Application of high resolution-continuum source flame atomic absorption spectrometry (HR-CS FAAS): determination of trace elements in tea and tisanes

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ABSTRACT

A new application of HR-CS FAAS (High Resolution-Continuum Source Flame Atomic Absorption Spectrometry) has been developed for the determination of several trace elements (Ca, Co, Cu, Fe, Mn, Ni, Na and Zn) in infusions made from tea, rooibos and *tea with seaweed* samples. The proposed methods are fast, inexpensive and show good performances: the mean analytical recovery was aproximately 100 %. The mean limit of detection was 29.4 µg/l, and the mean limit of quantification was 98.0 µg/l (both limits refer to the brewed samples). Due to the matrix effect observed, the standard addition method had to be applied. Preliminary classification (based on metal contents) using chemometric techniques such as PCA (Principal Component Analysis) and CA (Cluster Analysis), was successful for infusions made from rooibos and tea with seaweed, but inconclusive for black and green teas.

Keywords: HR-CS AAS, flame atomization, trace element, tea, tea with seaweed, brewed sample.

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1. Introduction

HR-CS AAS (High Resolution-Continuum Source Atomic Absorption Spectrometry) can be considered a significant innovation that improves the performance of AAS (Atomic Absorption Spectrometry). It is being applied to the analysis of many analytes in a great variety of samples, revealing the interest of researchers in exploring the potential and capabilities of this *new* technique.

According to the literature (Welz, Becker-Ross, Florek, Heitmann, & Vale, 2003; Welz, 2005; Welz, Morés, Carasek, Vale, Okruss, & Becker-Ross, 2010), when AAS was updated on the basis of the work by Walsh (1955), and Alkemade and Milatz (1955a; 1955b), the use of spectral line (LS) rather than continuum sources (CS) was necessary because the required spectral resolution could not be achieved with the monochromators available at that time. This technology-based decision produced one of the main limitations of AAS: the need to analyse one element at a time. However, during the last 60 years, the use of continuum sources has been explored by several authors, as reviewed by Becker-Ross, Florek, Heitmann, Huang, Okruss, & Radziuk (2006) and by Welz et al. (2010). All this research set the basis for manufacturing new spectrometers that, compared with "traditional" LS AAS instruments, show the following features:

- A xenon short-arc lamp, with an emission intensity higher (and over a broader wavelength range) than other continuum sources.
- A double Echelle monochromator (DEMON), which exhibits a much higher resolution power of resonant lines.
- A linear charge-coupled device (CCD), that provides an increased detection capability.

These improvements enable the performance of sequential multi-elemental analyses by HR-CS AAS, in shorter times than required by ICP-OES and make HR-CS AAS a step further towards absolute analysis.

Tea plants (Camellia spp.) are perennial evergreen shrubs with a number of varieties, among which *Camellia sinensis* is the most widely used. The leaves are most commonly used for tea, but other parts are also consumed, such as the apical buds. Different manufacturing processes result in four main classes of tea: black and green teas, pu-erh (the so-called "red tea") and brick tea. Moreover, within these main types, a great number of sub-types are consumed all over the world, resulting in some confusing classifications. Rooibos, or *Aspalathus linearis* (also known as "South African red tea", due to the colour of its brew), is a plant indigenous to South Africa and consumed as herbal tea.

Edible seaweeds have been consumed for hundreds of years. They have been considered a "novel food" in the EU (European Union) for nearly 17 years (European Union, 1997). This means increased consumption is anticipated in the EU in coming years, and further regulation is, therefore, necessary in the interests of public health.

Tea and seaweed mixtures, commercially denoted as *"tea with seaweed"*, have been sold in Spain for about five years. Tea with seaweed usually consists of mixtures of black and green teas and rooibos with the following seaweeds: wakame (*Undaria pinnatifida*), kombu (*Saccharina latissima*) and nori (*Porphyra umbilicales*). Such mixtures often include minor components, namely aromas and fruit peels. The nutritional properties of tea with seaweed result from the established characteristics of tea and those of algae: high fibre content, low amounts of lipids and relatively high protein content (7-24 % of dry weight) (Paz-Rodríguez, 2010).

Trace element determination and, more recently, speciation analysis in foods is a topic of interest for nutrition, toxicology and food safety. In the case of teas and tisanes, the research can have two complementary goals: on the one hand, the interest can be focused on the metallic profile of the vegetal materials used for brewing. This usually includes the digestion of such materials prior to the elemental determination (Narin, Colak, Turkoglu, Soylak, & Dogan, 2004; Al-Othman, Yilmaz, Sumayli, & Soylak, 2012; Shaltout, Abdel-Aal, Welz, & Castilho, 2013). On the other

hand, the research can be aimed at the determination of the metallic contents in the brewed samples but, to the best of our knowledge, HR-CS AAS has not been applied in the determination of trace metals in tea or tea-like samples, using either a flame or a graphite furnace as the atomizer. Hence, the work presented here describes a new application of HR-CS FAAS (using an air-acetylene flame) for the determination of total contents of several analytes in commercial black and green teas, rooibos and tea with seaweed as consumed (brewed samples). The authors intended to investigate the metallic content actually consumed, and not the levels of a series of elements in the raw materials used for brewing. A secondary objective of this work was to investigate the potential to classify the samples analysed, on the basis of their trace element profile, using unsupervised pattern-recognition chemometric techniques, such as Principal Component Analysis (PCA) and Cluster Analysis (CA).

According to the main objective, a procedure was defined, mimicking the preparation of tisanes by consumers. As it is well-known, a variety of components, minerals included, formerly present in the vegetal materials migrate to the aqueous phase. However, several authors (Nookabkaew, Rangkadilok, & Satayavivad, 2006; Shen, & Chen, 2008; Salahinejad, & Aflaki, 2010) have reported that the extraction efficiency of the elements in the preparation of tisanes is less than 100 %, and varies from one analyte to another. Thus, the concentration of minerals in the infusions depends on the extraction efficiencies, and the total concentration of those minerals in the vegetal material used for brewing (Salahinejad et al., 2010). Moreover, Natesan and Ranganathan outlined a classification of analytes depending on their extraction rates from black tea samples (Natesan, & Ranganathan, 1990). Consequently, the objective of this research was the assessment of trace element contents in brewed samples and to explore the possible classification of such samples.

2. Material and methods

2.1. Instrumentation

Measurements were carried out on a ContrAA^{TM} 300 high resolution-continuum source flame atomic absorption spectrometer from Analytik Jena AG (Jena, Germany). The oxidant flame was obtained with acetylene N52, purity 99.9992 % Alphagaz^{TM} from Air Liquide (Madrid, Spain) and compressed air.

2.2. Reagents

1000 mg/l stock standard solutions for atomic absorption (in 0.5 mol/l nitric acid) of each of the following elements Ca, Co, Cu, Fe, Mn, Ni, Na, and Zn were purchased from Merck (Darmstadt, Germany), except the Fe and Na solutions, which were purchased from Scharlau Chemie (Barcelona, Spain). A 36.5 % hydrochloric acid for analysis and a 65 % nitric acid for analysis (both from Merck) were utilized. Ultrapure water (resistivity 18 MΩ cm), obtained from a Milli-Q® water purification system (Millipore Corp., Bedford, MA, USA), was used for solutions, dilutions and rinsing.

2.3. Procedures

2.3.1. Cleaning of material

All material was washed and kept in 10 %(v/v) nitric acid solution for at least 48 h (to prevent trace metal contamination), and then rinsed with ultrapure water and allowed to dry before use.

2.3.2. Brewing

The preparation of the teas and tisanes for analysis was as follows: an accurately weighed 1 g of sample (vegetal material) was placed inside a tea sachet (commercially available tea filter, made of unbleached paper). The sachet was introduced into a glass beaker, 100 ml of boiling ultrapure water were added and the beaker was allowed to stand for 5 min to obtain the brewed sample. The tea sachet was removed, and the infusion left to cool to room temperature. Finally, 1.0 ml of 36.5 % hydrochloric acid was added to the infusion and the volume made up to 100.0 ml with ultrapure water. The resulting solution was subjected to HR-CS FAAS, except for sodium, which was measured by atomic emission using the same apparatus. The instrumental conditions are shown in Table 1. The standard addition method was applied to achieve quantitative results.

3. Results and discussion

3.1. Study of the effect of hydrochloric acid

Oliveira, Raposo, & Gomes Neto (2009) and Oliveira, Gomes Neto, Nóbrega, & Jones (2010) proposed the addition of a small amount of hydrochloric acid to help analyte determination in plant leaves. Accordingly, two series of 10.0 ml-standard solutions were prepared for each analyte: the solutions of one of the series contained 0.1 ml of 36.5 % hydrochloric acid (i.e., about 1 %(v/v) in the solution), whereas the solutions of the other series contained no hydrochloric acid. Each series produced a calibration line (linear regression equations presented in Table 2.a), and these results showed the addition of hydrochloric acid enhanced sensitivity for Ca, Co, Zn, and especially for Fe whereas it had no effect on the determination of Cu, Ni, and Na. A lower sensitivity as well as a poorer precision were observed for Mn. Thus, the addition of hydrochloric acid was chosen as a compromise for all the analytes.

3.2. Method validation

3.2.1. Calibration

In order to check whether the matrix of the samples affected the measurements, both aqueous calibration and standard addition lines were obtained for each analyte and type of brewed sample. Afterwards, the slopes of both lines were compared, applying when appropriate, a t hypothesis test ($\alpha = 0.05$). From those results, no matrix effect were observed in the following cases: for Ni in black teas; for Co, Mn, and Ni in green teas; for Ca, Cu, and Ni in rooibos and for Co and Ni in teas with seaweed tisanes. The standard addition method was applied throughout, i.e., for all analytes and infusions, again as a compromise condition.

Finally, the response was linear up to 0.5 μg/ml for all the analytes and tisanes, except for Ni, which showed a wider range of linear response (up to 1.5 μg/ml).

3.2.2. Accuracy

Due to the lack of commercially available certified reference materials, the accuracy of the proposed procedures could only be estimated by means of analytical recovery studies. From the corresponding results (see Table 2.b), the methods can be expected to show good accuracies. The mean analytical recovery value was 101.9 %.

3.2.3. Sensitivity

Defined as the slope of the calibration line of each method (Currie, 1995), the sensitivities achieved are included in Table 2.c. In addition, the sensitivities were also studied by calculating the

respective limits of detection (LODs) and quantification (LOQs), defined as follows (Currie, 1995):

$$LOD = \frac{3 \cdot s}{b} \qquad LOQ = \frac{10 \cdot s}{b}$$

("s" is the standard deviation of ten measurements of the blank and "b" the slope of the standard addition line). The LODs and LOQs obtained, referring to brewed sample, are included in Table 2.c. In general, these limits can be considered acceptable although, in the literature available, published data relating to the same (or similar) types of samples and technique were not found or provide little information regarding the calculation of LODs (Narin et al., 2004).

3.2.4. Precision

The precision of the developed methods was evaluated by studying the repeatability of both the measurements and the whole procedure.

The relative standard deviations (RSDs) of the measurements, for the different tisanes and analytes, are shown in Table 2.d. Values for Co and Ni are not shown, since they were less than the respective LODs. The RSDs are acceptable, and the poorest values were those obtained for analytes present at the lowest concentrations, as expected (Horwitz, Kamps, & Boyer, 1980).

The repeatability of the whole procedure was investigated by preparing seven replicate infusions with a given tea with seaweed sample, and analysing them following the developed procedures. The RSDs calculated for Ca, Cu, Fe, Mn, Ni, Na, and Zn were, respectively, 12.4, 9.7, 19.6, 15.4, 20.6, 14.1, and 21.3 %. The RSD for Co was not calculated since its concentration was less than the corresponding LOD.

3.3 Application

The method developed was applied to the determination of Ca, Co, Cu, Fe, Mn, Ni, Na, and Zn in 10 black ("B") and 10 green ("G") teas, 10 rooibos ("R") and five tea with seaweed ("TS") tisanes, which had been purchased at local markets in Galicia (Northwestern Spain) and China (in Shanghai and Nanchang –Jiangxi province).

The results are shown in Table 3.a; Co, Fe, and Ni could not be quantified in most of the samples (i.e., the concentration obtained was below the respective LOQs), as for Cu and Zn in the rooibos samples. In addition, even among samples of the same type, great variation were observed in the concentrations obtained for the different analytes.

3.4 Chemometric study

In order to examine the feasibility of categorizing the samples according to their metallic profile, unsupervised pattern recognition chemometric techniques were applied, namely PCA and CA. Table 3.a shows the concentrations obtained for the teas and tisanes analysed, where the following codes are also used: "n.m." (concentration not measured), "< LOD" (concentration lower than LOD) and "< LOQ" (concentration lower than LOQ, but higher than the LOD). However, only the data denoted as "n.m" were actually not available (27 out of 280).

To perform the chemometric study, the missing data in Table 3.a were replaced as follows: each "n.m." was substituted by the mean value of the corresponding variable within each type of infusion (black and green teas, rooibos and tea with seaweed); each "< LOD" was replaced by the LOD value obtained for each analyte and infusion and, finally, the respective measured value was included instead each "< LOQ". Additionally, all data were expressed as µg/l. The outcome of these changes are shown in Table 3.b, which represents the set of data subjected to chemometrics: 35

objects (brewed samples) on which eight variables were measured, resulting in a 35 x 8 data matrix. The software used was Excel®, Minitab® and IBM SPSS Statistics®.

First of all, the correlation between variables was investigated, in order to remove the correlated items. From the correlation matrix (Table 4.a), the corresponding draftsman plot (Fig. 1.a) and the respective dendrogram (obtained by CA on the original variables, with average linkage and correlation coefficient distance, Fig. 1.b), no correlation could be concluded between any pair of variables, except for Ca and Na, which exhibited a little correlation ($r = 0.9049$), not high enough for supporting the removal of any variable.

The following step was to apply PCA to the data matrix, using IBM SPSS Statistics®: the programme standardizes data (previously to PCA) by columns to eliminate scale effects and data are replaced by a quotient defined as the difference between that data-point and the mean value of the variable, divided by the standard deviation of the variable. No rotation was applied to the PCs (Principal Components) extracted. According to the results achieved (Table 4.b), Cu, Mn, and Zn, as well as Ca and Na explained the major part of the variance in PC 1. Similar deductions could be made regarding the other PCs. Furthermore, Fig. 1.c (the component plot of the first three PCs), besides showing graphically the information in Table 4.b, confirmed visually the slight Ca-Na correlation already mentioned. Table 4.c includes the coefficients needed for obtaining the scores of the objects in the multivariate space defined by the PCs.

A 75.56% of total variance was explained with the first three PCs (see Table 4.d). However, the shape of the scree plot (Fig. 1.d) might lead to the conclusion that the most significant part of the variance could be described by just two PCs or, perhaps, the variance explained by two PCs could be enough to describe the whole dataset. Additional investigation of this possibility produced Fig. 2.a (actually, a draftsman plot), which includes two-dimensional scatter graphs of the first four PCs. But, no well-defined separation or differentiation of the various types of objects could be concluded from those diagrams. Therefore, three-dimensional scatter plots of the first four PCs were prepared (Figs. 2.b and 2.c), but only a quite clear separation of the tea with seaweed samples was observed. Also, a less apparent separation of rooibos tisanes is shown in Figs. 2.b and 2.c (less distinct), where a black tea sample (pointed out with an arrow) was included in the same group. Somehow, these groups can already be conjectured from one of the scatter plots in Fig. 2.a, namely the one that depicts PC 1 vs PC 2 (reproduced at reduced size in Fig. 2.c). Hence, Figs. 2.b and 2.c suggest that rooibos and tea with seaweed are likely to be distinguished from black and green teas on the basis of their metallic contents. Some other PCA-experiments were done, including diverse PCs rotations, but no relevant information could be obtained.

Finally, CA was applied to the samples, using the Euclidean distance as a measure for similarity among samples and the average linkage as the linking method for the clusters. The results (Fig. 2.d) were consistent with those described for PCA: tea with seaweed infusions formed a distinct cluster, whereas in the cluster of rooibos samples a black tea sample was included and no definite clusters were found for black and green teas. Further investigation of a variety of distances and linking methods (not shown here) did not lead to any other valuable results.

4. Conclusions

This work demonstrates that HR-CS FAAS can be an useful tool for the determination of trace elements in the brewed samples analysed, providing suitable sensitivity, accuracy and precision, as well as other important characteristics (e.g., low cost and great simplicity) in comparison to other techniques such as ICP-OES.

Even when the results arising from the chemometric study of samples can only be regarded as preliminary, rooibos and tea with seaweed brewed samples were readily distinguishable on the basis of their metallic profile (at least, for the analytes considered). The origin of such assorted metallic contents may lie in characteristics such as the geographical origin of the samples, soils

where the plants grow, plant species, climate and, where applicable, type and amount of seaweed mixed with the tea. With the variables studied in this work, it was not possible to classify the black and green teas.

Future work should include more information about the samples (especially regarding the factors that affect the metallic profile), the study of the extraction rates of the brewing procedure (to enable the determination of analytes on the original vegetal materials), speciation analyses and, of course, the analysis of a larger number of samples.

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Table 1

Instrumental measurement conditions for the different analytes.

Analyte	Wavelength (nm)	Air (l/h)	C_2H_2 (l/h)	Burner height (mm)
Ca	422.6728	215	50	6
Co	240.7254	230	50	9
Cu	324.7540	210	50	6
Fe	248.3270	230	60	6
Mn	279.4817	230	55	8
Ni	232.0030	230	45	9
Na	588.9953	245	45	7
Zn	213.8570	230	50	6

No. of pixels used for measurements for all analytes: 3; principle: atomic absorption, except for Na, determined by atomic emission measurements.

Table 2

a. Effect of the addition of hydrochloric acid on the sensitivity.

Analyte	Slope (ml/μg) of the calibration line	
	Without hydrochloric acid	With hydrochloric acid
Ca	$3.824 \cdot 10^{-2}$	$4.258 \cdot 10^{-2}$
Co	$9.517 \cdot 10^{-2}$	$1.073 \cdot 10^{-1}$
Cu	$1.641 \cdot 10^{-1}$	$1.601 \cdot 10^{-1}$
Fe	$3.280 \cdot 10^{-2}$	$7.567 \cdot 10^{-2}$
Mn	$3.107 \cdot 10^{-1}$	$2.159 \cdot 10^{-1}$
Ni	$8.734 \cdot 10^{-2}$	$8.923 \cdot 10^{-2}$
Na	$3.630 \cdot 10^{-1}$	$3.424 \cdot 10^{-1}$
Zn	$2.819 \cdot 10^{-1}$	$3.407 \cdot 10^{-1}$

b. Analytical recoveries obtained for each analyte and type of brewed sample.

Analyte	Analytical recovery (%)				
	Black tea	Green tea	Rooibos	Tea with seaweed	Mean
Ca	90.3	108.8	102.9	102.4	101.1
Co	101.5	109.8	101.7	108.3	105.3
Cu	105.3	101.6	99.9	103.6	102.6
Fe	98.1	103.4	99.9	101.3	100.7
Mn	100.3	88.0	94.5	99.9	95.7
Ni	103.0	106.0	98.7	104.2	103.0
Na	107.6	107.1	102.6	103.9	105.3
Zn	94.4	105.0	104.3	103.2	101.7

c. Sensitivity (i.e., slope of the standard additions line) and limits of detection and quantification (both limits referred to brewed sample) obtained for each analyte and type of brewed sample.

Analyte	Black tea			Green tea			Rooibos			Tea with seaweed		
	Slope (ml/µg)	LOD (µg/l)	LOQ (µg/l)	Slope (ml/µg)	LOD (µg/l)	LOQ (µg/l)	Slope (ml/µg)	LOD (µg/l)	LOQ (µg/l)	Slope (ml/µg)	LOD (µg/l)	LOQ (µg/l)
Ca	$2.689 \cdot 10^{-2}$	36.2	120.5	$1.246 \cdot 10^{-2}$	77.8	259.3	$3.522 \cdot 10^{-2}$	27.6	92.1	$8.243 \cdot 10^{-3}$	118.6	395.3
Co	$1.053 \cdot 10^{-1}$	8.2	27.4	$1.091 \cdot 10^{-1}$	7.9	26.5	$1.078 \cdot 10^{-1}$	8.0	26.8	$1.082 \cdot 10^{-1}$	8.0	26.7
Cu	$1.493 \cdot 10^{-1}$	3.4	11.5	$1.695 \cdot 10^{-1}$	3.0	10.1	$1.610 \cdot 10^{-1}$	3.2	10.6	$1.690 \cdot 10^{-1}$	3.0	10.1
Fe	$4.462 \cdot 10^{-2}$	25.8	85.9	$5.307 \cdot 10^{-2}$	21.7	72.2	$4.194 \cdot 10^{-2}$	27.4	91.5	$4.518 \cdot 10^{-2}$	25.4	84.8
Mn	$1.310 \cdot 10^{-1}$	2.3	7.6	$2.525 \cdot 10^{-1}$	1.2	3.9	$1.973 \cdot 10^{-1}$	1.5	5.0	$2.540 \cdot 10^{-1}$	1.2	3.9
Ni	$8.152 \cdot 10^{-2}$	16.5	55.1	$8.922 \cdot 10^{-2}$	15.1	50.3	$8.116 \cdot 10^{-2}$	16.6	55.3	$8.016 \cdot 10^{-2}$	16.8	56.0
Na	$1.917 \cdot 10^{-1}$	35.8	119.2	$1.663 \cdot 10^{-1}$	41.2	137.4	$1.172 \cdot 10^{-1}$	58.5	195.0	$2.686 \cdot 10^{-2}$	254.9	849.6
Zn	$2.800 \cdot 10^{-1}$	21.2	70.8	$3.403 \cdot 10^{-1}$	17.5	58.2	$3.168 \cdot 10^{-1}$	18.8	62.6	$3.549 \cdot 10^{-1}$	16.8	55.8

d. Repeatability of the measurements for each analyte and type of brewed sample.

Type of sample	RSD (%)						
	Ca	Cu	Fe	Mn	Na	Zn	Mean
Black tea	2.0	5.7	1.3	1.7	1.3	3.3	2.6
Green tea	0.7	2.7	[a]	1.1	1.6	[a]	1.5
Rooibos	0.4	0.2	3.7	1.4	0.4	[a]	1.2
Tea with seaweed	3.6	3.5	15.1	2.6	0.3	3.2	4.7

[a] Data not available.

Table 3

a. Concentrations obtained for each analyte in the brewed samples).

Sample[a]	Concentration of analyte[b] (µg/l, unless otherwise stated) in the brewed samples							
	Ca	Co	Cu	Fe	Mn	Ni	Na	Zn
B5	< LOQ	< LOD	59.7	< LOQ	n.m.	< LOQ	n.m.	129.2
B7	n.m.	< LOD	51.9	< LOD	1.4[b]	< LOQ	373.7	111.2
B8	402.0	< LOD	73.4	< LOQ	n.m.	< LOQ	148.9	163.0
B13	n.m.	< LOD	55.5	< LOD	1.6[b]	< LOQ	n.m.	126.1
B14	822.6	< LOD	53.1	< LOD	n.m.	< LOQ	453.9	158.0
B15	167.5	< LOD	14.3	< LOD	0.6[b]	< LOD	n.m.	< LOD
B16	797.5	< LOD	40.0	< LOQ	n.m.	< LOQ	242.4	102.6
B18	811.6	< LOD	47.3	< LOD	n.m.	< LOQ	161.7	122.9
B30	355.2	< LOD	36.6	< LOQ	1.6[b]	< LOQ	< LOD	121.4
B34	< LOD	< LOD	57.6	< LOQ	1.9[b]	< LOQ	n.m.	< LOQ
G10	286.4	< LOD	64.0	74.4	n.m.	54.2	< LOQ	94.7
G11	533.1	< LOD	56.4	< LOQ	n.m.	< LOQ	< LOD	100.8
G17	469.5	< LOQ	< LOD	< LOD	0.3[c]	< LOD	< LOD	< LOD
G19	n.m.	< LOD	22.1	< LOD	1.1[c]	< LOD	< LOQ	116.6
G20	n.m.	< LOD	21.3	< LOD	1.1[c]	< LOQ	< LOQ	163.2
G21	868.8	< LOD	29.2	< LOD	1.0[c]	212.1	< LOQ	113.1
G22	n.m.	< LOD	26.8	< LOD	1.3[c]	313.0	< LOQ	188.2
G23	n.m.	< LOD	18.1	< LOD	1.1[c]	231.8	n.m.	100.7
G33	n.m.	< LOD	114.0	< LOQ	n.m.	612.9	n.m.	141.2
G35	< LOD	< LOQ	35.7	< LOQ	1.2[c]	85.2	< LOD	< LOQ
R36	2.4[c]	< LOD	< LOQ	< LOQ	155.0	< LOD	2.6[c]	< LOD
R37	0.6[c]	< LOD	14.9	< LOQ	460.3	< LOD	2.2[c]	< LOD
R38	1.4[c]	< LOD	< LOQ	< LOQ	250.7	< LOD	2.5[c]	< LOQ
R40	1.8[c]	< LOD	< LOQ	< LOQ	196.7	< LOD	2.2[c]	< LOD
R41	1.5[c]	< LOD	< LOQ	< LOQ	90.9	< LOD	2.3[c]	< LOD
R42	1.4[c]	< LOD	< LOQ	< LOD	153.5	< LOD	2.5[c]	< LOD
R43	0.8[c]	< LOD	< LOD	< LOD	80.8	< LOD	1.8[c]	< LOD
R44	1.4[c]	< LOD	< LOD	< LOD	82.0	< LOD	2.0[c]	< LOD
R45	1.5[c]	< LOD	< LOQ	< LOD	118.8	< LOD	1.9[c]	< LOD
R46	3.0[c]	< LOQ	< LOQ	< LOQ	448.5	< LOQ	2.3[c]	< LOQ
TS1	2.1[c]	< LOD	< LOQ	< LOQ	n.m.	< LOQ	9.7[c]	66.9
TS2	4.7[c]	< LOD	11.8	< LOD	n.m.	< LOQ	8.3[c]	< LOQ
TS3	n.m.	< LOD	< LOQ	< LOQ	62.4	< LOQ	n.m.	< LOQ
TS4	n.m.	< LOD	13.3	< LOQ	n.m.	< LOQ	8.6[c]	64.9
TS39	7.9[c]	< LOQ	16.4	< LOQ	75.8	59.1	11.3[c]	< LOQ

[a] "B": black tea; "G": green tea; "R": rooibos; "TS": tea with seaweed. The numbers are used for identification. [b] < LOD: the concentration measured is below the Limit of Detection; < LOQ: the concentration measured is between the Limits of Detection and Quantification; n.m.: not measured.

[c] Concentration expressed in µg/ml.

b. Data of Table 3.a completed by replacing "n.m." data by the mean value of the corresponding variable within each type of brewed sample (black tea, green tea, rooibos and tea with seaweed); "< LOD" data by the LOD value for each analyte and type of sample and "< LOQ" data by the measured values.

Sample[a]	Concentration of analyte (µg/l) in the brewed samples							
	Ca	Co	Cu	Fe	Mn	Ni	Na	Zn
B5	44.3	8.2	59.7	49.6	1421.9	35.4	236.1	129.2
B7	429.6	8.2	51.9	25.8	1413.9	37.4	373.7	111.2
B8	402.0	8.2	73.4	32.9	1421.9	39.5	148.9	163.0
B13	429.6	8.2	55.5	25.8	1620.8	28.8	236.1	126.1
B14	822.6	8.2	53.1	25.8	1421.9	32.2	453.9	158.0
B15	167.5	8.2	14.3	25.8	553.7	16.5	236.1	21.2
B16	797.5	8.2	40.0	37.2	1421.9	44.7	242.4	102.6
B18	811.6	8.2	47.3	25.8	1421.9	53.4	161.7	122.9
B30	355.2	8.2	36.6	51.8	1616.3	49.5	35.8	121.4
B34	36.2	8.2	57.6	57.0	1905.0	48.3	236.1	25.4
G10	286.4	7.9	64.0	74.4	1016.4	54.2	126.8	94.7
G11	533.1	7.9	56.4	22.8	1016.4	45.8	41.2	100.8
G17	469.5	21.8	3.0	21.7	287.7	15.1	41.2	17.5
G19	447.1	7.9	22.1	21.7	1128.4	15.1	94.5	116.6
G20	447.1	7.9	21.3	21.7	1051.2	37.8	74.5	163.2
G21	868.8	7.9	29.2	21.7	988.5	212.1	54.5	113.1
G22	447.1	7.9	26.8	21.7	1276.1	313.0	118.6	188.2
G23	447.1	7.9	18.1	21.7	1148.9	231.8	74.1	100.7
G33	447.1	7.9	114.0	31.8	1016.4	612.9	74.1	141.2
G35	77.8	14.8	35.7	38.1	1234.3	85.2	41.2	56.9
R36	2353.7	8.0	7.1	60.8	155.0	16.6	2582.3	18.8
R37	609.8	8.0	14.9	39.4	460.3	16.6	2186.3	18.8
R38	1357.1	8.0	8.4	90.3	250.7	16.6	2472.6	23.9
R40	1816.0	8.0	7.8	45.6	196.7	16.6	2188.5	18.8
R41	1534.7	8.0	5.8	32.7	90.9	16.6	2338.7	18.8
R42	1366.3	8.0	6.0	27.4	153.5	16.6	2513.8	18.8
R43	782.0	8.0	3.2	27.4	80.8	16.6	1815.7	18.8
R44	1379.5	8.0	3.2	27.4	82.0	16.6	1984.3	18.8
R45	1506.9	8.0	5.0	27.4	118.8	16.6	1919.2	18.8
R46	3023.4	9.5	4.2	90.1	448.5	44.9	2330.6	52.9
TS1	2087.5	8.0	9.4	36.6	69.1	20.6	9686.4	66.9
TS2	4696.6	8.0	11.8	25.4	69.1	23.4	8341.4	48.2
TS3	4887.9	8.0	4.3	43.0	62.4	20.3	9465.1	36.3
TS4	4887.9	8.0	13.3	62.5	69.1	19.5	8575.0	64.9
TS39	7879.7	9.8	16.4	44.6	75.8	59.1	11257.5	30.2

[a] "B": black tea; "G": green tea; "R": rooibos; "TS": tea with seaweed. The numbers are used for identification.

Table 4

a. Correlation matrix of variables.

	Ca	Co	Cu	Fe	Mn	Ni	Na	Zn
Ca	1.0000							
Co	- 0.0588	1.0000						
Cu	- 0.4123	- 0.1466	1.0000					
Fe	0.2545	- 0.0829	-0.0716	1.0000				
Mn	- 0.6101	- 0.0765	0.7398	- 0.1499	1.0000			
Ni	- 0.1713	- 0.0787	0.5274	- 0.1762	0.2527	1.0000		
Na	0.9049	- 0.0964	- 0.4380	0.2025	- 0.6579	- 0.2213	1.0000	
Zn	- 0.3649	- 0.2175	0.6596	- 0.2804	0.7360	0.4364	- 0.3997	1.0000

b. Component matrix of the PCs obtained by PCA. No rotation of PCs was applied.

Original variable	Component number							
	1	2	3	4	5	6	7	8
Ca	- 0.768	0.495	0.180	0.137	0.259	0.005	0.122	- 0.179
Co	- 0.094	- 0.674	0.283	0.613	0.279	0.040	- 0.026	0.009
Cu	0.808	0.334	- 0.068	0.250	0.116	-0.368	- 0.133	- 0.046
Fe	- 0.320	0.226	- 0.788	0.450	- 0.069	0.134	- 0.025	0.015
Mn	0.885	0.016	- 0.207	-0.006	0.313	-0.025	0.264	0.071
Ni	0.513	0.411	0.421	0.411	- 0.452	0.092	0.085	0.034
Na	- 0.795	0.473	0.198	0.042	0.234	- 0.071	- 0.037	0.206
Zn	0.794	0.339	0.116	- 0.085	0.316	0.334	- 0.146	- 0.015

c. Coefficient matrix for obtaining the scores of the objects on the PCs.

Original variable	Component number							
	1	2	3	4	5	6	7	8
Ca	- 0.210	0.361	0.178	0.164	0.418	0.017	0.914	-2.153
Co	- 0.026	-0.492	0.280	0.732	0.450	0.142	- 0.198	0.112
Cu	0.220	0.244	- 0.067	0.299	0.187	- 1.309	- 0.995	- 0.550
Fe	- 0.087	0.165	- 0.780	0.537	- 0.111	0.477	- 0.188	0.182
Mn	0.242	0.011	- 0.205	- 0.007	0.504	- 0.088	1.979	0.857
Ni	0.140	0.300	0.416	0.491	- 0.730	0.328	0.634	0.406
Na	- 0.217	0.345	0.196	0.051	0.377	- 0.253	- 0.276	2.467
Zn	0.217	0.247	0.115	- 0.101	0.510	1.190	- 1.096	- 0.185

40 **d.** Total variance explained after PCA.

Component	Variance explained		
	Eigenvalue	Proportion (%)	Cumulative (%)
1	3.664	45.80	45.80
2	1.370	17.13	62.93
3	1.011	12.63	75.56
4	0.838	10.47	86.03
5	0.620	7.75	93.78
6	0.281	3.51	97.29
7	0.133	1.67	98.96
8	0.083	1.04	100.00

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Fig. 1.a.

Draftsman plot for the correlation between variables (data included in Table 3.b) for the different brewed samples.(“B”: black tea; “G”: green tea; “R”: rooibos; “TS”: tea with seaweed).

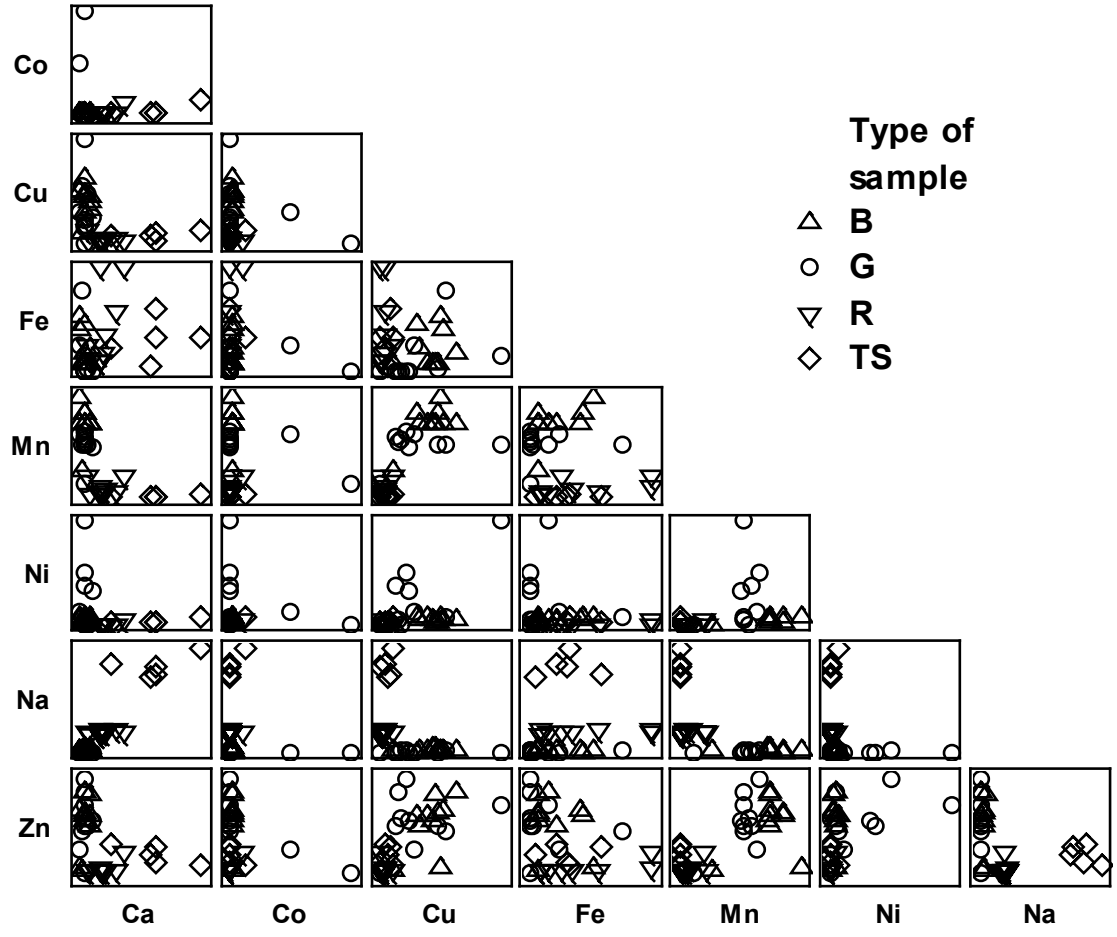

Fig. 1.b.

Dendrogram resulting from CA of the original variables, using average linkage method and the value of Pearson correlation coefficient to calculate the similarity.

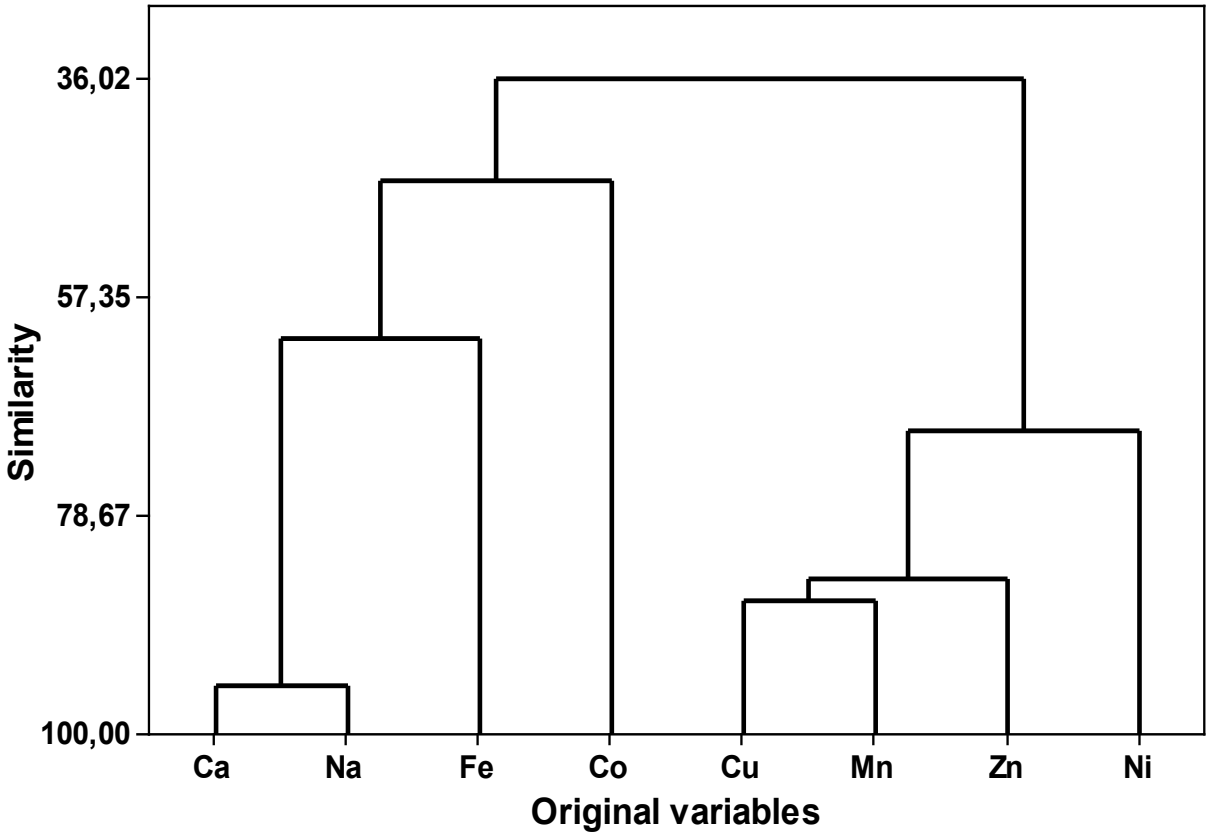

Fig. 1.c.

Component plot of the first three PCs.

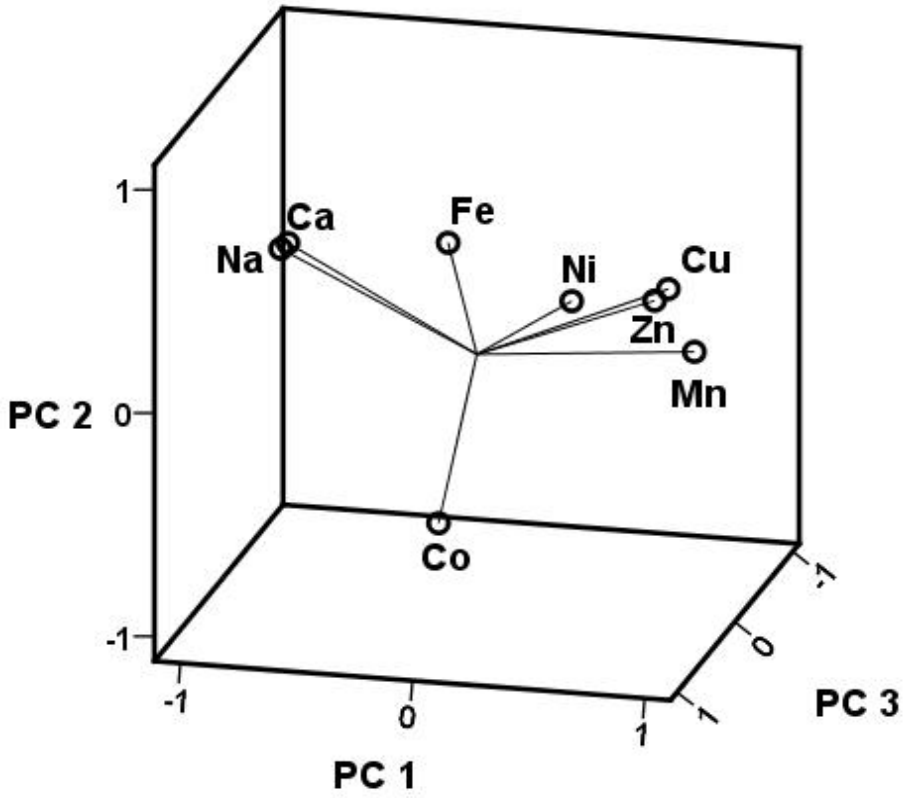

Fig. 1.d.

Scree plot of the first three PCs.

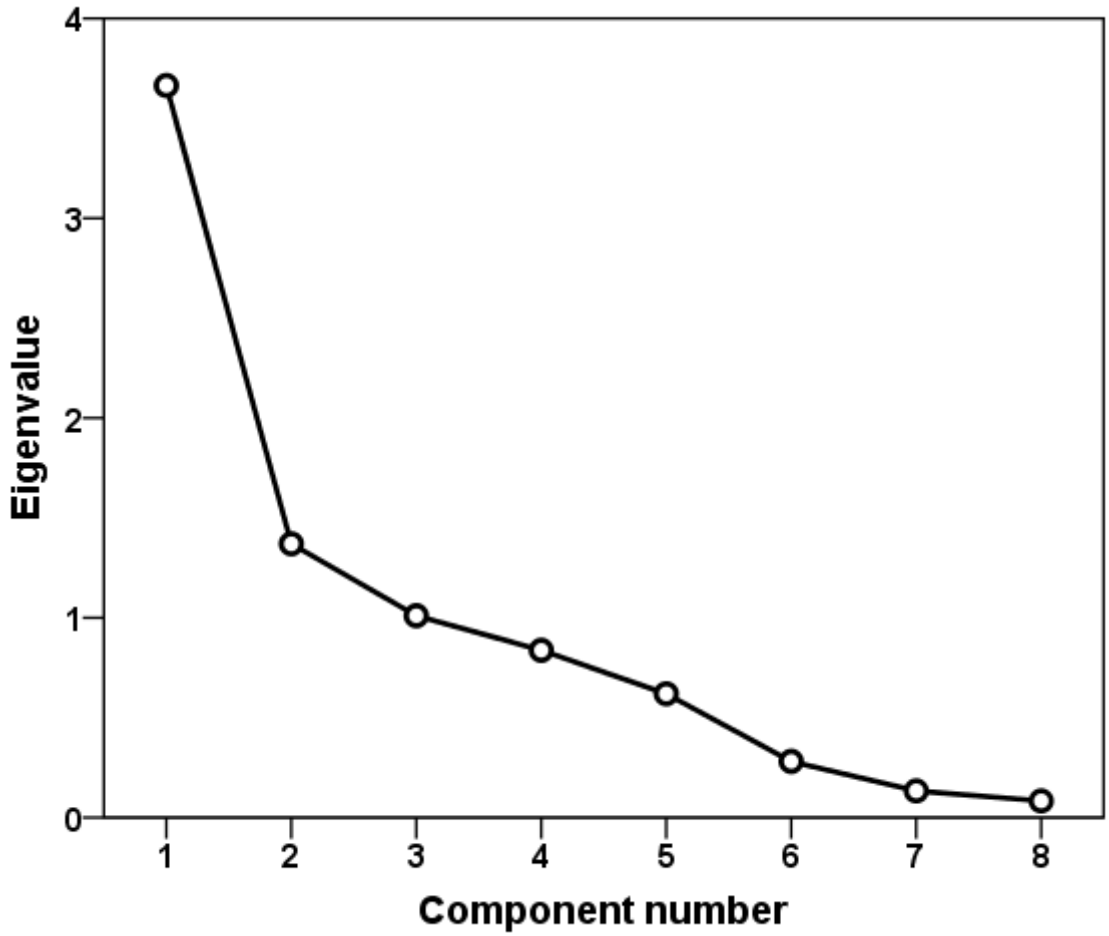

Fig. 2.a.

Two-dimensional scatter plots of the first four PCs. “B”: black tea; “G”: green tea; “R”: rooibos; “TS”: tea with seaweed.

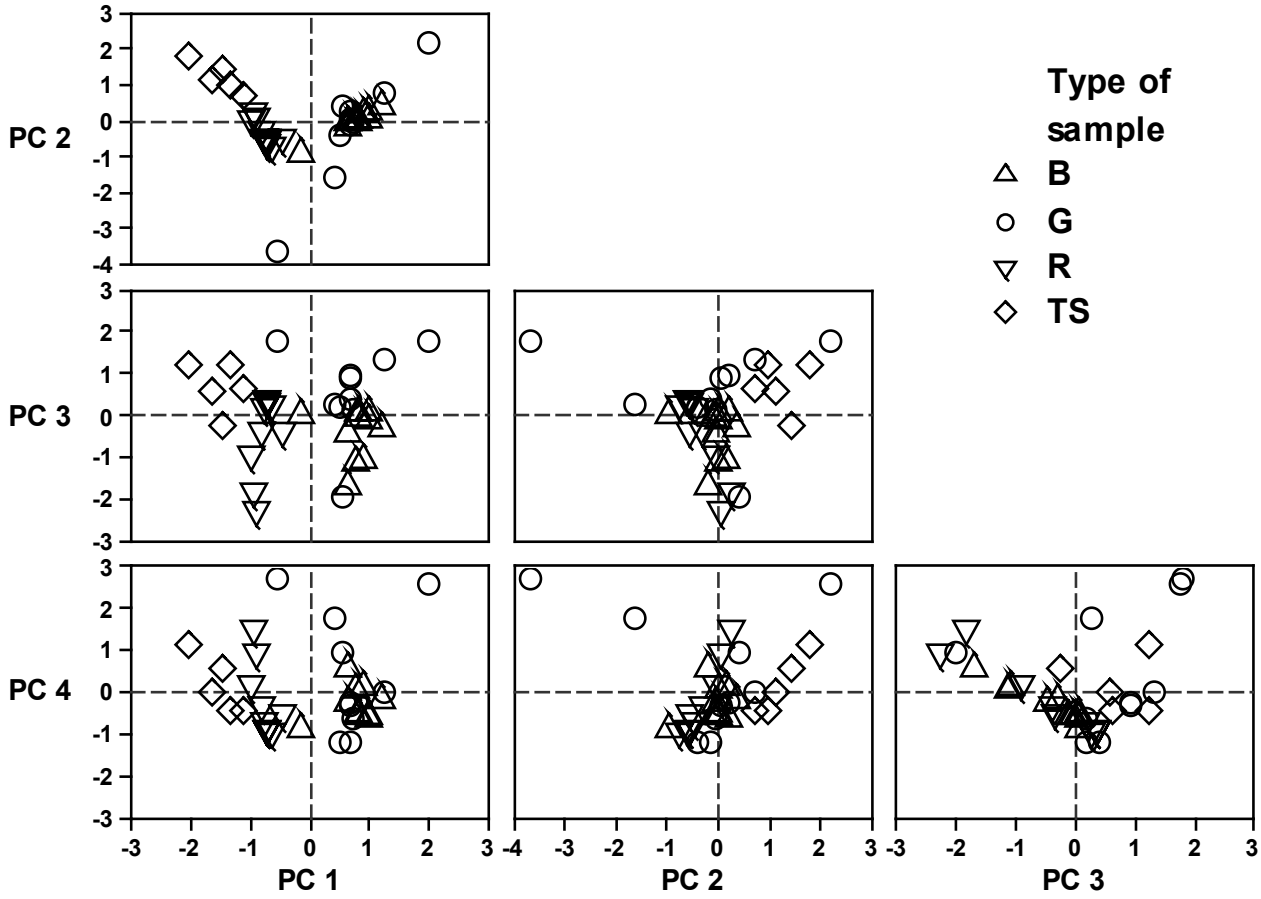

Fig. 2.b.

Three-dimensional scatter plot of the first three PCs. Dotted lines mark TS and R brewed sample groups.

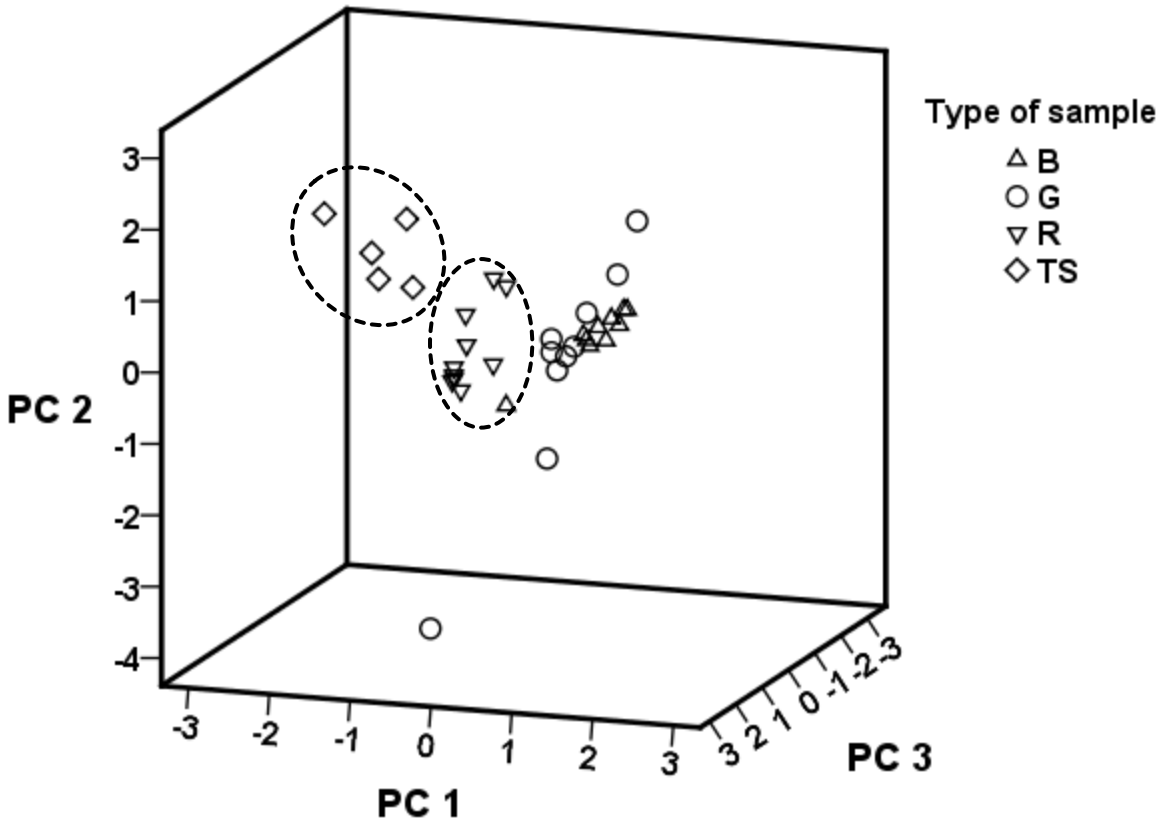

Fig. 2.c.

Three-dimensional scatter plot of PC 1, PC 2 and PC 4. Dotted lines mark TS and R brewed sample groups, and the arrow marks one B infusion among R infusions. Detail corresponds to PC 1 vs PC 2 scatter plot included in Fig. 2.a.

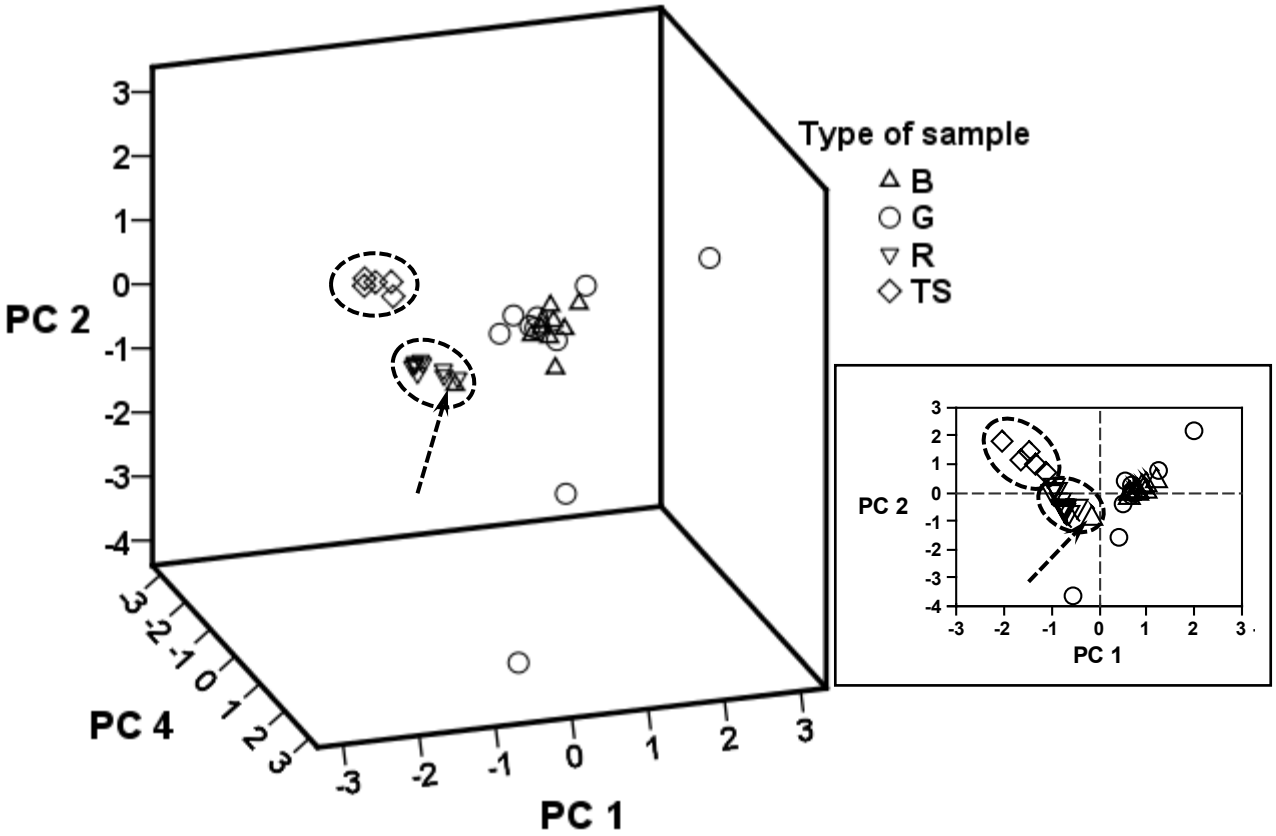

Fig. 2.d.

Dendrogram obtained by CA (Euclidean distance, average linkage) of the samples analysed. R and TS clusters are pointed out on x-axis.