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Differentiation and classification of beers with flame atomic spectrometry and molecular absorption spectrometry and sample preparation assisted by microwaves[☆,☆☆]

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Abstract

The characterization of beer samples has a lot of interest because their composition can affect the taste and stability of beer and consumer health. Flame atomic absorption spectrometry was used to determine Fe, Mn, Zn, Cu, Mg, Ca and Al. Sodium and K were determined by flame atomic emission spectrometry. A sample preparation method was developed, based on treatment with HNO_3 and H_2O_2 in a microwave oven. This has many advantages over the methods found in the literature. The combination of the results of atomic spectrometry and the spectrum obtained by molecular absorption spectrometry provides information on the inorganic and organic components of the samples. The application of chemometric techniques to chemical composition data could be extremely useful for food quality control. The metal concentrations, the molecular absorption spectrum, the pH and conductivity of each sample were subject to analysis of variance and linear discriminant analysis. Twenty-five different beer samples were used to differentiate and classify different types of beers. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Beer; Microwave digestion; Flame atomic absorption spectrometry; Flame emission; Molecular absorption; Chemometrics

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[☆☆] Dedicated to the memory of Dr. J. Hernandez-Cañavate.

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1. Introduction

Beer drinking has been steadily increasing in recent decades even in countries where alcoholic beverages are not traditional. Beer has indeed become an international drink, especially among young people [1]. Furthermore, it is now being recognised that there may be some health benefits associated with moderate consumption of beer. The health implications of trace elements are now well recognised and trace element concentrations are subject to legislation [2]. Trace metals in beers may originate from natural sources (soil, water, cereal, hops and yeast) as well as from environmental contamination, fertilisers, pesticides, industrial processing and containers [3]. There are several reasons for the determination of trace metals in beers. They are essential or toxic in the human body and they can also influence the brewing process [2]. Moreover, the compounds responsible for the flavour of beer are most likely oxidation products which have been linked to the presence of oxygen and metal ions [4] such as Fe and Cu. On the other hand, beer composition is very complex and many parameters need to be known for complete characterization. However, it may be enough to determine only some of these parameters for classification and differentiation. Trace metal elements have recently been found to be good indicators for determining wine origin [5].

Flame atomic spectrometry is one of the most useful techniques for trace metal analysis thanks to high sensitivity and selectivity, good accuracy, adequate precision, large element coverage, moderately priced equipment and a well-established methodology [6]. Direct aspiration of the beer causes flame fluctuations and solid deposits on the burner head. Various authors have suggested the need for sample pretreatment [3,7–9]. The digestion procedures entail the use of HNO_3 [7], HNO_3 and $HClO_4$ [3], or HNO_3 and H_2O_2 [8,9]. All of these are time consuming and are prone to analyte loss and contamination. In general, only a few measurements can be achieved without problems of burner clogging.

This paper describes microwave-assisted sample treatment in closed reactors in order to determine Fe, Mn, Cu, Zn, Ca, Mg and Al by flame atomic absorption spectrometry (AAS) and Na and K by flame atomic emission spectrometry (AES). Other elements of toxicological interest can be determined using the proposed sample treatment method and using more sensitive techniques such as graphite furnace AAS or hydride generation AAS. The UV-VIS molecular absorption spectra were used as an index for the organic matter content. Conductivity and pH were measured directly in the samples.

Twenty-five samples of beers of different types, countries, containers and raw materials were analysed. Statistical analysis was performed for all the data obtained from flame atomic spectrometry, molecular absorption spectrometry, pH and conductivity. Analysis of variance (ANOVA) was used to establish the differences among samples, and linear discriminant analysis (LDA) was used to classify the beers according to the traditional scheme. The influence of molecular absorption spectrometry data in classification is also demonstrated.

2. Experimental

2.1. Instrumentation

A microwave oven (CEM MDS.2000) equipped with Teflon vessels (PFA, 120 ml, 220 psi) and pressure regulator; Unicam 929 atomic absorption spectrometer; Milton Roy spectronic 3000 array spectrophotometer; cryogenic bath (Haake E 1); ultrasonic bath (Selecta); Philips PW 9526 digital conductivity meter with a conductivity cell 9550; Crison micro pH 2002; and a P Selecta centrifugator.

2.2. Reagents

Nitric acid, 70% (Panreac; pro analysis); hydrogen peroxide, 30% (Panreac; pro analysis); ICP multi-element standard solution IV (Merck); sodium chloride (Merck, GR); potassium chloride (Merck, GR); and Milli-Q water.

2.3. Sampling

The samples were obtained from a local store and represent the types of beers readily available to consumers.

2.4. Procedure for trace metal analysis by flame AAS

This procedure is described for three perfluoroalkoxy Teflon vessels in a microwave oven. A sample volume of approximately 23 ml was transferred to the closed vessels. A programme was run at 40% power, maximum pressure 100 psi and 6 min duration for the purpose of degassing the beer sample.

A second programme with two stages was selected to digest the sample. In the first stage, 20 ml degassed beer was placed in each vessel and 3 ml HNO_3 was added. The vessels were closed and a three-step programme was set, each step at 40% power, 15 psi maximum pressure and 2 min. The vessels were left to cool in a cryogenic bath at 5°C for 5 min. The reactors were opened, and in a second stage 2 ml of 30% H_2O_2 was added. The setting was 40% power, 20 psi maximum pressure, 3 min. After cooling at room temperature, the vessels were opened and the product was transferred to a 25-ml volumetric flask and diluted to volume with Milli-Q water. After centrifugation, the metals were measured in the supernatant liquid.

The procedure is summarized in Table 1. The standard solutions for the calibration graph were prepared by dilution of the ICP multi-element standard solution.

2.5. Statistical analysis

ANOVA and linear discriminant analysis (LDA) were used for data treatment. All statistical analyses were performed with the computer software Statistica version 5.1.

Table 1
Microwave program for three reactors

Stage	Reagent added	Time (min)	Power (%)	Pressure (psi)
1	3 ml HNO_3 (c)	2	40	15
		2	40	15
		2	40	15
2	2 ml H_2O_2	3	40	20

3. Results and discussion

3.1. Development of a procedure for sample treatment in order to determine metals by flame atomic spectrometry

The traditional dry ashing procedure used for beers [10] was discarded because it is time consuming and gives imprecise results, especially for volatile elements. Other procedures for beers [2,7–9] based on wet digestion in open vessels were tested with Spanish beers. In all cases drawbacks were found, such as time-consuming sample pretreatment, risk of loss and contamination of trace elements and burner clogging, in spite of the apparent improvements obtained when H_2O_2 is added. We concluded that a fast method was needed which would avoid analyte loss, contamination and burner clogging. The procedure chosen was microwave digestion in closed vessels. There are hardly any references in the literature to acid treatment of beers in a microwave oven for trace metal analysis, except for Pb determination by flow injection-hydride generation AAS [11], but the hydride generation technique avoids problems concerning the matrix.

Several methods of beer degassing were tested: paper filtration, vacuum filtration, ultrasonic bath and microwave heating. The latter gave the fastest and most effective results. Therefore, degassing in a microwave oven was selected. The next step was to examine the treatment of 20 ml of beer with a small volume of HNO_3 in closed reactors in a microwave oven. Two drawbacks can be expected when comparing with use of a hot plate [2] or with conventional dry ashing: (1) there is a risk of explosions due to the rapid formation of gases (the reactors can withstand pressures up to 220 psi); and (2) higher detection limits.

Several variables were studied using a one-step quick-digestion programme: the HNO_3 volume added, the magnetron power, maximum pressures and duration. It was observed that after several minutes, the pressure exceeded the specified maximum reactor pressure with the risk of explosions. In these cases, the samples were completely clarified and suitable for measuring by flame spectrometry without burner clogging, but this procedure was discarded in view of the hazards involved. The requirement, then, was a very low maximum pressure and a minimum programme time. The volume of HNO_3 was fixed at 3 ml, because higher volumes increased the risk of explosions. A programme with three reactors at 40% power, 15 psi maximum pressure and 5 min duration was required for digestion. However, it was not considered suitable for flame atomic spectrometry due to the heterogeneous aspect of the resulting sample solution.

Next, the possibility of using a programme in several steps was examined. This was the same as described before but divided into three steps lasting 2 min each. In this way, no explosions were produced and the digested samples always showed the same slight turbidity. These samples were aspirated into the flame to test their suitability for measuring the metal content by flame atomic spectrometry. After a short time, the flame showed fluctuation because small solid particles were deposited in the burner slot. The separation of the solid particles from the digested beer by centrifugation was not successful. The absorbance signals for trace metals in the supernatant liquid were not reproducible because a good separation between solid and liquid phase was not achieved. A second stage with 2 ml H_2O_2 30% was then investigated at 40% W of power, 20 psi maximum pressure and 3 min. The digested samples were transferred to 25-ml volumetric flasks and diluted to volume with Milli-Q water. The expected clarification effect was not observed, but in this case the separation of solids by centrifugation was improved and it was possible to analyse a large number of samples without burner clogging. Following this procedure it is possible to carry out approximately 45 measurements with an air–acetylene flame. When a nitrous oxide–acetylene flame was required, the number of measurements allowed was reduced by approximately 10.

In order to check that metals are not discarded with the solid particles, a comparison was performed between the signals obtained when metals are added to the supernatant liquid after separating the solid, and those obtained with spiked beer samples with the same metal content. The results were very similar, and we therefore concluded that the proposed procedure was satisfactory for analysis of the metal content in beers. The procedure is summarised in Table 1.

In order to increase the sensitivity of certain heavy metals using flame AAS, the slotted tube atom trap was tested with the digested samples. Improvements were obtained for spiked samples for Zn, Cu, Pb and Cd. For the unspiked samples there were no significant improvements, i.e. Pb and Cd were not found and Cu and Zn were still low.

3.2. Determination of metals by flame atomic spectrometry

In the selection of the metals to be determined, several factors were taken into account: presence in the raw materials, environmental, brewing and container contamination, interferences in the brewing process, influence on the quality and taste of beer, essential trace metal contribution and consumer health implications. The experimental work described below was performed with two different types of beers: Cruzcampo (amber colour) and Beamish (black colour). The metals were: Fe, Mn, Cu, Zn, Cr, Al, Ca, Mg, Pb and Cd by flame AAS; and Na and K by flame emission spectrometry. An air–acetylene flame was used for Fe, Mn, Cu, Zn, Ca, Mg, Pb, Cd, Na and K, and a nitrous oxide–acetylene flame for Cr and Al. An ionisation buffer was used for Na and K. After preparing the samples, the following parameters were optimised for each metal: burner height, acetylene flow rate and impact bead position. Other parameters were according to established operating conditions for this matrix [12]. To determine the metal concentration, the calibration curve method with inorganic aqueous standards was compared with the standard addition method. In both cases the results were as expected and similar to the results previously obtained by other authors [9]. This shows that there were no significant interferences from the

Table 2
Results obtained for metal concentrations (mg l^{-1}), absorptivity coefficient ε_{269} (cm^{-1}), pH and conductivity (mS cm^{-1}) (mean and % RSD*)

Beer name	Fe	Mn	Cu	Zn	Mg	Ca	Na	K	ε_{269}	pH	Cond.
Cruzcampo (B)	0.110	0.163	0.033	0.457	94.13	33.86	13.23	146.7	18.16	4.02	1.34
	3.7*	1.1*	0.0*	32.9*	1.7*	1.5*	1.8*	5.9*	20.6*	0.2*	0.4*
Beamish (C)	0.234	0.077	0.025	0.025	55.85	32.44	4.136	144.9	40.15	3.93	1.45
	5.4*	4.7*	16.0*	8.7*	2.0*	2.3*	19.4*	0.5*	4.5*	0.1*	0.3*
San Miguel (B)	ND^{T}	0.096	ND	ND	47.34	65.05	39.03	54.63	19.31	4.04	1.21
	–	1.8*	–	–	2.1*	7.1*	8.2*	3.0*	0.7*	0.2*	0.1*
Dab (C)	0.065	0.140	0.052	0.024	90.94	11.45	32.71	190.9	31.97	4.48	1.74
	24.0*	2.0*	5.8*	0.0*	1.9*	0.6*	4.2*	4.4*	0.7*	0.2*	0.9*
Heineken (B)	0.064	0.085	0.046	0.038	96.18	69.78	47.62	124.4	26.90	4.35	1.77
	20.1*	4.7*	0.0*	34.8*	0.9*	2.5*	0.6*	2.4*	12.1*	0.2*	0.2*
Draught (C)	0.062	0.150	ND	ND	68.31	50.28	7.604	138.1	36.46	3.91	1.60
	16.8*	1.5*	–	–	3.5*	5.6*	5.0*	3.7*	19.3*	0.1*	0.4*
Carlsberg (C)	0.545	0.105	ND	ND	86.84	95.17	30.21	121.1	20.16	3.92	1.98
	0.0*	2.2*	–	–	2.1*	24.6*	0.6*	21.2*	0.5*	0.1*	0.3*
Kaliber sin (C)	0.174	0.088	0.030	ND	42.01	37.96	16.26	150.0	13.81	4.49	1.02
	15.6*	2.6*	0.0*	–	2.8*	11.8*	10.6*	6.4*	0.2*	0.2*	0.4*
Sagres (C)	0.077	0.059	ND	0.020	72.53	25.06	24.75	219.0	21.63	4.24	1.50
	35.9*	3.9*	–	0.0*	1.4*	0.0*	4.2*	0.6*	1.7*	0.1*	0.1*
Heineken (C)	0.088	0.091	0.068	ND	97.65	44.85	24.50	265.8	26.73	4.45	1.88
	13.8*	6.2*	22.4*	–	4.5*	17.6*	5.5*	2.1*	0.7*	0.1*	0.0*
Paulaner (C)	0.494	0.064	0.050	ND	87.69	26.58	4.771	89.83	30.94	4.28	1.78
	2.0*	7.2*	8.0*	–	3.7*	10.5*	32.1*	9.1*	3.4*	0.1*	0.7*
Cruzcampo (C)	0.151	0.167	0.035	ND	89.49	39.91	10.06	22.88	21.55	4.21	1.43
	5.6*	1.0*	0.0*	–	0.9*	5.3*	17.8*	23.5*	3.7*	0.1*	0.2*
Tangle Foot (B)	0.163	0.151	0.036	0.031	78.83	101.5	53.55	134.6	28.05	3.98	1.87
	13.8*	1.2*	7.1*	36.5*	0.3*	4.4*	3.5*	4.3*	1.7*	0.0*	0.0*
Marston's (B)	0.116	0.169	0.041	0.057	127.6	100.0	25.91	207.3	37.43	4.12	2.25
	9.9*	2.5*	13.5*	79.4*	4.6*	2.5*	5.8*	2.1*	1.1*	0.0*	1.0*
Altenmünster (B)	0.101	0.080	0.064	ND	86.64	35.52	1.188	174.1	30.21	4.60	1.91
	6.23*	0.0*	1.1*	–	1.0*	18.4*	13.7*	2.2*	1.6*	0.0*	0.0*
Negra Modelo (B)	0.153	0.197	0.034	0.064	111.7	111.3	39.62	90.73	29.13	4.18	1.94
	8.7*	1.5*	24.8*	24.1*	2.4*	7.0*	13.5*	6.7*	0.7*	0.0*	0.1*
Miller (B)	0.059	0.097	0.022	ND	63.12	59.27	5.729	17.50	17.85	3.91	1.36
	10.8*	3.0*	0.2*	–	3.5*	0.0*	0.6*	7.9*	2.3*	0.1*	0.0*
San Miguel (C)	0.096	0.121	ND	ND	65.59	86.25	40.62	83.07	23.55	4.12	1.71
	6.6*	0.0*	–	–	1.4*	0.0*	1.5*	9.9*	1.2*	0.1*	0.2*
Carlsberg (B)	0.086	0.089	0.019	0.031	84.00	74.90	46.11	46.72	21.19	3.96	2.00
	6.7*	0.0*	26.3*	22.4*	1.7*	6.9*	4.8*	3.4*	2.6*	0.1*	0.0*
Kaliber sin (B)	0.102	0.044	0.038	ND	45.85	37.24	3.948	100.1	15.00	4.63	1.17
	24.6*	7.9*	32.2*	–	2.2*	16.4*	11.5*	0.3*	4.4*	0.0*	0.2*
Pilsner Urquell (B)	0.136	0.153	0.030	0.077	97.31	9.833	4.156	112.1	30.78	4.45	1.83
	4.2*	1.2*	9.7*	35.9*	2.7*	1.2*	18.2*	4.4*	2.2*	0.4*	0.0*
Franziskaner (C)	0.145	0.207	0.061	0.102	96.23	28.18	2.677	142.5	23.13	4.15	1.98
	6.9*	3.8*	38.0*	7.4*	1.11*	24.7*	7.4*	8.9*	0.9*	0.1*	0.1*
Bohemia (B)	0.063	0.116	0.025	0.114	67.96	31.72	45.21	21.46	16.61	4.09	1.37
	15.2*	5.3*	51.5*	45.5*	4.5*	0.0*	1.4*	11.6*	2.7*	0.5*	0.1*
Öko Krone (B)	0.134	0.116	0.033	0.050	23.66	41.09	8.823	245.3	33.26	4.46	2.36
	15.2*	4.0*	5.3*	17.4*	1.0*	11.0*	63.3*	3.5*	1.1*	0.0*	0.0*
Sagres (B)	0.120	0.056	ND	0.067	67.68	16.49	14.46	72.73	20.86	4.13	1.56
	19.7*	7.2*	–	51.5*	1.7*	13.2*	0.5*	17.1*	1.97*	0.1*	0.2*

*RSD in %. ND^{T} refers to < limit of detection. B, Bottle; C, Can.

sample matrices. The calibration curve method was therefore chosen. The stability of the beer samples analysed was determined by measuring the absorbance signals periodically. They are stable for at least 1 week. The repeatability of the results was assessed on the basis of the relative standard deviation (R.S.D.). Under the experimental conditions, 25 beer samples were analysed in triplicate. When a concentration was higher than the highest standard, the sample solution was diluted with Milli-Q water or a secondary wavelength was used. The accuracy of this method is supported by other published methods [8,9] using the same reagents but with higher risks of analyte loss and contamination, and also by the results obtained in this work after the chemometric treatment. Unfortunately, no reference materials are available. The results (mean and R.S.D.) are shown in Table 2.

3.3. Determination of absorbance by UV-VIS molecular absorption spectrometry

UV-VIS molecular absorption spectrometry was used in this work to obtain information on the organic matter content. The amount of organic compounds in beers is enormous and analytical determination cannot be performed without prior separation of groups of similar compounds. Nevertheless, the absorbance of the UV-VIS radiation by organic compounds affords useful information for differentiation and classification, since these compounds are related to taste and stability, factors determining the quality of beer. This technique is used in the brewing industry for various different purposes: to assess wort colour by measuring absorption at 430 nm, to determine the amount of resins by measuring at 275 nm and to determine diacetyl which is related to unpleasant flavour [13].

The beer samples were diluted appropriately with Milli-Q water and the absorption spectrum between 200 and 400 nm was registered at different dilutions. The range was from 0.3 to 3 ml of beer diluted to 25 ml. In all cases, two absorption bands were obtained with maxima at approximately 213 nm and 269 nm and absorbance values depending on the beer analysed (Fig. 1). Shifts depending on the sample type and the dilution were mainly found in the 213-nm absorption band. For each beer, the absorbance signal obtained at approximately 213 nm was a function of the dilu-

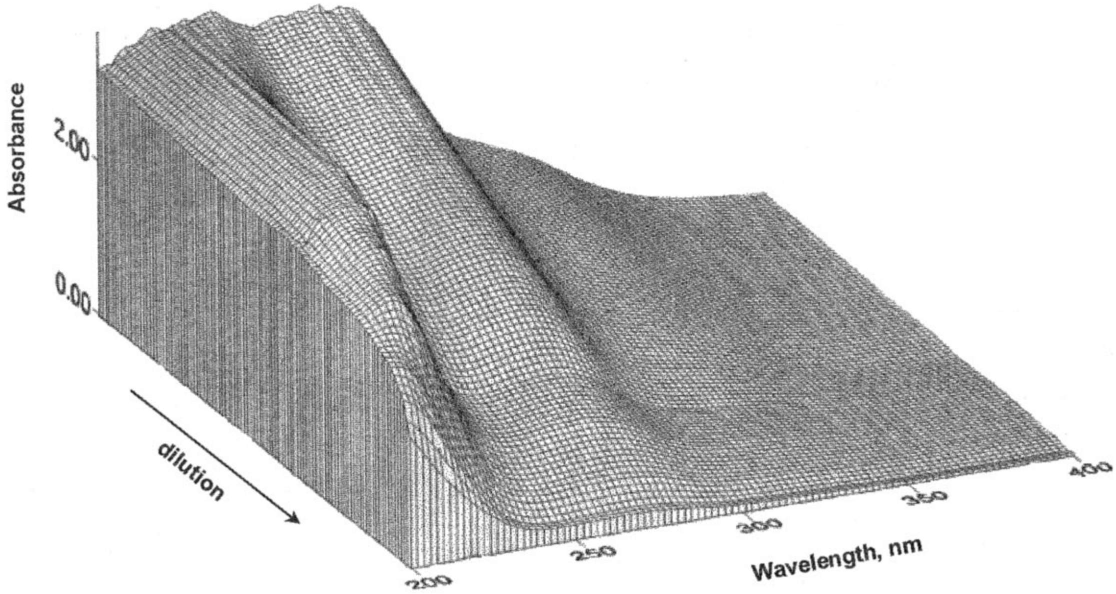

Fig. 1. Molecular absorption spectra for a lager beer at different dilutions (0.3–3 ml of beer diluted to 25 ml).

Table 3
F and *P*-values of ANOVA and Tukey test for containers

	F	*P*-Values	Main effect
Fe	10.012	0.0023	B < C
Cu	0.219	0.0095	B < C

tion and of the absorptivity coefficient, whereas the absorbance signal obtained at approximately 269 nm was only a function of the dilution factor. Therefore, the ε_{269} values were similar and characteristic for each beer.

Since our aim was to obtain an index for use in combination with the inorganic parameters, we selected only the 269-nm absorption band. The absorptivity coefficients for three dilutions (1/25, 1.5/25 and 2/25) were used as a chemometric index. The results are summarized in Table 2.

3.4. Determination of the pH and conductivity

The pH of beer has a strong influence on product quality, primarily through the flavour and the suppression of microbial growth. The protons impart sourness, and, in addition, the relative proportion of charged and uncharged forms of many organic compounds depends directly on pH. The pH of each sample was measured in triplicate. The resulting pH values were between 3.9 and 4.6 and were consistent with the values reported in the literature. They are summarized in Table 2.

The conductivity measurements are of interest because certain salts contribute to beer flavour. The water used for brewing is an important source of inorganic salts, and it must have the correct balance of ions. The conductivity values obtained in triplicate for each beer varied from 1 to 2.4 mS/cm (see Table 2).

Table 4
F and *P*-values of ANOVA and Tukey test for beer type

	F	*P*-Values	Main effect
Fe	5.16	0.0028	W > L,S,A
Cu	2.79	0.0400	W > L,S,A
Mn	6.97	0.0004	W,S,A > L
Mg	2.97	0.0370	A > L,S
Ca	9.67	0.0000	A > L,S,W
Na	5.38	0.0021	L,A,S > W A > L,S,W
Ω	4.68	0.0048	A > L,S
MAS	20.43	$< 10^{-7}$	A,S > L

Table 5
Matrix classification for type of beers[a]

Group	Percent correct	L	S	W	A
L	100.00	36	0	0	0
S	66.67	0	6	0	3
W	100.00	0	0	6	0
A	100.00	0	0	0	6
Total	94.74	36	6	6	9

[a] Grouping variables: L, lager; S, stout; W, wheat; A, ale.

3.5. Statistical analysis

Table 2 provides a summary of all the analytical results from 25 samples. The analysis was performed in triplicate. The values found in this study were within the range reported in the literature [2,9]. There was notable dispersion of the Zn results, whereas high coefficients of variation for Zn in beers and other samples by AAS have been reported elsewhere [14]. Therefore, this variable will not be considered. Copper was found in 19 samples. Aluminium at low levels was only found in three samples, which can be explained by the fact that Al cans were used. As expected, Cr, Cd and Pb were not found. The analysis of these four metals can be performed by graphite furnace AAS using the proposed procedure. All the results obtained by molecular absorption, pH and

Table 6
Matrix classification for raw materials[a]

Group	Percent correct	B	BC	W
B	92.8	39	3	0
BC	100.00	0	9	0
W	100.00	0	0	6
Total	94.74	39	12	6

[a] Grouping variables: B, barley; BC, barley + other cereals; W, wheat.

conductivity were considered appropriate for the statistical analysis.

3.5.1. Analysis of variance

ANOVA is used to determine statistically significant differences among arithmetical means of a variable. This method has recently been used to differentiate wines on the basis of their trace metal content [5]. In the present work it was used to establish the influence of several factors on the results. The factors studied were the container (can and bottle) and the type of beer (stout, ale, lager and wheat beer). A value of $P = 0.05$ was established as critical. The results for the containers showed significant differences only for Fe and Cu. The Tukey homogeneity test was applied to establish these differences (Table 3). Iron and Cu concentrations are higher in canned beers, and this could influence the taste and stability of canned and bottled beers. These results are of interest for quality control [15]. In a similar study but with a smaller number of beer samples, no differences were found between canned and bottled beers for Fe and Cu [8].

Statistically significant differences among the four type of beers studied were found for Fe, Cu, Mn, Mg, Ca and Na content, conductivity and molecular absorption spectrum (Table 4). No differences were found for K and pH. Wheat beer showed the highest content of Fe and Cu. In ale beer the content of Mg, Ca and Na was highest, which is consistent with the low Ca content in Pilsen beers [13].

3.5.2. Linear discriminant analysis

LDA is usually the method of choice for classifying samples into different groups. The method supplies a number of linear discriminant functions in order to provide a method for predicting the group into which a new case will most likely fall.

Fundamentally, beers may be divided into ales, lagers or stouts. Lately, however, there has been a considerable blurring of the boundaries that divide these beer types so that it is difficult to classify beers. New product development ideas characterised the brewing industry in recent years,

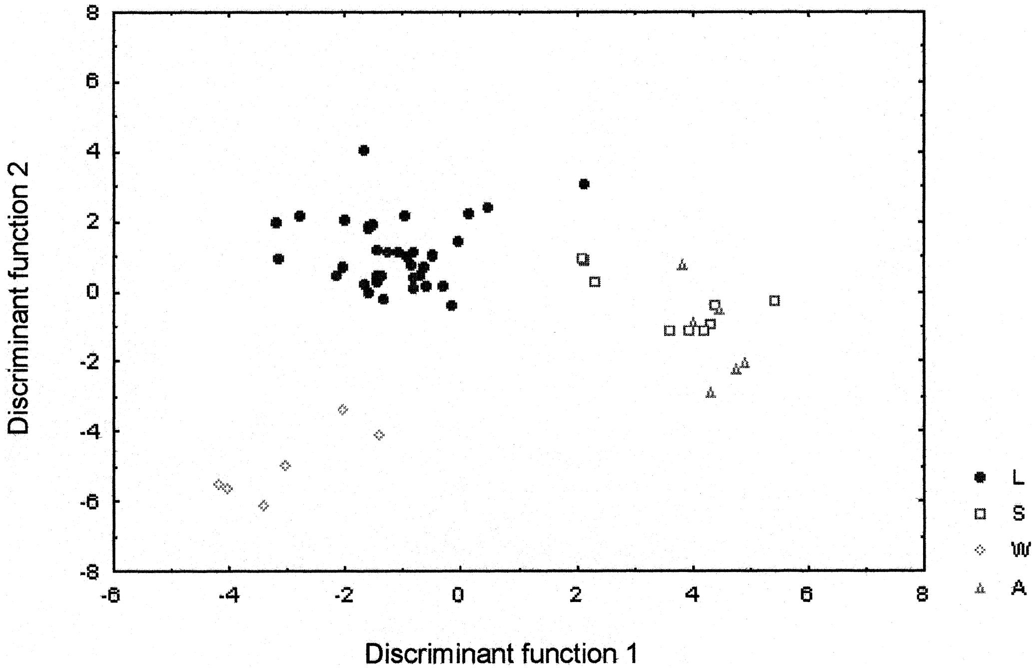

Fig. 2. Plot of the first discriminant functions vs. the second for type of beers: L, lager; S, stout; W, wheat beer; A, ale.

i.e. the British market has sampled and rejected black lagers [13].

Discriminant analysis was applied to the data obtained in triplicate for all the variables except Zn. The grouping variables used were: types of beer, containers and raw materials. Four types of beer were considered: stout (S), ale (A), lager (L) and wheat beer (W). First of all, LDA was performed with all the independent variables. After that, the same analysis was performed but without the molecular absorption spectrum variable, in order to detect the influence of this index in relation to the organic matter content. The result for the first case is shown in Table 5 and Fig. 2. As these show, each type of beer was correctly classified. Only in the case of stout was there some confusion with ale. However, this is consistent with the traditional classification based on the brewing process [13], which classifies ales and stouts in the same group. Without the molecular absorption spectrum variable, classification is poorer, because the stout is incorrectly classified and is confused with the lager. Three raw materials were considered: barley (B), barley plus other cereals as adjuncts of malt (BC), and wheat (W). In the first instance, LDA was performed with all the variables. The results are shown in Table 6 and Fig. 3. The classification in this case was satisfactory. Elimination of the molecular absorption spectrum variable did not alter the results.

The results of the ANOVA model encouraged us to attempt a classification based on the two types of containers studied: bottle (B) and can (C). The results for all the variables are given in Table 7. The two groups are well classified.

4. Conclusions

The sample treatment proposed is much shorter than other published methods and avoids contamination and loss of trace elements when comparing with other procedures that are not assisted by microwaves in closed reactors. Flame atomic spectrometry following this procedure is appropriate for essential metal determinations when beer is considered as a foodstuff, but it is not sensitive enough for trace metals of interest in toxicology.

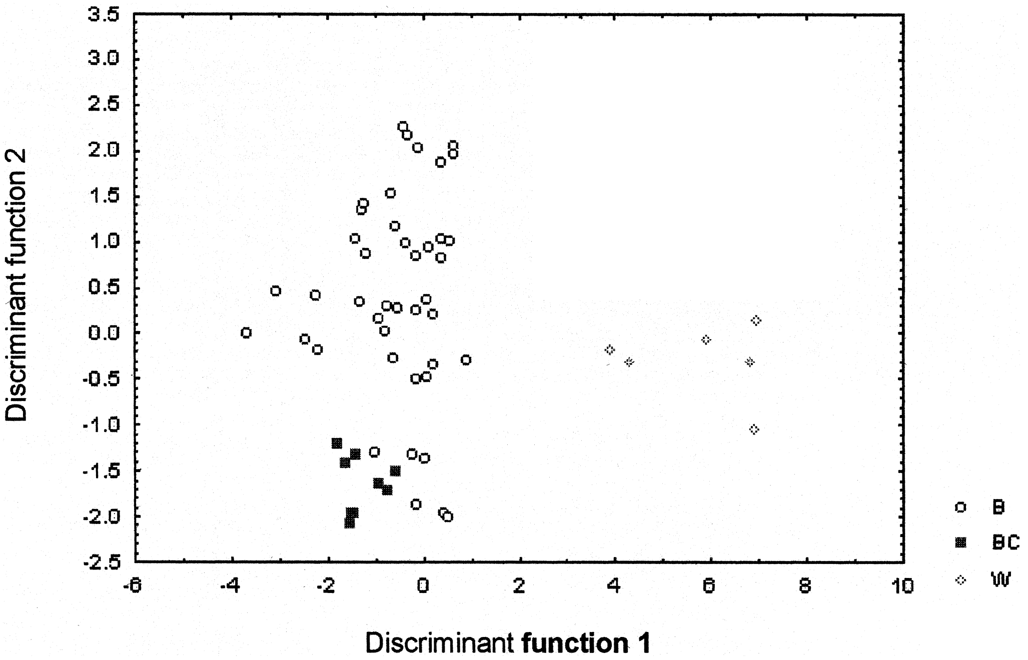

Fig. 3. Plot of the first discriminant functions vs. the second for raw materials: B, barley; BC, barley plus cereals; W, wheat.

Table 7
Matrix classification for containers[a]

Group	Percent correct	B	C
B	100.00	33	0
C	83.33	4	20
Total	92.98	37	20

[a] Grouping variable: B, bottle; C, can.

However, this methodology could be suitable for detecting ultratrace elements by graphite furnace AAS and hydride generation AAS.

ANOVA revealed differences between canned and bottled beers relating to taste and stability. This method also demonstrated that the intake of essential elements varies according to the type of beer. LDA proved capable of grouping the beers analysed in spite of the new beer styles being produced lately by the brewing industry.

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