels was used for the microwave (MW) digestion of the samples. The instrument is also equipped for the control of temperature and pressure during the mineralization steps.

Before use and between each sample digestion, the vessels were cleaned by adding nitric acid (50% v/v) and heated in microwave.

(*d*) *Acid Dispenser.* A 2−10-mL reagent dispenser (Dispensette model supplied from Brand GMBH, Germany) was used for all the Suprapur reagents.

(*e*) *Electronic Balance.* A Mettler PM4800 Δ-range (Mettler Toledo AG, Greifensee, Switzerland) with a sensitivity of ± 0.01 g was used.

(*f*) *Muffle Furnace.* A commercial electronic muffle furnace, Nannetti PK3 model distributed from Opto-Lab (Italy), capable of varying the temperature from 50 to 1100 °C was used. The ashing temperature was set at 500 °C.

All chemicals were analytical reagent grade. Pure water (ASTM Type IV) produced by a Millipore Q 185 Plus equipment was used whenever water is specified. All other chemicals, Pd(NO₃)₂, Mg(NO₃)₂, NH₄H₂-PO₄, HNO₃ 65%, and H₂O₂ 30% were Suprapur reagents grade and were supplied by Merck. The same firm supplied the atomic absorption reference solutions, (1000 ± 2) μg mL^{-1} of Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, and Pb.

The multi-element reference solution, Certipur Reference Material (Lot no. OC039495, product code 15474 ICP Multi Element Standard I) was also supplied by Merck. All element concentrations refer to NIST Standard Reference Materials and are reported on the supplied Certificate of Analysis.

Matrix Modifier Solutions. The matrix modifier solution for Cd was prepared by diluting with pure water a stock solution of Pd(NO₃)₂ (c(Pd) = 10.0 ± 0.2 g L^{-1}) to a final concentration of 5000 μg mL^{-1} Pd standard. The matrix modifier solution for Co, Mn, and Cr was prepared in the same way by diluting a stock solution of Mg(NO₃)₂ (c(Mg) = 10.0 ± 0.2 g L^{-1}) to a final concentration 10000 μg mL^{-1} Mg(NO₃)₂.

Metals Determination in ABTM

J. Agric. Food Chem., Vol. 52, No. 13, 2004   **4049**

Lead was determined by GFAAS using either $Pd(NO_3)_2$ and $NH_4H_2PO_4$ as matrix modifiers. The $NH_4H_2PO_4$ solution was prepared to a final concentration of 10000 μg mL^{-1} PO$_4^{3-}$.

Preparation of Standards. *Atomic Absorption Working Standards.* Working standards of Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, and Pb were prepared by diluting 1000 μg mL^{-1} stock reference solutions to produce concentrations suitable for the calibration curve of each species. The standards were diluted with 0.2% (v/v) HNO$_3$. Final calibration standards were prepared with the AS70 auto sampler in GFAAS determinations.

Sampling. The samples analyzed in the present work were collected from a unique system of 10 contiguous wooden casks called S1–S10 (barrels set), where S1 contains the oldest vinegar sample (the marketable product) and S10 the youngest one. From each barrel, of different size, different wood, and different product aging, 5 aliquots of 100 mL of product were taken at different depth and homogenized to obtain a bulk sample. Successively, an aliquot of 100 mL of test sample was taken from the bulk sample and stored in polystyrene boxes. Samples were successively stored at 4 °C to prevent any matrix modification such as fermentation or other chemical modifications. Before analysis, samples were kept at room temperature with continuous mixing to return the product to their initial condition.

In particular, going from sample S10 (youngest vinegar) to the marketable ABTM, S1, whose density is greater than 1.30 g mL^{-1}, the water concentration decreases approximately from 70 to 20% w/w (*17*). Thus, each determination was carried out by weight to avoid any volume correction of the final results.

Sample Preparation. *Dry Ashing Technique* (DA). This technique probably represents the most used approach for the desegregation of organic-based sample (*16, 19*).

An aliquot of 3 mL for each sample was weighed into a platinum crucible, placed in a low-temperature oven (105 °C) and heated overnight. The residues obtained after the vaporization of the water and of the most volatile organic compounds were transferred to a high-temperature muffle furnace and ashed at 500 ± 20 °C. The following morning samples were cooled, and the residues were treated with a diluted, 0.2% (v/v), nitric acid with a gentle warming on a hot plate until the ashes become colorless. White ashes were successively dissolved in 2 mL of 10% (v/v) nitric acid, and the resulting solutions were brought to a final weight of 50.00 g with pure deionized water. If necessary, solutions were filtered with a 0.45-μm disposable filter membrane. To improve the precision of the measured metal concentration, all the samples were replicated twice.

Wet Ashing Technique (WA). A 3-mL sample portion was weighed into a 250-mL Pyrex beaker, and concentrated nitric acid (20 mL) was carefully added. Following overnight predigestion at room temperature, samples were placed on a sand bath at (60 ± 10) °C and digested until a dark orange color appeared. The volume was reduced to near dryness by gentle warming. Charring of the samples was avoided, to minimize the possibility of analyte loss. An additional 10–20 mL of concentrated nitric acid was added to sample, and the digestion process was continued until approximately 5 mL of solution remained. The flasks were rinsed with 10–20 mL of deionized water, and 1–2 mL hydrogen peroxide were added to all samples. The solutions were heated for an additional 10–15 min. Mineralization was considered complete when the cooled solution was colorless or slightly pale yellow.

After cooling, the solutions were brought to a final weight of 50.00 g in a polystyrene disposable container. Reagent blanks were always run for each sample treatment. Also, in this case, all the measured samples were prepared in duplicate.

Microwave Digestion (MW). The sample aliquots (2 mL) were accurately weighed into the PFA (Poly-Fluoro-Alkoxy) vessels, and 8 mL concentrated nitric acid and 4 mL hydrogen peroxide were added.

Digestions of the samples were carried out with a multistep program varying the time of exposition and the power of the microwave generated by the klystron. **Table 2** reports the parameters set up on the programmable microwave oven for each step. Optimization of the digestion steps was carried out by considering the trend of the monitored variables, temperature, and pressure inside the control vessel. In fact, as a general rule, the mineralization procedure is considered complete when the pressure reaches its maximum value during the last step of

Table 2. Closed-Vessel Microwave Digestion Program

stage	1 deg step	2 deg step	3 deg step	4 deg step
power/watt %	40	50	60	80
pressure/psi	50	80	150	200
time/min.	10.0	10.0	15.0	10.0
time at P, T	2.00	3.00	3.00	3.00
T/ deg C	120	150	170	200

the digestion program. If this is the case, it is possible to assume that all the organic materials are degraded to water and CO_2. To have the maximum efficiency of the mineralization process during the microwave treatment, the samples were left in the vessels containing the nitric acid overnight. In fact, for organic rich matrixes, the gas evolution during the first minutes of contact between the oxidizing acid mixture and the sample may strongly reduce the capabilities of the microwave closed vessels technique because the system reaches the limiting pressure value already at low temperature. In this case, the mineralization procedure is pressure controlled, and the resulting sample solutions may present untreated materials.

A maximum of six samples was prepared per batch. Each batch consists of five samples and a reagent blank. A cleaning procedure with concentrated nitric acid and hydrogen peroxide was run between two mineralization batches. Maximum digestion temperature of 140–150 °C was reached in the last step of the digestion program outlined in **Table 2**. The cooled samples were transferred to polystyrene containers and brought to a final weight of 50.00 g.

Without Treatment (WT). For a limited number of elements, Cr, Co, Ni, Cu, and Pb, the metal concentration has also been evaluated by using the GFAAS capability on the diluted vinegars.

In this case, after an appropriate dilution of each sample, the solution was inserted in the furnace, and the thermal program was run. With this technique, it is possible to have a direct reading of the metal concentration without any further manipulation of the sample. This approach is available only when the density of the final solution is close to the water value. For all the other cases, a more complicated compensation of the density of the sampling volume of the autosampler is necessary.

Determination. The spectrometers operating parameters are outlined in **Table 1**. The rinse solution used in the AS70 auto sampler was 0.2% HNO$_3$ with the addition of few drops of Triton-×100. Measurements were all performed after instrument calibration with aqueous standards. Sample concentration was calculated from the corresponding regression curves taking into account dilution factors (weight to weight) as well.

The standard addition method was used throughout this work for evaluation of the recovery of the investigated metals (*20, 21*). The main requirements for a correct use of this approach are the absence of any kind of sample contamination, and in general, of additive interference and/or systematic errors that cannot be compensated by the addition technique. Only in this case, it is possible to identify the recovery value with the accuracy of the analytical method (*22, 23*).

The standard addition method was applied on the entire procedure by using four points (the sample and three spiked solutions) on twice replicated samples. In particular, samples for the recovery evaluation were always spiked before digestion. The determination of the recovery function allows the evaluation of the influence of the entire analytical procedure on the experimental data.

At first, the calibration function of the analytical procedure is determined by calibrating with aqueous standards, obtaining the following function:

$$y = a_w + b_w \cdot x_w$$

where y represents the value of the measured property at a concentration (x_w) of the specimen and a_w and b_w are the intercept and the slope of the calibration function, respectively.

Successively, the standard addition technique is applied to the sample of the investigated matrix obtaining the standard addition curve according to the equation

Table 3. Graphite Furnace AAS Temperature Cycle Used for Metal Atomization

metal	Cr				Co				Ni			
step	T (deg C)	ramp (s)	hold (s)	gas flow (mL/s)	T (deg C)	ramp (s)	hold (s)	gas flow (mL/s)	T (deg C)	ramp (s)	hold (s)	gas flow (mL/s)
1	120	10	15	300	120	10	15	300	120	10	15	300
2	150	25	20	300	150	25	25	300	150	25	25	300
3	1650	35	15	300	850	25	10	300	800	20	20	300
4	50	5	5	300	1400	5	10	300	1400	10	10	300
5	2500	0[a]	6	0	2500	0[a]	5	0	2500	0[a]	5	0
6	2700	1	5	300	2700	1	5	300	2750	1	5	300
7	30	5	5	300	30	5	5	300	30	5	5	300

metal	Cu				Mn				Cd				Pb			
step	T (deg C)	ramp (s)	hold (s)	gas flow (mL/s)	T (deg C)	ramp (s)	hold (s)	gas flow (mL/s)	T (deg C)	ramp (s)	hold (s)	gas flow (mL/s)	T (deg C)	ramp (s)	hold (s)	gas flow (mL/s)
1	120	10	15	300	120	10	15	300	120	10	30	300	120	10	30	300
2	150	15	30	300	150	25	25	300	150	25	20	300	150	25	20	300
3	500	10	20	300	650	15	10	300	750	30	20	300	500	10	20	300
4	1000	10	15	300	1350	10	5	300	1750	0[a]	6	300	900	10	20	300
5	2400	0[a]	5	0	2200	0[a]	4	0	2600	1	5	0	1850	0[a]	5	0
6	2700	1	5	300	2600	1	5	300	30	5	5	300	2600	1	5	300
7	30	5	5	300	30	5	5	300					30	5	5	300

[a] Reading step.

$$y_{sa} = a_{sa} + b_{sa} \cdot C_{sa}$$

where y_{sa} is the value of the measured property for the C_{sa} concentration added, and a_{sa} and b_{sa} are the intercept and slope of the standard addition curve. Now, from the y_{sa} value and the aqueous calibration curve, it is possible to calculate the concentration C_{calc} for the spiked samples.

$$C_{calc} = \frac{y_{sa} - a_w}{b_w}$$

if the concentration data C_{calc} are plotted on the ordinate versus the original added concentration data, C_{sa}, on the abscissa, the recovery curve is obtained

$$C_{calc} = a + b\, C_{sa}$$

In the ideal case, the value of the slope b of the regression curve is equal to 1. For all the investigated metals, the spiking solutions were prepared for single element use to avoid any possible interference due to the matrix that should remain constant except for the element being tested (*24*).

The required standards were prepared by the auto sampler, with 4 μL as the minimum volume intake for the GFAAS determinations. Calibrations and instrument performance were verified during the measurement operations on the basis of the correlation coefficient, slope, and characteristic mass, M_0, for each element. A multi-element reference solution, Certipur Reference Material, was used as quality control solution to test the correct functioning of the furnace, spectrometer, and the concentration of the single element solution data. In particular, the calculated characteristic masses were always close to the tabulated values (*25*).

All analyses were performed at least by triplicate firings on duplicate samples. **Table 3** reports the optimized graphite furnace program used for each element. Drying, ashing, and atomizing steps in the heating programs for the investigated metals were optimized to perform measurements directly on the diluted samples. The same procedure was used for the Fe and Zn determinations carried out by flame FAAS technique. Metal concentrations were measured on three replicas of each sample.

Statistical Analysis. The comparison among the different dissolution methods was done by applying different statistical and chemometric tools. Principal component analysis was performed on the matrix of the concentration values of all the analyzed metals, as obtained by the different dissolution methods, measured on each cask from S1 to S10

of the studied barrels set. To compare the within-method variability with the between-methods variability, each series of data (a given metal with a given method) on replicated measurements has been considered as a different variable; this led to a data matrix composed by 64 variables (variables listed in **Tables 4−12** replicated measurements) for each one of the 10 samples, which was autoscaled prior to PCA analysis. Two principal components (PCs) were selected on the basis of the Scree-plot, accounting for the 88.44% of the total variance.

To compare the results obtained from the different dissolution methods for each single metal, both two-way ANOVA with interaction and linear regression analysis have been used (*26*).

ANOVA allows the isolation and estimation of the different sources of variation present in the analyzed data. In our case, two-way-ANOVA with interaction was used to separate the between-casks (relative to the studied barrels set) from the between-methods of dissolution sources of variation, with the aim of evaluating if the effects of the between-methods variation and/or of the variation due to interaction between those two factors were significant. ANOVA has been performed for each analyzed metal separately, thus significant variation effects indicate that for the given metal the results obtained with the diverse dissolution methods are not coincident at the given probability level ($\alpha = 0.05$). This approach allows easily identifying the cases where all the different dissolution methods are consistent, but on the other hand, when significant differences are observed, it is quite complicated to identify the method to which the inconsistency of the data has to be attributed. In fact, the classical multi-comparison methods (e.g., LSD, Tuckey, Duncan, etc) are not easily mastered when, for instance, a significant interaction effect is observed, nor is the analysis of the residuals straightforward when treating a relatively large amount of levels for each factor.

In this case, the comparison between the dissolution methods is better carried out by applying the classical least-squares method, testing if the intercept, a, and the slope, b, of the regression model calculated between each pair of dissolution methods are equal to 0 and 1, respectively (*26*). Obviously, for all the considered regression models, the regression coefficient has been checked to be significantly different from zero.

RESULTS AND DISCUSSION

Tables 4−12 report the experimental results, corrected for the corresponding recovery factor (*27*), together with the error of the determinations relative to each investigated metals in the vinegar (S10 to S2) and ABTM (S1) samples. At first, the
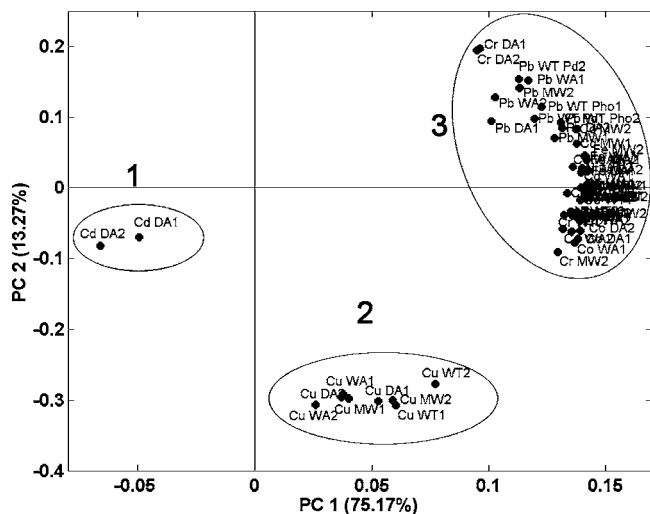
Metals Determination in ABTM

J. Agric. Food Chem., Vol. 52, No. 13, 2004   **4051**

Figure 1. Lodings plot of PC1 vs PC2 obtained by autoscaled PCA matrix relative to the metal concentration values.

Figure 2. Zoom of group 3 of **Figure 1**.
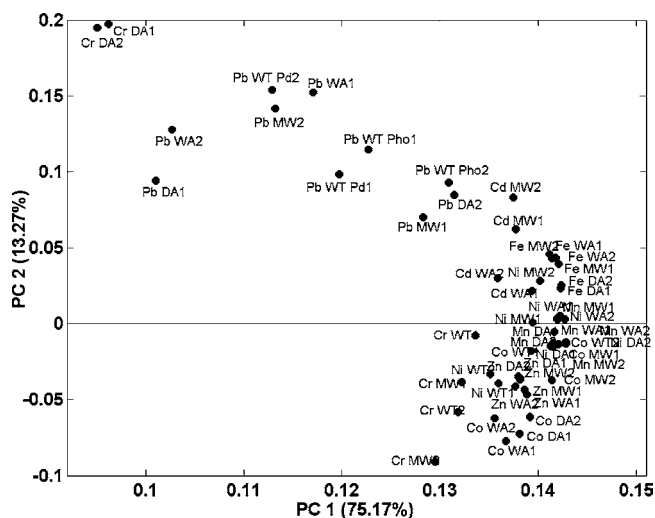

Figure 3. Scores plot of PC1 vs sample number.

Table 4. Chromium Content, Expressed as ng g^{-1}, with the Associated Uncertainty, Determined in the ABTM Samples and Recovery Function Parameters Relatively to Dry Ashing (DA), Microwave (MW), and Without Treatment (WT) Methods

	Cr/ng g^{-1}		
sample	DA	MW	WT
S10	80 ± 15	40 ± 9	40 ± 5
S9	50 ± 20	35 ± 5	30 ± 5
S8	90 ± 10	40 ± 14	30 ± 10
S7	250 ± 25	35 ± 13	35 ± 4
S6	240 ± 20	70 ± 7	80 ± 6
S5	190 ± 16	60 ± 3	60 ± 5
S4	320 ± 25	60 ± 10	55 ± 5
S3	600 ± 20	70 ± 15	80 ± 10
S2	270 ± 20	70 ± 6	80 ± 9
S1	330 ± 20	120 ± 4	125 ± 10

	recovery function		
parameter	DA	MW	WT
$b \pm s_b$	0.99 ± 0.01	0.96 ± 0.01	0.98 ± 0.01
$a \pm s_a$	0.09 ± 0.01	0.10 ± 0.01	0.09 ± 0.01
r^2	0.999	0.999	0.999

overall results will be discussed, then a brief summary for each metal will be given.

From **Figure 1**, reporting the loadings plot of the first two PCs, it is evident that all the variables are grouped in three different regions, namely: Cd data as evaluated by the DA method (marked as group 1); the Cu data obtained by all the dissolution methods (marked as group 2); and all the other variables (marked as group 3). In particular, Cd-DA values are differentiated from the Cu data obtained by the other methods probably because of the inadequacy of the DA method for the determination of this metal. The different position of the Cu data with respect to the other metals of group 3 reflects the slightly oscillating, almost constant, behavior of the concentration of this metal going from barrel S10 to barrel S1. At variance, the group 3 variables show a systematic increase of their concentration with the product aging. Zooming on the variables of group 3, as reported in **Figure 2**, three further subgroups can be observed: Cr measured by DA; all Pb data; and the remaining variables. The different position of these variables in the first two PCs loadings space seems to be due to the more or less marked dependence of the corresponding metal concentration values going from S1 to S10. In fact, PC1 essentially reflects the cask-related variance, as it can be clearly observed in **Figure 3**, where the score values for PC1 are reported versus
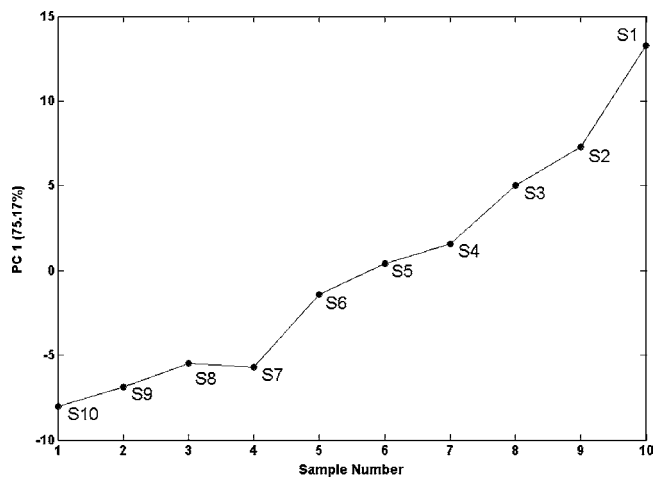
the sample number. This experimental observation will be discussed in detail in the copper section.

The results of both the ANOVA and linear regression analyses are reported in **Table 13**, where for the sake of clearness, all the significant differences according to Student's *t*-test for *a* and *b* and all the significant effects of the between-methods and interaction variation sources have been reported in bold style. The results obtained with the two different statistical techniques are coherent; in fact, significant effects (between-methods and/or interaction), according to two-way ANOVA, always correspond to significant differences in some of the corresponding couples of variables, according to Student's *t*-test applied to the regression parameters. The only discrepancy concerns the Mn data. In fact, according to ANOVA, no significant effects are present, while the regression analysis shows a systematic difference between the MW and the DA methods ($a \neq 0$). Anyway, it has to be told that this is not a marked difference, because the calculated *t* value is very close to the tabulated one ($t_{calc} = 2.5$, $t_{tab} = 2.1$).

Chromium (Table 4). The data reported in **Table 4** show that the evaluated metal concentration is always in the ng g^{-1} range for the aged product too (i.e., the ABTM marketable balsamic vinegar). Data evaluated for this particular casks set show an increase of the metal concentration with the increasing

4052 *J. Agric. Food Chem.,* Vol. 52, No. 13, 2004

Cocchi et al.

Table 5. Manganese Content, Expressed as μg g^{-1} with the Associated Uncertainty, Determined in the ABTM Samples and Recovery Function Parameters Relative to Dry Ashing (DA), Wet Ashing (WA), and Microwave (MW) Methods

	Mn/μg g^{-1}		
sample	DA	WA	MW
S10	2.0 ± 0.2	2.1 ± 0.2	2.1 ± 0.2
S9	2.5 ± 0.2	2.5 ± 0.2	2.5 ± 0.2
S8	3.1 ± 0.1	3.0 ± 0.1	3.2 ± 0.2
S7	3.1 ± 0.1	3.1 ± 0.2	3.2 ± 0.1
S6	3.5 ± 0.1	3.6 ± 0.1	3.7 ± 0.2
S5	4.5 ± 0.1	4.4 ± 0.2	4.4 ± 0.2
S4	4.1 ± 0.2	4.3 ± 0.1	4.2 ± 0.2
S3	5.5 ± 0.1	5.6 ± 0.2	5.5 ± 0.1
S2	6.6 ± 0.2	6.6 ± 0.2	6.5 ± 0.1
S1	8.0 ± 0.1	7.9 ± 0.1	7.9 ± 0.1
	Recovery Function		
parameter	DA	WA	MW
$b \pm s_b$	0.99 ± 0.04	1.02 ± 0.03	1.01 ± 0.02
$a \pm s_a$	6.6 ± 0.1	6.4 ± 0.2	6.5 ± 0.2
r^2	0.999	0.996	0.990

Table 6. Iron Content, Expressed as μg g^{-1} with the Associated Uncertainty, Determined in the ABTM Samples and Recovery Function Parameters Relatively to Dry Ashing (DA), Wet Ashing (WA) and Microwave (MW) Methods

	Fe/μg g^{-1}		
sample	DA	WA	MW
S10	10.0 ± 0.5	9.0 ± 0.5	6.1 ± 0.5
S9	16.0 ± 0.5	20.0 ± 1.0	15.0 ± 1.0
S8	35.0 ± 1.0	33.0 ± 1.0	31.2 ± 1.0
S7	34.0 ± 0.5	38.0 ± 2.0	33.0 ± 1.5
S6	55.0 ± 1.0	69.0 ± 1.5	54.0 ± 1.0
S5	67.0 ± 1.0	88.0 ± 1.0	69.0 ± 1.0
S4	80.0 ± 0.5	97.0 ± 1.0	80.0 ± 1.0
S3	86.0 ± 1.5	117 ± 1.0	91.0 ± 1.0
S2	97.0 ± 1.0	117 ± 1.0	94.0 ± 1.0
S1	125 ± 1.0	156 ± 1.0	121 ± 1.0
	Recovery Function		
parameter	DA	WA	MW
$b \pm s_b$	0.85 ± 0.02	0.77 ± 0.03	0.86 ± 0.01
$a \pm s_a$	66 ± 3	94 ± 3	66 ± 1
r^2	0.999	0.997	0.999

age of the vinegars of roughly of a factor three. This probably only reflects the natural loss of water occurring during the aging period (*28*, *29*), as already described in the literature (*17*, *30*). As regards the different analytical procedures, all the methods give good recovery values as testified by the slope data ($b \pm s_b$) of the recovery function (**Table 4**). **Table 13** shows that both by ANOVA and regression approaches, (*26*) the dry ashing digestion treatment (DA) always gives different results when compared with the MW and WT ones. Although the difference between the MW and WT is statistically significant, the t_{calc} values for both slope and intercept are very close to the tabulated one (i.e., $t_{b=1} = 2.6$, $t_{a=0} = 2.3$, and $t_{tab} = 2.1$). In the case of trace and ultra trace metals, as for Cr determination, owing to the peculiar chemical and physical properties of the ABTM matrix to some extent, high errors associated to the analytical determinations have to be expected. The high dilution factor employed in the WT procedure (needed to lower the density and the viscosity of the samples) and the low mass introduced in the closed vessels for the MW technique are the main limiting factors of the analytical procedures.

Manganese (Table 5). Manganese concentration is in the trace range, and from sample S10 to sample S1, it is possible to observe a concentration factor of 4, (loss of water of the product) (*17*). All the analytical methods are characterized by a high recovery value, confirming the good capabilities of all the pretreatment approaches for the investigated metal, **Table 5**. Moreover, the analytical data are also statistically equivalent according to ANOVA.

Iron (Table 6). This metal was investigated by flame AAS. Owing to this fact, the WT data are missing. In fact, the dilution factor that would have been needed to lower the density and viscosity of the oldest samples would have led to a concentration value of the iron lower than the detection limit of the technique. All the methods are characterized by good recovery ranging from 0.8 (WA treatment) to 0.9 (DA and MW treatments).

Table 13 shows that the WA method lies outside the confidence limits of the *t*-test and gives significant different results when compared with the DA and MW. The concentration factor (order of 10) passing from sample S10 to S1 is significantly higher than the one observed in the case of the previous metals Cr and Mn, and therefore the loss of water alone seems insufficient to give reason of this aspect. The formation

Table 7. Cobalt Content, Expressed as ng g^{-1} with the Associated Uncertainty, Determined in the ABTM Samples and Recovery Function Parameters Relatively to Dry Ashing (DA), Wet Ashing (WA), Microwave (MW), and Without Treatment (WT) Methods

	Co/ng g^{-1}			
sample	DA	WA	MW	WT
S10	3 ± 5	18 ± 6	7 ± 2	2 ± 5
S9	7 ± 2	19 ± 1	7 ± 1	14 ± 1
S8	10 ± 1	15 ± 2	12 ± 1	16 ± 1
S7	12 ± 1	19 ± 1	10 ± 1	11 ± 2
S6	20 ± 3	25 ± 1	16 ± 2	19 ± 3
S5	26 ± 3	40 ± 1	21 ± 2	33 ± 2
S4	26 ± 1	32 ± 1	23 ± 4	28 ± 2
S3	30 ± 4	35 ± 2	25 ± 2	37 ± 3
S2	34 ± 1	49 ± 3	36 ± 2	41 ± 2
S1	67 ± 1	72 ± 6	45 ± 2	61 ± 2
	Recovery Function			
parameter	DA	WA	MW	WT
$b \pm s_b$	1.12 ± 0.02	0.96 ± 0.02	1.02 ± 0.09	1.00 ± 0.02
$a \pm s_a$	0.016 ± 0.001	0.011 ± 0.001	0.015 ± 0.008	0.021 ± 0.002
r^2	0.990	0.990	0.990	0.995

of stable complexes in solution could be the most probable explanation for this concentration increasing. In fact, the high values of the stability constants of the iron-ligands species (mainly organic acids) permit an increase of the quantity of metal in solution also at low pH. Furthermore, the increase of the total acidity of the product (*30*) (mainly tartaric, citric, and malic acids) is in good agreement with this observation.

Cobalt (Table 7). Cobalt is present in vinegar and ABTM samples at ultra trace level. ANOVA indicates as significant both between-methods and interaction terms, outlining the discrepancies existing among digestion methods. The comparison of each pairs of methods by regression shows that only DA-WT methods are equivalent. Whatever the correct method may be, it appears clear that the whole analytical approach should be revised to account for the low metal concentration for this element.

Nickel (Table 8). All the methods give good recovery values for Ni. As far as the increasing concentration of the metal in this series of samples is concerned, a concentration factor of 4

Metals Determination in ABTM

J. Agric. Food Chem., Vol. 52, No. 13, 2004   **4053**

Table 8. Nickel Content, Expressed as ng g^{-1} with the Associated Uncertainty, Determined in the ABTM Samples and Recovery Function Parameters Relative to Dry Ashing (DA), Wet Ashing (WA), Microwave (MW), and Without Treatment (WT) Methods

	Ni/ng g^{-1}			
sample	DA	WA	MW	WT
S10	60 ± 5	110 ± 5	140 ± 20	90 ± 10
S9	50 ± 5	130 ± 5	110 ± 15	70 ± 10
S8	80 ± 6	150 ± 10	130 ± 10	60 ± 10
S7	80 ± 6	160 ± 12	140 ± 10	100 ± 15
S6	120 ± 10	240 ± 15	200 ± 20	150 ± 12
S5	140 ± 10	250 ± 15	260 ± 20	110 ± 10
S4	140 ± 14	280 ± 20	280 ± 20	140 ± 10
S3	210 ± 18	320 ± 22	310 ± 25	220 ± 15
S2	280 ± 20	420 ± 25	430 ± 30	310 ± 20
S1	330 ± 25	460 ± 30	450 ± 30	360 ± 25

	Recovery Function			
parameter	DA	WA	MW	WT
$b \pm s_b$	0.94 ± 0.02	1.03 ± 0.02	0.98 ± 0.02	0.99 ± 0.03
$a \pm s_a$	0.11 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	0.14 ± 0.01
r^2	0.99	0.998	0.997	0.994

Table 9. Copper Content, Expressed as μg g^{-1} with the Associated Uncertainty, Determined in the ABTM Samples and Recovery Function Parameters Relative to Dry Ashing (DA), Wet Ashing (WA), Microwave (MW), and Without Treatment (WT) Methods

	Cu/μg g^{-1}			
sample	DA	WA	MW	WT
S10	2.4 ± 0.1	2.8 ± 0.1	2.4 ± 0.2	2.5 ± 0.1
S9	2.8 ± 0.1	3.3 ± 0.1	2.7 ± 0.2	2.6 ± 0.2
S8	2.4 ± 0.1	3.0 ± 0.2	2.5 ± 0.1	2.3 ± 0.1
S7	2.2 ± 0.1	2.7 ± 0.1	2.2 ± 0.3	2.2 ± 0.1
S6	2.3 ± 0.1	3.2 ± 0.1	2.3 ± 0.1	2.4 ± 0.1
S5	1.9 ± 0.1	2.7 ± 0.1	2.0 ± 0.1	2.3 ± 0.1
S4	2.9 ± 0.1	3.3 ± 0.1	2.4 ± 0.3	2.5 ± 0.2
S3	1.8 ± 0.2	2.1 ± 0.1	1.9 ± 0.1	1.9 ± 0.2
S2	2.3 ± 0.2	2.5 ± 0.1	2.6 ± 0.1	2.5 ± 0.1
S1	3.4 ± 0.1	4.0 ± 0.1	3.5 ± 0.1	3.6 ± 0.1

	Recovery Function			
parameter	DA	WA	MW	WT
$b \pm s_b$	0.96 ± 0.01	0.912 ± 0.001	1.00 ± 0.01	0.95 ± 0.02
$a \pm s_a$	2.53 ± 0.03	2.78 ± 0.03	2.7 ± 0.1	2.9 ± 0.1
r^2	0.999	0.999	0.999	0.997

Table 10. Zinc Content, Expressed as μg g^{-1} with the Associated Uncertainty, Determined in the ABTM Samples and Recovery Function Parameters Relative to Dry Ashing (DA), Wet Ashing (WA), and Microwave (MW) Methods

	Zn/μg g^{-1}		
sample	DA	WA	MW
S10	5.0 ± 0.5	6.0 ± 0.5	6 ± 0.5
S9	13.0 ± 0.5	13.0 ± 0.5	12 ± 0.5
S8	12.0 ± 1.0	13.0 ± 0.5	12 ± 1.0
S7	13.0 ± 1.0	13.0 ± 0.5	12 ± 0.5
S6	16.0 ± 0.5	16.0 ± 0.5	16 ± 0.5
S5	19.0 ± 0.5	19.0 ± 0.5	17 ± 0.5
S4	18.0 ± 0.5	19.0 ± 0.5	19 ± 0.5
S3	29.0 ± 0.5	31.0 ± 0.5	30 ± 0.5
S2	43.0 ± 0.5	43.0 ± 1.0	44 ± 1.0
S1	50.0 ± 0.5	54.0 ± 0.5	50 ± 0.5

	Recovery Function		
parameter	DA	WA	MW
$b \pm s_b$	0.96 ± 0.04	0.95 ± 0.04	0.98 ± 0.04
$a \pm s_a$	15 ± 1	17 ± 1	15 ± 2
r^2	0.997	0.996	0.997

among the different analytical digestion procedures while all effects tested by ANOVA are significant, the comparison by regression method indicates that only the MW-WA ($t_b = 2.5$, $t_a = 1.1$, $t_{tab} = 2.1$) are statistically different, see **Table 13**. As regards the concentration of Cu in the studied samples, it is almost constant, passing from the youngest vinegar, S10, to the oldest ABTM S1, at variance with the trend observed for all the other metals that, to a different extent, underwent concentration phenomena. The presence of an almost constant amount of copper could be tentatively explained by considering the possibility of precipitation phenomena induced by the progressive loss of water.

Zinc (Table 10). At first glimpse, the recovery shows a good agreement between the three methods. The statistical comparison carried out both by ANOVA and the regression method highlights the scarce agreement between the data obtained with WA-DA and MW-WA methods, as reported in **Table 13**. Furthermore, contrary to expectations, the DA method gives very good results if compared with the MW ones, although in some cases, it is possible to have loss of analyte during the drying processes. Although zinc is present at high concentration in sample S1 (old ABTM), even considering a daily intake of tenths of grams of product, the metal assumption is extremely lower than the 14 mg/day reported in the literature as the dietary daily intake value for human health (*32*).

Cadmium (Table 11). Cadmium is a toxic metal for human health, and the determination of its concentration in foods and environment represents a quite intriguing task. **From Table 11**, some considerations may be drawn, taking into account either the metal concentration or the trend through this series of casks. As regards the metal concentration, Cd is present in the ultra trace range, and even in the oldest sample, S1, its concentration may be considered under the risk level of disease for human body. In fact, the daily intake via food has been well documented (*33*), and besides local individual variations depending on habits, the mean value ranges between 10 and 30 μg/day.

Actually, considering a daily intake of a few mL, 1−2 mL, of ABTM, the assumption of Cd would be about 40−80 ng/day, which is an extremely low quantity.

The trend observed along this particular casks set from the youngest to the oldest product increase with a concentration

going from sample S10 to S1 due to the loss of water is observed. **Table 8** shows a different behavior of the metal depending on the condition of disgregation: dry or wet ashing. In fact, both the DA and WT approaches always give lower values for the same sample when compared with the WA and MW methods. The statistical comparison (**Table 13**) shows that the MW-WA and DA-WT methods are equivalent and all the other methods are significantly different. A tentative explanation of this fact may be the formation of volatile compounds during the ashing of the organic matrix at temperature above 500 °C. The use of modifiers is not strictly necessary when atomizing Ni from l'Vov platform (because the Ni determination is virtually free of interference under STPF conditions) (*25, 31*), and the use of 50μg of Mg(NO$_3$)$_2$ as matrix modifier has not given any improvement of the experimental results. Similar considerations may be drawn for DA determinations.

Copper (Table 9). The statistical parameters of the recovery function confirm that all the tested digestion procedures give good analytical results. Considering the statistical comparison

Table 11. Cadmium Content, Expressed as ng g^{-1} with the Associated Uncertainty, Determined in the ABTM Samples and Recovery Function Parameters Relative to Dry Ashing (DA), Wet Ashing (WA), and Microwave (MW) Methods

	Cd/ng g^{-1}		
sample	DA	WA	MW
S10	7.0 ± 1.0	3.0 ± 0.5	3.0 ± 0.5
S9	5.0 ± 0.5	6.0 ± 1.0	5.0 ± 1.0
S8	3.0 ± 0.5	9.0 ± 1.0	8.0 ± 3.0
S7	3.0 ± 0.5	7.0 ± 1.0	8.0 ± 2.0
S6	4.0 ± 0.5	21.0 ± 1.0	19.0 ± 1.0
S5	4.0 ± 0.5	20.0 ± 1.5	22.0 ± 1.0
S4	5.0 ± 0.5	15.0 ± 1.0	18.0 ± 1.0
S3	3.0 ± 0.5	23.0 ± 2.0	27.0 ± 3.0
S2	3.0 ± 0.5	29.0 ± 2.0	29.0 ± 1.0
S1	3.0 ± 0.5	33.0 ± 3.0	31.0 ± 1.0

	Recovery Function		
parameter	DA	WA	MW
$b \pm s_b$	0.55 ± 0.02	0.93 ± 0.02	0.98 ± 0.02
$a \pm s_a$	(64 ± 1)10^{-4}	(102 ± 1)10^{-4}	(124 ± 3)10^{-4}
r^2	0.990	0.994	0.98

factor of 10 times. Cadmium undergoes a concentration effect greater than the one due to a simple loss of water, and to a different extent, similar to the trend of Zinc. As concerns the analytical aspects of the metal determination, the WA and MW methods give good recovery values, as reported in **Table 11**. Regarding the DA method, the low calculated recovery confirms the inadequacy of the dry ashing technique in the determination of metals characterized by a high vapor pressure such as Cd. The inadequacy of the DA approach is also confirmed by the experimental data evaluated on the vinegar sample. In fact, as shown by the PCA analysis, **Figure 1**, and as reported in **Table 11**, the DA values are always lower than those determined with WA and MW methods, although the experimental data have been corrected for the analytical recovery of the method. The loss of Cd observed with the DA approach is not completely evaluated by the recovery function correction, confirming the high influence of the sample matrix in the whole analytical method. The results of the statistical analysis reported in **Table 13** relative to the methods of comparison for Cd still confirms the experimental evidences.

Lead (Table 12). Lead is known for its toxicity effects in living organisms. Depending on its chemical form, either organic or inorganic, different toxic effects and different organs of the human body are concerned and involved with this heavy metal (*34*). Many effects due to Pb presence in foods occur at levels of 15−50 μg of Pb/dL blood. As a matter of fact, it is not clear what level of Pb in blood has no effect. For these reasons, health concerns regarding presence and concentration of lead in grape-derived products, such as ABTM, have been raised enormously during the last years. **Table 12** reports the metal concentration corrected for the analytical recovery and evaluated by the different approaches.

From the data, it is possible to observe that the metal concentration is somewhat higher with respect to the reported law limit, at least for the older samples. In fact, the lead concentration ranges from 90 to 730 μg kg^{-1}. The metal concentration of the elder ABTM sample is at least two times higher than the stated law limit set at 300 μg kg^{-1}. It is possible to justify this relatively high concentration of lead on the basis of the natural loss of water occurring during the aging period in the barrel set.

As reported in **Table 12**, when the WT method was applied, different matrix modifiers (i.e., palladium nitrate (Pd(NO$_3$)$_2$) and ammonium di-hydrogen phosphate (NH$_4$H$_2$PO$_4$)) were tested. In fact, different effects should be expected during atomization due to the sample matrix, and therefore, different modifiers were tested to get the lowest background interference on the analytical signal. The statistical comparison between the different dissolving techniques shows a significant between-methods variance (ANOVA test). On the other hand, the comparison by regression analysis shows agreement among all the methods pairs, with the exception of the WT(PO$_4$$^{3-}$)-WTPd(NO$_3$)$_2$ ($t_b = 3.86$, $t_{tab} = 2.1$). The DA approach, as expected, gives a poor analytical recovery value, but contrary to the cadmium determinations, the calculated value is less dependent from the Pb concentration, and therefore, the correction of the experimental data by the recovery is more efficient. In fact, the lead concentrations evaluated with the DA approach on the S1−S10 series are closer to the ones of the other methods.

The results of this study show the importance of the digestion techniques of organic samples when determining metals at trace and ultra trace levels. In particular, closed vessel microwave

Table 12. Lead Content, Expressed as μg g^{-1} with the Associated Uncertainty, Determined in the ABTM Samples and Recovery Function Parameters Relative to Dry Ashing (DA), Wet Ashing (WA), Microwave (MW), Without Treatment with Phospate Modifier (WT/(PO$_4$$^{3-}$)), and Without Treatment with Palladium Modifier (WT/(Pd)) Methods

	Pb/μg g^{-1}				
sample	DA	WA	MW	WT/(PO$_4$$^{3-}$)	WT/(Pd)
S10	0.16 ± 0.06	0.32 ± 0.04	0.24 ± 0.01	0.21 ± 0.02	0.25 ± 0.03
S9	0.09 ± 0.02	0.27 ± 0.12	0.21 ± 0.01	0.22 ± 0.02	0.24 ± 0.04
S8	0.21 ± 0.02	0.32 ± 0.22	0.18 ± 0.00	0.31 ± 0.03	0.27 ± 0.02
S7	0.26 ± 0.12	0.21 ± 0.04	0.18 ± 0.05	0.20 ± 0.01	0.19 ± 0.04
S6	0.42 ± 0.11	0.30 ± 0.07	0.28 ± 0.01	0.35 ± 0.02	0.32 ± 0.03
S5	0.38 ± 0.03	0.50 ± 0.05	0.54 ± 0.20	0.45 ± 0.04	0.40 ± 0.01
S4	0.59 ± 0.12	0.57 ± 0.01	0.56 ± 0.02	0.63 ± 0.03	0.53 ± 0.04
S3	0.49 ± 0.03	0.73 ± 0.00	0.58 ± 0.12	0.68 ± 0.04	0.59 ± 0.06
S2	0.45 ± 0.01	0.62 ± 0.27	0.52 ± 0.02	0.54 ± 0.06	0.43 ± 0.03
S1	0.53 ± 0.02	0.61 ± 0.04	0.56 ± 0.02	0.64 ± 0.04	0.49 ± 0.04

	Recovery Function				
parameter	DA	WA	MW	WT/(PO$_4$$^{3-}$)	WT/(Pd)
$b \pm s_b$	0.67 ± 0.01	0.91 ± 0.01	1.01 ± 0.03	0.97 ± 0.03	1.01 ± 0.03
$a \pm s_a$	0.17 ± 0.01	0.31 ± 0.01	0.30 ± 0.02	0.26 ± 0.1	0.29 ± 0.01
r^2	0.999	0.997	0.998	0.990	0.990

Metals Determination in ABTM

J. Agric. Food Chem., Vol. 52, No. 13, 2004   **4055**

Table 13. Results of the Statistical Comparison, Two-Way ANOVA, and Linear Regression Tests for the Reported Analytical Methods

Compared Methods	Element							
	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd
MW-DA	$b \neq 1$	$b = 1$	$b = 1$	$b \neq 1$	$b \neq 1$	$b = 1$	$b = 1$	$b \neq 1$
	$a \neq 0$	$a \neq 0$	$a = 0$	$a \neq 0$	$a \neq 0$	$a = 0$	$a = 0$	$a \neq 0$
MW-WA		$b = 1$	$b \neq 1$	$b \neq 1$	$b = 1$	$b \neq 1$	$b \neq 1$	$b = 1$
		$a = 0$	$a = 0$	$a = 0$	$a = 0$	$a = 0$	$a = 0$	$a = 0$
MW-WT	$b \neq 1$			$b \neq 1$	$b = 1$	$b = 1$		
	$a \neq 0$			$a = 0$	$a \neq 0$	$a = 0$		
DA-WT	$b \neq 1$			$b = 1$	$b = 1$	$b = 1$		
	$a = 0$			$a = 0$	$a = 0$	$a = 0$		
WA- WT				$b = 1$	$b = 1$	$b = 1$		
				$a \neq 0$	$a \neq 0$	$a = 0$		
WA-DA		$b = 1$	$b \neq 1$	$b = 1$	$b \neq 1$	$b = 1$	$b \neq 1$	$b \neq 1$
		$a = 0$	$a = 0$	$a \neq 0$	$a \neq 0$	$a = 0$	$a = 0$	$a \neq 0$
			two way ANOVA[a]					
betw-meth	sign.	no sign.	sign.	sign.	sign.	sign.	sign.	sign.
interaction	sign.	no sign.	sign.	sign.	sign.	sign.	sign.	sign.

Compared Methods	Pb
MW-DA	$b = 1$
	$a = 0$
MW-WA	$b = 1$
	$a = 0$
MW-WT(PO_4^{3-})	$b = 1$
	$a = 0$
MW-WTPd(NO_3)$_2$	$b = 1$
	$a = 0$
DA-WT(PO_4^{3-})	$b = 1$
	$a = 0$
DA-WTPd(NO_3)$_2$	$b = 1$
	$a = 0$
WA-WT(PO_4^{3-})	$b = 1$
	$a = 0$
WA-WTPd(NO_3)$_2$	$b = 1$
	$a = 0$
WA-DA	$b = 1$
	$a = 0$
WT(PO_4^{3-})-WTPd(NO_3)$_2$	$b \neq 1$
	$a = 0$
two way ANOVA[a]	
betw-meth	sign.
interaction	no sign.

[a] The effect of between-barrels (**S10** to **S1**) variance is not reported because it is always significant (significance values range between 6.4E−53 for Fe and 3.8E−26 for Cd).

digestion technique has always shown good recovery values relative to the investigated metals.

On the other hand, the main limits of the closed vessel approach are the low mass of sample that may be processed and consequently the high pressure generated by the organic samples during the digestion process. However, the possibility of repeated analyses and the drastic reduction of process times are elements that contribute to the large diffusion of this technique in the chemical and biological laboratories. Although at the moment it is not possible to perform a comparison among the different methods on the basis of a reference procedure or to make a method validation by using a certified reference material (which is lacking), both PCA and linear regression analysis suggested that the MW and WT sample treatment methods seem to be the most reliable ones.

On the basis of these results, our future interests in this research field will be mainly focused on the metals determination in different casks set, for example, sample replications, to evaluate if the "concentration effect" observed in this particular *batteria* has to be considered as a "product characteristic" or not. While the ABTM matrix undergoes a concentration process due to water evaporation to a final volume of roughly one-third of the starting one, some metals (Fe, Cu, and Cd) follow a different dynamic. Although it is quite difficult to investigate the chemical equilibria of these elements in the vinegar matrix due to the presence of several species such as organic acids, sugars polyphenols, furfurals, etc., among different hypotheses, we may suggest that some metals may be present in solution in different chemical forms, as complexes or free ions, and therefore a different behavior of the species should be expected as well.

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