

Differentiation of Tea (*Camellia sinensis*) Varieties and Their Geographical Origin According to their Metal Content

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The metal content of 46 tea samples, including green, black, and instant teas, was analyzed. Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, Sr, Ti, and Zn were determined by ICP–AES. Potassium, with an average content of 15145.4 mg kg⁻¹ was the metal with major content. Calcium, magnesium, and aluminum had average contents of 4252.4, 1978.2, and 1074.0 mg kg⁻¹, respectively. The average amount of manganese was 824.8 mg kg⁻¹. There were no clear differences between the metal contents of green and black teas. Pattern recognition methods such as principal component analysis (PCA), linear discriminant analysis (LDA), and artificial neural networks (ANN), were applied to differentiate the tea types. LDA and ANN provided the best results in the classification of tea varieties. These chemometric procedures were also useful for distinguishing between Asian and African teas and between the geographical origin of different Asian teas.

Keywords: Tea; *Camellia sinensis*; atomic spectroscopy; metals; chemometrics; pattern recognition; artificial neural networks

INTRODUCTION

Tea is one of the most consumed beverages in the world and is prepared from the leaves of the shrub *Camellia sinensis*. Green and black teas are the two most popular types. Drying and roasting the leaves produces green tea; black tea is obtained after a fermentation process. Considering that an estimated amount of 18–20 billion teacups are consumed daily in the world (1) its economic and social interest is clear. The therapeutic value of tea for the prevention and treatment of many diseases has become more and more commonly known (2–5). Tea contains flavonoids, minerals, and trace elements that are essential to human health, and, consequently, tea drinking could be an important source of some essential minerals such as manganese, which activates numerous essential enzymes (6). Other food stuffs contain relatively small amounts of manganese. Because of the great importance of the minerals present in tea, many studies have been carried out to determine their levels in tea leaves and their infusions. Several techniques such as inductively coupled plasma–atomic optical emission spectrometry (ICP–AES) (7, 8), ICP–mass spectrometry (9), electrothermal atomic absorption spectroscopy (9), and total-reflection X-ray fluorescence (6) have been used for determining metals in tea samples. Though the chemical composition of the tea varieties is similar, it is possible to find parameters that may differentiate them. A better discrimination may be obtained by using multivariate analysis in combination with some chemometric procedures. Several attempts have been made to apply this to tea samples (1, 10, 11). The metal content can be very adequate for these purposes. The main sources of trace metals to plants are their growth media; consequently, some differences in the metal

content of samples with different origins could be inferred.

In the present paper the content of Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, Sr, Ti, and Zn in tea samples has been determined by using acid digestion followed by ICP–AES. Principal component analysis (PCA), linear discriminant analysis (LDA), and artificial neural networks (ANN) trained by back-propagation have been applied to classify green, black, and instant teas according to their metal content. The supervised pattern recognition methods LDA and ANN permit the differentiation of tea varieties according to their metal content. The metal profile in combination with LDA also has been proved to be useful to differentiate the geographic origins of the studied tea samples.

MATERIALS AND METHODS

Apparatus. A Fisons-ARL 3410 inductively coupled plasma atomic emission spectrometer (FISONS Instruments, Valencia, CA) equipped with a Minitorch burner and a Meinhard nebulizer was used for metal determinations. The operating parameters are shown in Table 1.

Reagents and Standard Solutions. Nitric and perchloric acids (Merck, Darmstadt, Germany) were of analytical grade. A multi-element standard (1000 mg L⁻¹) for all the analyzed metals except titanium was obtained from Merck. Titanium 1000 mg L⁻¹ solution was prepared according to the Perkin-Elmer Pure Atomic Spectroscopy Standards guidelines (Perkin-Elmer Corp., Norwalk, CT). Working standard solutions were prepared by serial dilution of the standards. All aqueous solutions and dilutions were prepared with ultrapure water (Milli-Q, Millipore, Bedford, MA).

Samples. Forty-six commercial tea samples were selected for this study. All of them were obtained from markets and herbalists. Their types and countries of origin, when known, as well as their corresponding sample codes, are shown in Table 2. These tea samples comprised 21 green, 23 black, and 2 instant teas. The geographic origin of 24 samples was known: 12 were from China, 3 were Japanese, 3 were Indian, 2 were Kenyan, and 4 were from Sri Lanka. Accordingly, an identification code was assigned to each sample. The code

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Table 1. Operating Parameters for ICP–AES

RF frequency	27.12 MHz
operating power	650 w
coolant argon flow rate	7.5 L min ⁻¹
plasma argon flow rate	0.8 L min ⁻¹
burner type	Minitorch
nebulizer type	Meinhard
sample flow rate	2.3 mL min ⁻¹
Detection wavelengths/nm	
Al	396.152
Ba	455.403
Ca	393.366
Cu	324.754
Fe	259.940
K	766.490
Mg	279.553
Mn	257.610
Na	589.592
Sr	407.771
Ti	334.941
Zn	213.856

Table 2. Analyzed Tea Samples

code	origin	class ^a
1G	unknown	green
2G	unknown	green
3GCh	China	green
4G	unknown	green (Mint)
5GCh	China	green
6G	unknown	green
7G	unknown	green
8GCh	China	green (Jasmine)
9GCh	China	green (Gunpowder)
10GJ	Japan	green
1BI	India	black (Darjeeling)
11G	unknown	green (Earl Grey)
12GCh	China	green (Chum Mee)
13GJ	Japan	green (Sencha)
14GJ	Japan	green
15GCh	China	green (Gunpowder)
16GCh	China	green (Genmaicha)
17G	unknown	green
18G	unknown	green
19GCh	China	green (Gunpowder)
20GCh	China	green (Jasmine)
21GCh	China	green (Jasmine)
2B	unknown	black (Earl Grey)
3BCh	China	black
4B	blend	black (English breakfast)
5B	unknown	black (Teekane)
6BS	Sri Lanka	black (Ceylon)
7BI	India	black (Assam)
8BS	Sri Lanka	black (Ceylon)
9B	blend	black
10BK	Kenya	black
11B	blend	black
12B	blend	black (Earl Grey)
13B	unknown	black
14B	unknown	black (Lemon)
15B	unknown	black
16B	unknown	black
17B	blend	black (Teekanee)
18B	blend	black
19BCh	China	black
20BS	Sri Lanka	black (Ceylon)
21BS	Sri Lanka	black (Ceylon)
22BI	India	black (Assam)
23BK	Kenya	black
1I	unknown	Instant
2I	unknown	Instant

^a Some additional known characteristics of the teas are included in parentheses.

consisted of a correlative number followed by a letter (G for green teas, B for the black ones, and I for instant teas; Ch for Chinese teas, J for Japanese teas, K for Kenyan teas, and S for teas from Sri Lanka).

Analytical Procedure. Dried tea samples were mineralized according to a modification of the Lamble and Hill method

(8) as follows: assay portions of 0.50 g of tea sample were accurately weighed into a clean 100-mL beaker. Concentrated nitric acid (10 mL) was added, the beaker was covered with a watch glass, and the material was boiled gently on a laboratory sand bath coupled with a tuneable thermostat until digestion was complete. This process took approximately 1 h. A 1-mL portion of 70% perchloric acid was then added, and heating was continued for 1 h. Small aliquots of milli-Q purified water were added to prevent dryness evaporation. After the digest was cooled, it was filtered and transferred to a 100-mL volumetric flask that had been rinsed with ultrapure water. This digestion procedure was validated by using the reference certified material NIES No. 7 ("Tea leaves") (8). Accordingly, with the digestion procedure being validated, no further studies about the possible matrix effects were carried out. Three replicate digestions were made for each tea. Three blanks were prepared in an identical way but omitting the sample. The average of blank ICP–AES signals was subtracted from analytical signals of digested samples after interpolation on calibration graphs. To express the results on a dry basis the moisture of the samples was determined before analysis.

Data Analysis. Each tea sample was considered as an assembly of twelve variables (i.e., the contents of the analyzed metals) which constitute their chemical descriptors. A data matrix of 44 rows (tea samples) and twelve columns, which are the mentioned descriptors, was built for use in the chemometric calculations. The two samples of instant teas have been considered as outliers and have not been included in the data matrix because of their difference with the rest of the tea samples. Pattern recognition methods have been applied to the data set, including visualization methods such as principal component analysis (PCA) (12) and supervised learning methods for classification such as linear discriminant analysis (LDA) (13) and artificial neural networks (ANN) trained by back-propagation (14). The statistical package STATISTICA 99 from StatSoft (15), including the neural network module, was used for pattern recognition calculations.

RESULTS AND DISCUSSION

Metal Content. The contents of the metals mentioned in the previous section were determined in the 46 tea samples. The results, expressed as mg kg⁻¹ on a dry basis, are shown in Table 3. It can be seen that potassium is the metal with major content in the analyzed samples, with an average content of 15145.4 mg kg⁻¹; and in the case of instant teas, its content is higher, with an average value of 38670.3 mg kg⁻¹. Calcium, magnesium, and aluminum have average contents of 4252.4, 1978.2, and 1074.0 mg kg⁻¹, respectively. Lower values for these metals were obtained for instant teas, namely 295.0, 3577.5, and 614.6 mg kg⁻¹, respectively. Another important element present in tea is manganese with an average amount of 824.8 mg kg⁻¹. The other analyzed metals in tea leaves mostly appear with values less than those mentioned above. As can be seen, clear differences appear in the metal contents of the tea leaves with respect to the instant teas, meanwhile no apparent distinction appears between green and black teas. Thus, to achieve a more reliable tea differentiation, pattern recognition procedures are applied to the data matrix.

Principal Component Analysis. For a better understanding of the discriminating efficiency of the descriptors, a preliminary study based on PCA has been carried out, despite the a priori knowledge of the class membership of the tea samples.

The most important use of this chemometric method is to represent the *n*-dimensional data set in a smaller number of dimensions, usually two or three. This allows the observation of groupings of cases, which can define

Table 3. Metal Content (mg kg⁻¹ dry basis) of Tea Samples^a

sample	Zn	Mn	Fe	Mg	Cu	Ti	Al	Sr	Ca	Ba	Na	K
1GCh	31.7	667.9	321.8	2080.4	16.4	19.8	1099.2	11.5	4044.5	6.6	80.2	15031.2
2G	34.9	804.1	269.6	2241.3	17.8	15.5	1258.9	18.3	6324.0	10.6	164.1	14776.1
3GCh	26.6	1595.4	259.1	2160.0	19.4	14.3	2230.7	23.1	5630.2	6.6	42.6	12600.8
4G	33.0	675.4	2036.7	4272.5	24.0	262.9	2560.4	72.4	Nd ^b	8.8	1760.0	15088.3
5GCh	28.7	977.5	345.2	2148.3	21.7	19.6	1695.8	34.9	5044.2	8.0	81.2	14209.9
6G	30.6	869.0	352.2	2008.3	14.1	33.4	928.1	12.2	3055.3	9.0	64.0	16111.9
7G	26.6	881.0	247.4	1968.1	15.6	20.8	1063.9	17.1	4691.7	9.5	100.1	14996.2
8GCh	36.8	683.1	239.3	1780.7	20.0	15.9	796.5	10.4	2893.3	9.9	63.2	15660.6
9GCh	31.7	774.7	324.7	1899.8	14.3	23.2	904.3	12.4	2905.0	7.6	53.2	15885.1
10GJ	21.0	660.7	193.0	2066.4	9.4	12.3	927.6	12.0	4029.1	1.3	130.9	14610.0
1BI	37.7	460.7	170.9	1872.9	24.4	8.1	465.9	10.7	3844.3	10.2	54.5	16359.8
11G	22.8	744.7	147.6	2041.7	12.5	7.7	850.5	10.6	3760.8	12.8	77.3	14948.4
12GCh	26.8	838.8	290.9	1950.6	16.4	18.2	991.6	11.8	3730.4	10.4	201.7	14865.6
13GJ	24.6	1069.7	144.5	1778.5	10.2	5.7	952.5	11.8	3984.4	9.7	33.9	13284.3
14GJ	25.2	928.9	140.9	1814.2	9.7	5.2	919.5	13.6	3618.1	7.3	37.7	11900.5
15GCh	25.2	1317.2	196.6	1489.5	13.5	5.9	1122.3	13.9	3641.3	9.2	66.5	14031.5
16GCh	18.7	971.1	108.5	2021.8	19.2	0.6	1472.5	10.7	5193.3	10.4	44.3	12242.6
17G	24.6	1225.0	257.5	1933.7	14.8	13.0	1177.8	18.7	4297.8	2.9	46.3	14194.8
18G	22.8	1496.6	272.9	1691.0	15.9	14.0	1327.2	12.5	4932.4	9.2	60.1	12758.6
19GCh	28.5	972.6	239.6	1770.9	14.8	14.3	828.8	16.9	3637.3	12.3	59.1	14274.3
20GCh	45.3	691.6	204.0	1894.0	25.4	10.7	745.3	16.4	3998.1	15.0	109.4	16994.1
21GCh	51.0	777.0	245.0	2210.0	37.0	16.0	911.0	17.0	4536.0	15.0	117.0	15778.0
2B	43.2	1004.1	1000.1	2131.1	31.0	60.7	2065.7	17.3	5475.0	5.7	249.2	14330.5
3BCh	49.6	1114.4	946.2	2184.5	37.5	65.2	2210.1	17.8	5203.3	6.5	349.1	14227.6
4B	35.8	982.6	388.8	1987.3	17.6	26.8	972.7	18.9	4040.5	5.8	69.6	16527.5
5B	25.8	813.5	454.9	1957.2	15.0	69.8	1286.1	25.5	4366.5	6.0	51.4	16899.2
6BS	34.8	225.9	107.1	2009.3	24.6	8.3	793.5	12.9	4642.9	14.0	53.6	16398.0
7BI	26.4	431.4	90.3	2126.6	16.3	2.7	510.8	18.5	4540.5	5.2	76.4	17654.0
8BS	31.5	530.9	121.7	1917.4	17.6	6.5	915.2	23.3	4486.9	7.4	49.4	15939.8
9B	21.9	599.3	182.1	2002.7	14.2	12.0	624.1	24.8	4076.0	7.3	35.1	16335.7
10BK	26.9	874.6	282.4	1738.0	11.8	22.8	704.1	38.7	3212.2	12.8	42.9	17196.2
11B	26.7	462.6	209.3	1778.4	19.8	15.5	724.1	24.9	3596.9	13.3	50.0	16354.2
12B	23.7	843.4	415.7	2028.0	16.9	15.6	1180.5	22.6	5245.4	6.9	65.0	15955.8
13B	39.6	1281.4	759.3	2217.6	37.2	42.3	1891.8	21.0	5525.5	7.1	166.0	15433.9
14B	21.2	756.1	149.5	1737.8	13.3	13.2	861.4	21.9	4359.2	5.4	138.0	16682.7
15B	28.0	1051.6	302.5	1946.7	18.6	22.3	662.1	13.1	4309.9	5.9	47.5	16304.9
16B	23.3	778.8	254.6	1751.1	12.4	24.6	1341.2	33.7	4852.7	5.4	43.4	14133.6
17B	20.8	1003.6	296.7	1809.3	11.4	49.3	1223.5	29.9	4620.4	5.3	75.1	14788.3
18B	23.7	1001.3	285.0	1810.5	15.7	30.3	1135.6	20.9	4550.7	6.7	70.1	13835.5
19BCh	35.8	664.4	176.0	1699.5	22.5	14.5	798.4	11.3	4150.5	7.7	84.0	14840.4
20BS	20.0	315.6	141.5	1717.0	18.2	10.4	549.1	11.5	3335.2	10.7	69.7	16700.3
21BS	36.8	148.0	74.0	1879.4	26.5	2.8	509.8	7.2	3619.4	7.3	43.8	14626.8
22BI	23.9	527.0	108.3	1905.9	15.5	2.9	474.1	15.9	3862.2	7.4	37.9	16022.5
23BK	24.4	797.6	247.2	1611.3	11.1	20.5	593.0	38.0	2990.6	13.3	31.7	14606.7
1I	39.8	1096.9	14.3	3565.8	7.6	0.2	612.6	3.5	338.4	3.5	109.0	38772.4
2I	38.4	1243.3	28.2	3589.1	10.3	0.5	616.5	2.5	251.5	1.5	80.2	38568.1

^a Average of triplicate determinations. % RSD < 8. ^b Nd: not detected.

the structure of the data set. PCA finds the maximum variations in the data set and forms new variables known as principal components (PCs) (16), such that each successive PC accounts for as much of the remaining variability as possible and each new variable must be totally independent of all other variables. In this case, PCA was applied to our data. At a glance, no clear differentiation can be appreciated in the tea samples. Another type of information that can be obtained from PCA is which are the most discriminating of the studied variables. Considering the loadings of the variables for PC1, iron, titanium, sodium, and aluminum are the metals with the most contribution and, hence, the most discriminating power.

Classification by Linear Discriminant Analysis.

As it has been explained in the description of the samples, the data set is composed of 21 green teas and 23 black teas. Because of the a priori knowledge of the class membership of the samples, it is possible to apply supervised pattern recognition methods to the data. In our case, we have 44 samples and two classes: green (G) and black (B). By using linear discriminant analysis (LDA), suitable classification rules for assigning categories to samples can be calculated. By performing linear combinations of the selected descriptors the so-called discriminant functions are obtained which best

separate the classes according to the maximization of the *F*-ratio of between classes sum of squares and within classes sum of squares (17). After applying LDA to our data, one discriminant function, DF1, was calculated as follows:

$$\text{DF1} = -1.16 \text{ Zn} - 0.88 \text{ Mn} + 1.72 \text{ Fe} - 2.18 \text{ Mg} + 1.78 \text{ Cu} + 1.43 \text{ Ti} - 1.80 \text{ Al} + 1.25 \text{ Sr} + 1.19 \text{ Ca} - 0.75 \text{ Ba} - 0.65 \text{ Na} + 0.16 \text{ K}$$

Looking at the a posteriori probabilities, all samples were correctly classified except sample 5GCh, which represented a recognition ability of 97.8%. To evaluate the classification performance, the leave-one-out method (18) was used as a validation procedure. A prediction ability of 93.5% was obtained. LDA being applied strictly speaking to separation of classes with linear boundaries may fail in cases where the distribution pattern is nonlinear. In such a way, ANN methods proved to be very useful.

Classification by Artificial Neural Networks.

Artificial neural networks such as multi layer perceptrons (MLPs) trained by back-propagation (BPNN) are efficient tools for classifying and discriminating food products. The MLP consists of formal neurons and connections (weights) between them. The neurons are

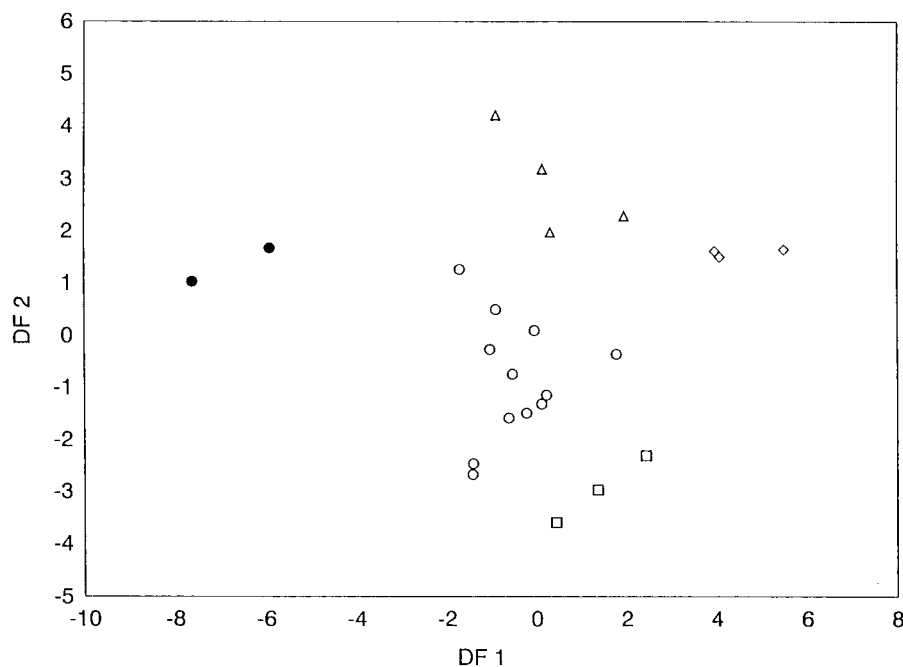


Figure 1. Scatter plot of the two discriminant functions for tea samples with known geographical origin: China (○), Japan (□), India (◇), Sri Lanka (△), and Kenya (●).

arranged in layers (an input layer, one or more hidden layers, and an output layer) and the connections are unidirectional from the input to the output. Adjacent layers are fully connected and no connections between neurons within the same layer exist.

A formal neuron sums up incoming signals (multiplied by the connection weights), subtracts a threshold value (called bias), and calculates the output signal using the so-called transfer function. Neurons can use different transfer functions. Input ones simply distribute the elements of the data matrix row to the hidden layer neurons without any further computation. Hidden layer neurons have a sigmoidal transfer function which limits the neuron's output signal to values between 0 and 1. The output neurons have also a sigmoidal transfer function. Training objects are taken randomly. After each input, all the weights are changed according to the "delta rule". In the back-propagation of errors learning scheme, one pass of all the training objects through the network is called an iteration cycle or an epoch.

In our case we have used a MLP with three layers according to the architecture $12 \times 2 \times 2$, consisting of 12 neurons (the content of metals) in the input layer, a hidden layer with two neurons, and an output layer with two neurons corresponding to the classes to be assigned (B and G).

An important step in the development of a classification strategy is the splitting of the data set into a training data set (utilized to estimate the recalling efficiency) and a validation or test set (used to evaluate the performance of the prediction ability for new samples). This strategy is more complex for the case of neural networks because of the overfitting problem (19). Thus, an additional monitoring set is necessary to stop the training at the suitable number of epochs in order to avoid the learning of idiosyncrasies present in the training data, which leads to overtraining. Accordingly, although the aim of the use of MLP is for illustrative purposes only (because there is no validation) it is possible to select some samples to establish a verification or monitoring set. In our case several verification

sets of samples were selected by considering two class G samples and two class B samples.

When performing our MLP, initial weights were taken randomly within the interval $-0.1, 0.1$. The learning rate (η) and the momentum (μ) were fixed to 0.2 and 0.5, respectively. Target outputs were normalized to 0–1 and written in binary form: 1 0 (class G) and 0 1 (class B). Both training and monitoring errors (as RMS) decreased monotonically with the increasing number of cycles up to 1000 epochs without showing overfitting. The recalling ability of the training sets led to 100% hits. The classification procedure was then validated by applying the leave-one-out method by selecting 1000 epochs and the prediction ability was of 95.6% hits on average. These results are slightly better than those obtained from LDA, and are suitable enough to perform tea classification.

Geographical Classification of Tea Samples.

Considering the tea samples with a known origin, LDA was applied in order to get a separation attending to the geographical origin. In this case, the data set was constituted by 24 samples. Five categories were considered: Kenyan, Chinese, Japanese, Indian, and teas from Sri Lanka. From LDA calculations four discriminant functions were obtained. Figure 1 represents the scatter plot of samples obtained when using as axes DF1 and DF2. There is a clear separation between the African (Kenya) and the Asian teas (China, Japan, Sri Lanka, and India), which appear as two well-differentiated clusters. When observing the Asian group of samples, it can be seen that they are grouped according to their country of origin. All the Chinese teas appear together, well separated from the rest of the Asian samples, and the same occurs for the other three classes of Japanese, Sri Lankan, and Indian teas. According to the a posteriori probabilities, the method has a recognition ability of 100%. In this case and because of the smaller number of samples in this particular data set, it is worthless to apply the leave-one-out method to calculate the prediction ability. Many more samples would be needed to evaluate the prediction ability of the metal contents in

relationship with the geographical origin, though it has been established that these metal parameters are good chemical descriptors to recognize the country of origin of tea samples.

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