Chapter

Elemental Classification of Tea Leaves Infusions: Principal Component, Cluster and Meta-analyses

Francisco Torrens and Gloria Castellano

Abstract

The elemental analysis of 11 teas consumed in Turkey is clustered by principal component analyses (PCAs) of metals and plant cluster analyses (CAs), which agree. Samples group into four classes. Elemental PCA and tea CA allow classifying them and concur. The first PCA axis explains 45%; the first two, 71%; the first three, 85% variance; etc. Different behaviours of teas depend on Cu, etc. They are considered as a good source of Mn, etc. Two elemental classes are distinguished: Cu-K-Mn and Fe-Na-Zn. Teas present adequate elemental contents, good antioxidant capacity and may be used as a functional beverage. They represent plants useful as a natural source for nutraceutical formulations.

Keywords: tea leaf, tea infusion, green tea, black tea, element, phytochemical, cytochemical

1. Introduction

Tea is the second popular beverage and plays a role in intake of nutritional/toxic trace elements [1]. It is used in folk medicine for headache, digestion, diuresis, enhancement of immune defence, as an energizer and to prolong life [2]. Epidemiological and pharmacological studies link its consumption to a risk reduction of cardiovascular diseases (CVDs), high cholesterol, arthritis, osteoporosis and dental caries [3]. Leaves' metallic composition is different according to the type and geological source [4]. The chemical composition of tea and its leaves is object of medical and toxicological studies [5]. Investigations were carried out to determine leaves and infusion mineral levels [6–8]. Its elemental contents were determined via analytical methods (e.g. atomic absorption spectrometry (AAS) [9], inductively coupled plasma (ICP)-atomic emission spectrometry (AES) [10], ICP-mass spectrometry (MS) [11], thermal neutron activation analysis (TNAA) [12], ion chromatography [13]). Microwave digestion less contaminates a sample, minimises volatile analyte losses, uses small acids amounts and shortens digestion times [14–16].

Tea trace elements were determined in producing countries [17–19]. Aksuner et al. reported elemental analysis of teas consumed in Turkey (cf. **Table 1**) [20]. Potassium was suggested to be incorporated within a binding ligand in tea leaves. Sodium content showed variability. Because of its biochemical importance, Mn was the most analysed element in tea leaves. Zinc is responsible for enzymatic processes and involved in the working of genetic materials, proteins and immune reactions. Copper is a micronutrient, but it is phytotoxic at high concentrations. Iron is essential, necessary for haemoglobin formation and oxidative processes of living tissues. Nickel is moderately toxic, but it leads to problems, e.g. respiratory system cancer.

Effects of chronic ingestion of catechin-rich green tea were reported on hepatic gene expression of gluconeogenic enzymes in rats [21]. Effects of a catechin-free fraction derived from green tea on gene expression of enzymes related to lipid metabolism in the mouse liver were informed [22]. Beneficial effects of tea and green tea catechin epigallocatechin-3-gallate on obesity were published [23]. Epigallocatechin-3-gallate was identified as an inhibitor of phosphoglycerate mutase 1 (PGAM1) [24]. The relationship between the phytochemical profile of different teas with relative antioxidant and anti-inflammatory capacities was shown [25]. Antimicrobial activity of tea tree oil vs. pathogenic bacteria and comparison of its effectiveness with eucalyptus oil, lemongrass oil and conventional antibiotics were informed [26]. Earlier publications in *Nereis* classified yams [27], lactic acid bacteria (LABs) [28], fruits [29], food spices [30], chlorogenic acids (CGAs) in coffee [31], methylxanthines, cotinine [32], caffeine (caff), its metabolites, nicotine metabolite [33] and tea compounds [34] by PCA, CA and meta-analysis. The main aim of the present report is to develop code learning potentialities, and since tea elements are more naturally described via varying size-structured representation, find general approaches to information processing. In view of tea ethnomedicinal and nutritional benefits, the objective was to cluster them with PCA/CA, which differentiated metals. Section 2 describes the computational method. Sections 3 and 4 illustrate and discuss the calculation results. Finally, the last section summarises our conclusions.

Sample		Cu[a]	Fe	K	Mn	Na	Ni	Zn
1.	Brand A black tea	0.112	0.240	198	8.17	0.322	<0.200	0.148
2.	Brand C black tea	0.102	0.378	180	6.49	0.380	<0.200	0.130
3.	Brand D black tea	0.143	0.460	194	6.95	0.432	<0.200	0.165
4.	East Black Sea black tea	0.130	0.344	173	7.75	0.298	<0.200	0.137
5.	Pure Ceylon tea	0.126	0.291	167	7.08	0.287	<0.200	0.197
6.	Green tea	0.108	0.270	149	5.41	0.657	<0.200	0.152
7.	Sage tea	0.078	2.85	179	0.552	1.08	<0.200	0.204
8.	Herbal mixture tea	0.071	1.17	171	3.09	4.39	<0.200	0.168
9.	Linden tea	0.090	1.11	185	1.10	0.575	<0.200	0.171
10.	Rosehip tea	<0.060	1.15	86	2.47	0.611	<0.200	0.114
11.	Apple tea	<0.060	0.240	188	1.28	0.598	<0.200	0.102

[a]*Elements:* i_1, *Cu;* i_2, *Fe;* i_3, *K;* i_4, *Mn;* i_5, *Na; and* i_6, *Zn.*

Table 1.
Elemental analysis of tea infusions ($mg{\cdot}L^{-1}$) (n = 3).

2. Computational method

The PCA is a dimension reduction technique [35–40]. From original variables X_j, PCA builds orthogonal variables $\tilde{P}_j$ linear combinations of mean-centred ones $\tilde{X}_j = X_j - \overline{X}_j$ corresponding to eigenvectors of sample covariance matrix $S = 1/(n-1)\sum_{i=1}^{n}(x_i - \overline{x})(x_i - \overline{x})'$. For every loading vector $\tilde{P}_j$, matching eigenvalue $\tilde{l}_j$ of S tells how much data variability is explained: $\tilde{l}_j = \mathrm{Var}\left(\tilde{P}_j\right)$. Loading vectors are sorted in decaying eigenvalues. First k PCs explain most variability. After selecting k, one projects p-dimensional data on to subspace spanned by k loading vectors and computes co-ordinates vs. $\tilde{P}_j$, yielding scores

$$\tilde{\mathrm{t}}_i = \tilde{P}'(\mathrm{x}_i - \overline{\mathrm{x}}) \tag{1}$$

for every $i = 1, \ldots, n$ having trivially zero mean. With respect to original co-ordinate system, projected data point is computed fitting

$$\hat{\mathrm{x}}_i = \overline{\mathrm{x}} + \tilde{P}\tilde{\mathrm{t}}_i \tag{2}$$

Loading matrix $\tilde{P}$ $(p \times k)$ contains loadings column-wise and diagonal one $\tilde{L} = \left(\tilde{l}_j\right)_j$ $(k \times k)$, eigenvalues. Loadings k explain variation:

$$\left(\sum_{j=1}^{k}\tilde{l}_j\right) \Big/ \left(\sum_{j=1}^{p}\tilde{l}_j\right) \geq 80\% \tag{3}$$

The CA encompasses different classification algorithms [41, 42]. The starting point is $n \times p$ data matrix $\mathbf{X}$ containing p components measured in n samples. One assumes data were preprocessed to remove artefacts and missing values imputed. The CA organises samples into small number of clusters such that samples within cluster are similar. Distances l_q between samples $x, x' \in \Re^p$ are

$$\left\|x - x'\right\|_q = \left(\sum_{i=1}^{p}\left|x_i - x_i'\right|^q\right)^{1/q} \tag{4}$$

(e.g. Manhattan, l_1; Euclidean, l_2 distances). Comparing samples, *Pearson's correlation coefficient* (PCC) is advantageous:

$$r\left(x - x'\right) = \frac{\sum_{i=1}^{p}(x_i - \overline{x})\left(x_i' - \overline{x}'\right)}{\left[\sum_{i=1}^{p}(x_i - \overline{x})^2\sum_{i=1}^{p}\left(x_i' - \overline{x}'\right)^2\right]^{1/2}} \tag{5}$$

where $\overline{x} = \left(\sum_{i=1}^{p}x_i\right)/p$ is a measure of mean value for sample x [43–49].

3. Calculation results

Elemental contents of 11 teas from Aksuner et al. were used as data. The PCC matrix $\mathbf{R}$ was computed between plants, and the upper triangle turns out to be

$$\mathbf{R} = \begin{pmatrix} 1.000 & 1.000 & 1.000 & 1.000 & 1.000 & 1.000 & 0.999 & 0.999 & 0.999 & 1.000 & 0.999 \\ & 1.000 & 1.000 & 1.000 & 1.000 & 1.000 & 0.999 & 0.999 & 1.000 & 1.000 & 1.000 \\ & & 1.000 & 1.000 & 1.000 & 1.000 & 0.999 & 0.999 & 1.000 & 1.000 & 1.000 \\ & & & 1.000 & 1.000 & 1.000 & 0.999 & 0.999 & 0.999 & 1.000 & 0.999 \\ & & & & 1.000 & 1.000 & 0.999 & 0.999 & 0.999 & 1.000 & 0.999 \\ & & & & & 1.000 & 0.999 & 1.000 & 1.000 & 1.000 & 1.000 \\ & & & & & & 1.000 & 1.000 & 1.000 & 1.000 & 1.000 \\ & & & & & & & 1.000 & 1.000 & 1.000 & 1.000 \\ & & & & & & & & 1.000 & 1.000 & 1.000 \\ & & & & & & & & & 1.000 & 1.000 \\ & & & & & & & & & & 1.000 \end{pmatrix}$$

Correlations are maximum between teas {1–6} and {7–11} (e.g. $R_{1,2} = R_{7,8} = 1.000$), slightly greater than combining both types, e.g. $R_{1,7} = 0.999$. All are illustrated in the partial correlation diagram (PCD) that could contain high ($r \geq 0.75$), medium ($0.50 \leq r < 0.75$), low ($0.25 \leq r < 0.50$) and zero ($r < 0.25$) partial correlations. All 55 pairs of teas show high partial correlations (cf. **Figure 1**, *red*). The corresponding interpretation is that all teas present similar composition. The PCD is in qualitative agreement with previous results.

The dendrogram of teas according to elemental analysis (cf. **Figure 2**) shows different behaviour depending on metals Cu, Fe, K, Mn, Na and Zn. Four classes are clearly recognised:

(1,4,5)(2,3,6)(7,9,11)(8,10)

Plants in classes 1–3 are clearly distinguished: brand A, East Black Sea black (BTs) and Pure Ceylon teas present high contents of metals K and Mn and are grouped into class 1; brand C/D BTs and green teas (GT) show high heavy metal Cu and are

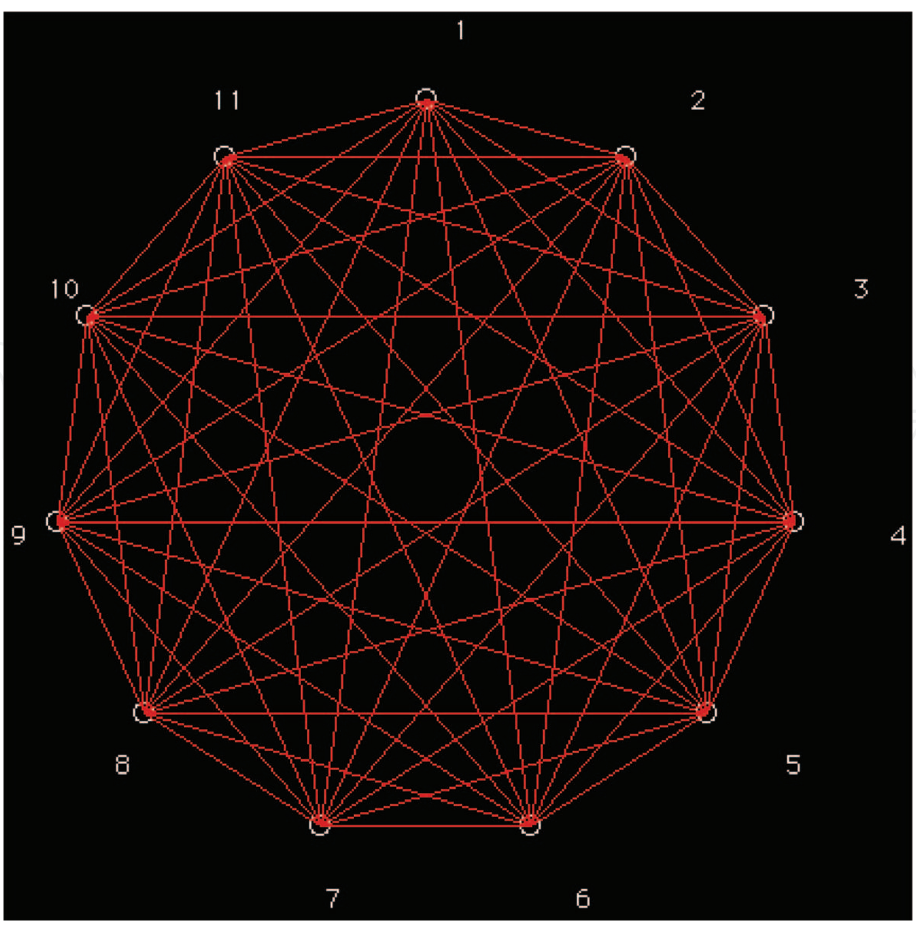

Figure 1.
Partial correlation diagram showing all 55 high (red) *partial correlations.*

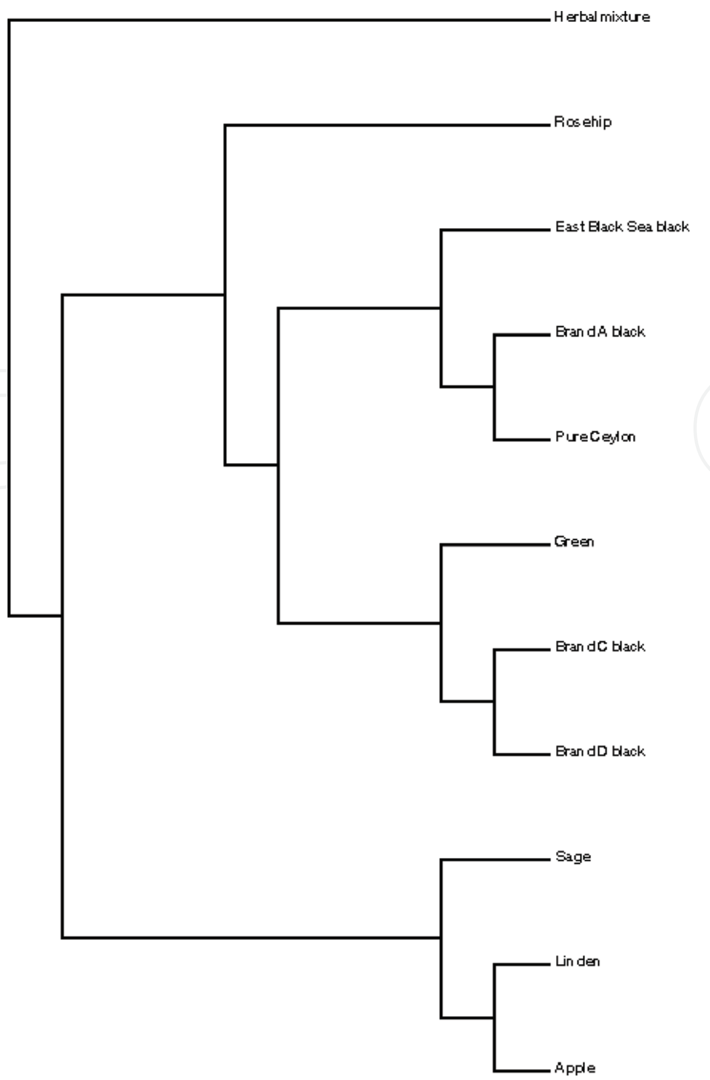

Figure 2.
Dendrogram of teas according to elemental analysis.

included in cluster 2; sage, Linden and apple teas have high heavy metals Fe and Zn and are taken as class 3; herbal mixture and rosehip teas present high alkaline Na and form cluster 4. Manganese level results higher in BT than herbal and GT infusions. Content of Mn is greatest for brand A BT. The plants in the same class appear highly correlated in PCD (**Figure 1**).

The radial tree (cf. **Figure 3**) shows different behaviour of teas depending on Cu, etc. The same classes above are clearly recognised in agreement with PCD and dendrogram (**Figures 1** and **2**). Again, plants with high K and Mn are grouped into cluster 1, etc.

The split graph for 11 teas in **Table 1** (cf. **Figure 4**) shows that teas 1, 4 and 5 as well as 2, 3 and 6 collapse. It reveals conflicting relationships between classes because of interdependences [50]. It indicates spurious relations between groupings 3 and 4 resulting from base composition effects. It illustrates different behaviours of plants depending on Cu, etc. It is in qualitative agreement with PCD and binary/radial trees (**Figures 1**–**3**).

Principal components (PCs). PCA allows *summarising* information contained in **X**-matrix. It decomposes **X**-matrix as product of matrices **P** and **T**. *Loading matrix* **P** with information about variables contains a few vectors: PCs that are obtained as linear combinations of original *X*-variables. In *score matrix* **T** with information about objects, every object is described by projections on to PCs instead of original variables: $\mathbf{X} = \mathbf{TP}' + \mathbf{E}$, where′ denotes transpose matrix. Information not contained in matrices remains *unexplained* X-*variance* in *residual matrix* **E**. Every PC_i is a new

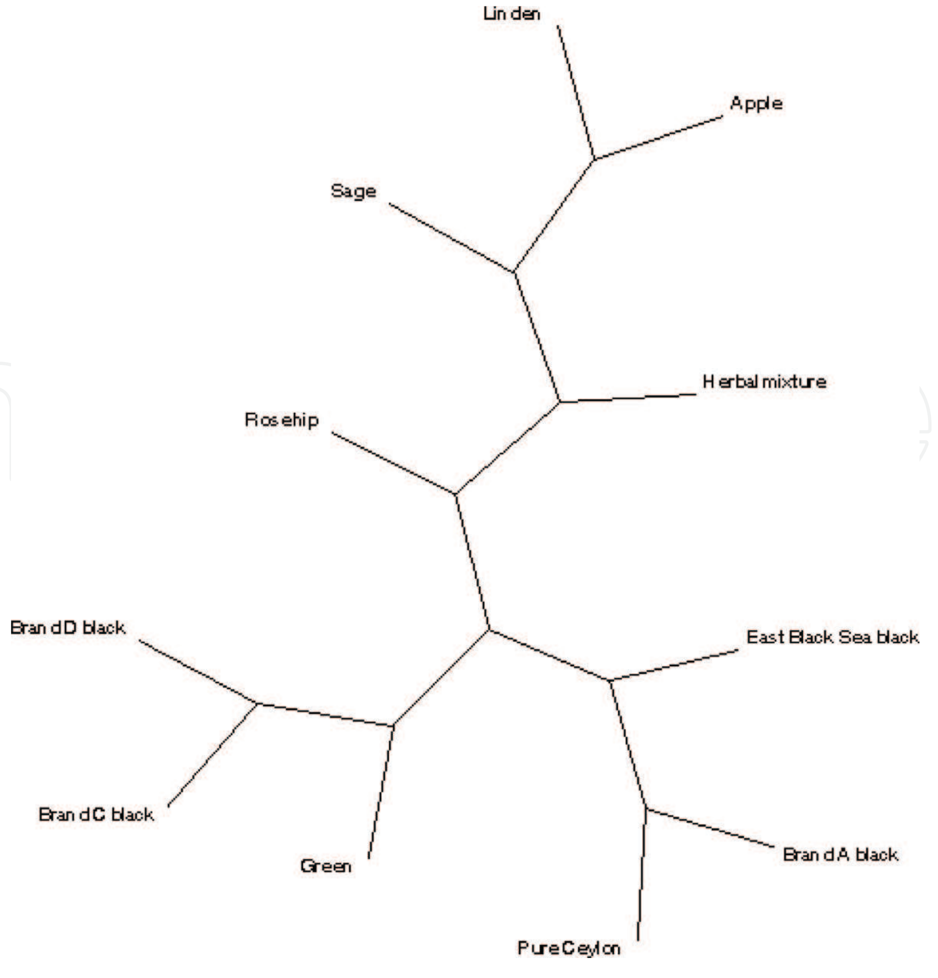

Figure 3.
Radial tree of teas according to elemental analysis.

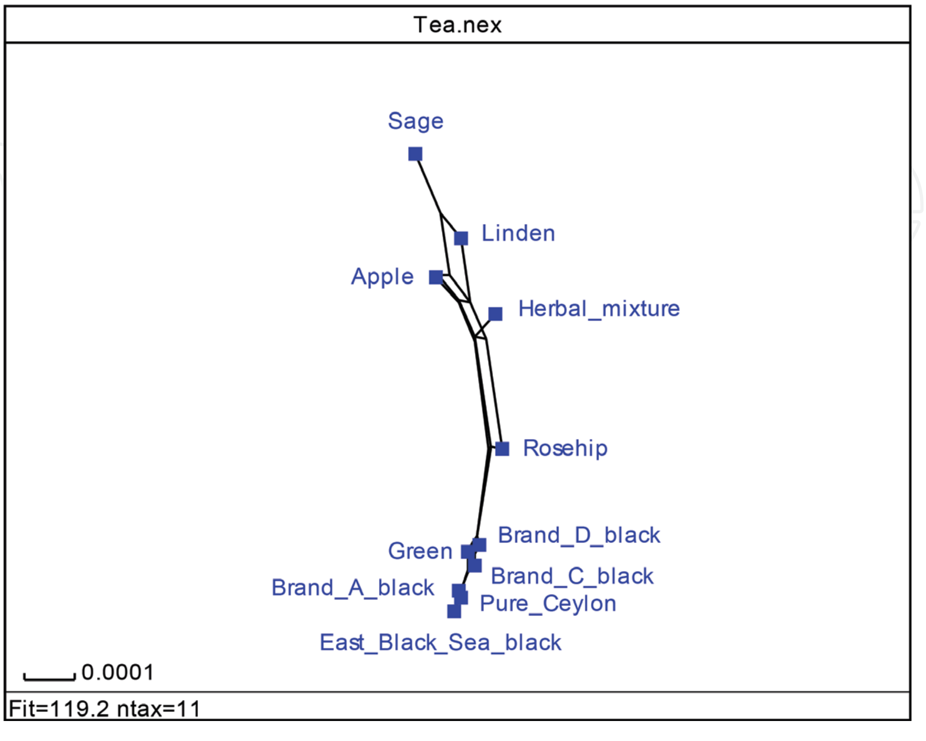

Figure 4.
Split graph of teas according to elemental analysis.

co-ordinate expressed as linear combination of old x_j: $PC_i = \Sigma_j b_{ij} x_j$. New co-ordinates PC_i are *scores* (*factors*), while coefficients b_{ij} are *loadings*. Scores are ordered according to information content vs. total variance among objects. *Score-score plots* show compound positions in new co-ordinate system, while *loading-loading plots* show location of features that represent compounds in new coordination. Properties of PCs follow: (1) they are extracted by decaying importance; (2) every PC is orthogonal to each other. A PCA was performed for teas. Importance of PCA factors $F_{1–6}$ for elements (cf. **Table 2**) shows that both F_1 and F_2 present the corresponding eigenvalue greater than one. Factor F_1 explains 45% variance (55% error); $F_{1/2}$, 71% variance (29% error); $F_{1–3}$, 85% variance (15% error); etc. Nickel was not included because it cannot distinguish teas. For F_1, variable i_4 shows greatest weight; however, F_1 cannot be reduced to two variables $\{i_1,i_4\}$ without a 40% error. For F_2, variable i_6 presents greatest weight; notwithstanding, F_2 cannot be reduced to two variables $\{i_3,i_6\}$ without a 26% error. For F_3, variable i_5 assigns greatest weight; nevertheless, F_3 cannot be reduced to two variables $\{i_3,i_5\}$ without 23% error, etc.

Scores plot of PCA F_2–F_1 for teas (cf. **Figure 5**) illustrates different behaviours depending on Cu, etc. The four clusters above are clearly distinguished: class 1 with

Factor	Eigenvalue	Percentage of variance	Cumulative percentage of variance
F_1	2.67023632	44.50	44.50
F_2	1.60820005	26.80	71.31
F_3	0.81249949	13.54	84.85
F_4	0.68779941	11.46	96.31
F_5	0.15511961	2.59	98.90
F_6	0.06614511	1.10	100.00

Table 2.
Importance of PCA factors for the elemental analysis of tea infusions.

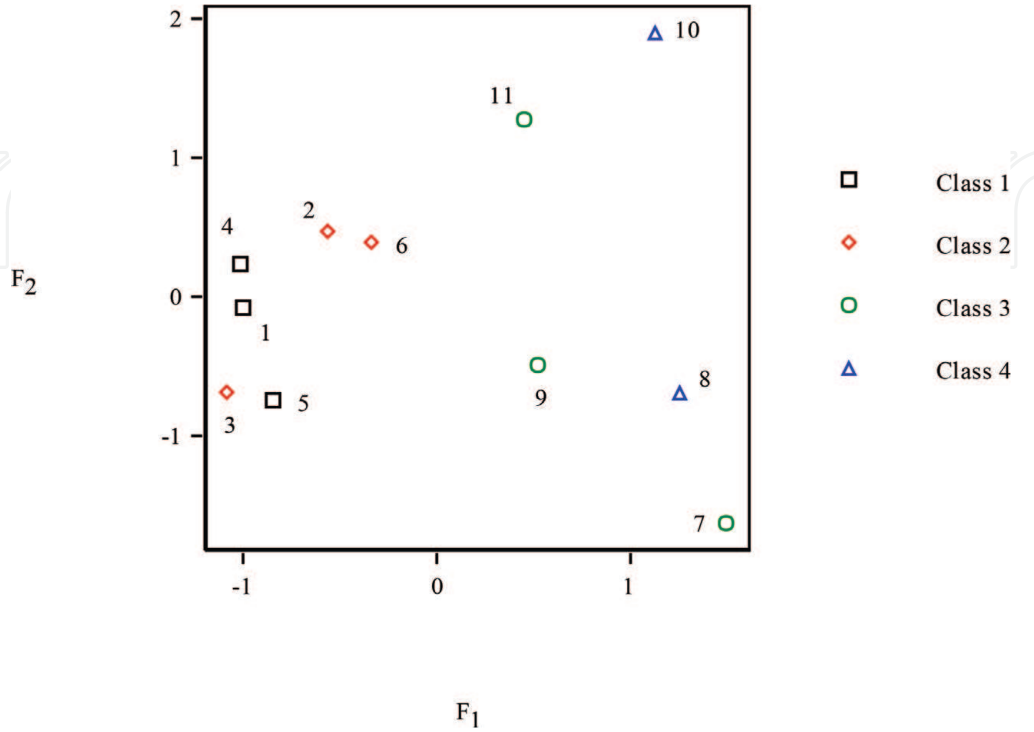

Figure 5.
PCA scores plot of teas according to elemental analysis.

three teas ($F_1 < < F_2 \approx 0$, *left*), grouping 2 with three plants ($F_1 < F_2 \approx 0$, *middle*), cluster 3 with three samples ($F_1 > F_2 \approx 0$, *bottom right*) and class 4 with two teas ($F_1 > > F_2 \approx 0$, *top*). The diagram is in qualitative agreement with PCD, binary/radial trees and split graph (**Figures 1**–**4**).

From PCA factors loading of teas, F_2–F_1 loadings plot depicts six elements (**Table 1**). Two clusters are clearly distinguished: class 1 with three metals {1,3,4} ($F_1 < F_2 < 0$, cf. **Figure 6** *left*) and grouping 2 with three elements {2,5,6} ($F_1 > > F_2$, *bottom right*). Heavy metals such as Cu and Zn, Fe and Mn and alkalines K and Na split into classes 1 and 2. Copper is closer to Mn than Zn; Fe is closer to Zn than Mn; and Na is closer to Fe than K. In addition, as a complement to scores diagram for loadings, it is confirmed that teas in class 1, located in the left side, present a more pronounced contribution from elements in grouping 1 situated in the same side of **Figure 5**. Metals in cluster 3 in the bottom right show a contribution from contents in class 2 found in the same side of **Figure 5**. The plot agrees qualitatively with PCD, binary/radial trees and split graph (**Figures 1**–**4**).

Instead of 11 teas in the space $\mathfrak{R}^6$ of six elements, consider six components in the space $\mathfrak{R}^{11}$ of 11 teas. Matrix **R** upper triangle results

$$R = \begin{pmatrix} 1.000 & -0.462 & 0.393 & 0.835 & -0.419 & 0.321 \\ & 1.000 & -0.134 & -0.677 & 0.324 & 0.496 \\ & & 1.000 & 0.209 & -0.023 & 0.290 \\ & & & 1.000 & -0.318 & -0.016 \\ & & & & 1.000 & 0.197 \\ & & & & & 1.000 \end{pmatrix}$$

High correlations appear between pairs of heavy metals Cu–Mn $R_{1,4} = 0.835$. Correlation between heavy metals Cu–Zn $R_{1,6} = 0.321$ is low. Correlation between heavy metals Fe and Mn $R_{2,4} = -0.677$ and alkalines K and Na $R_{3,5} = -0.023$ is negative. Correlation between Cu and Mn is greater than between Cu and Zn.

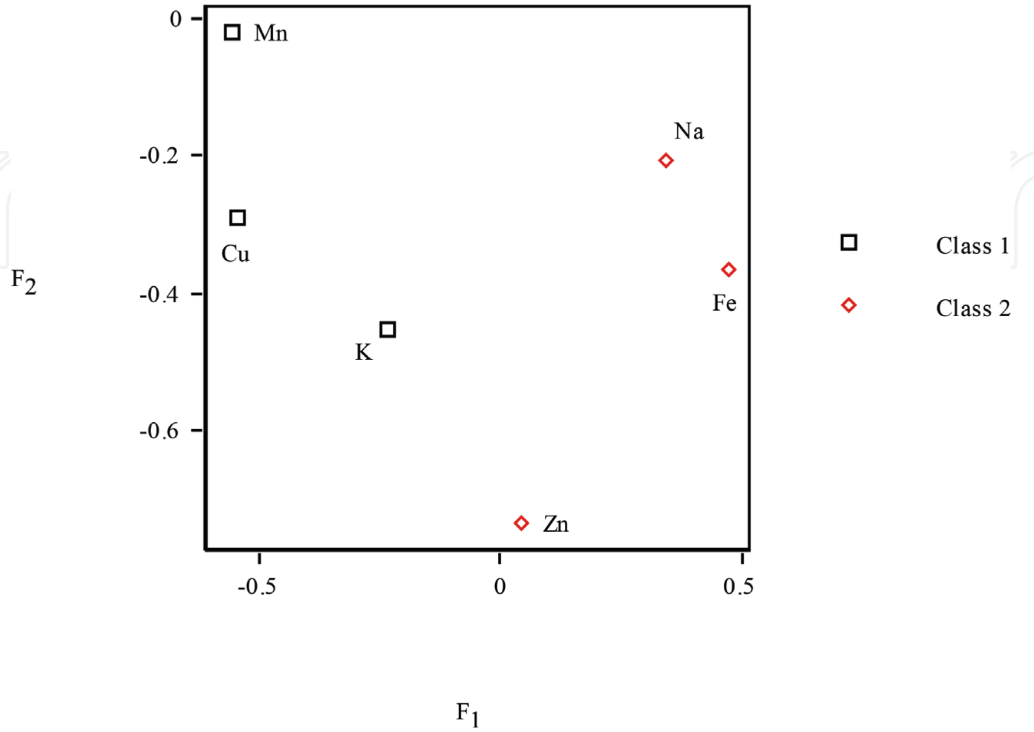

Figure 6.
PCA loadings plot of teas according to elemental analysis.

Correlation between Fe and Zn is greater than between Fe and Mn. Correlation between Fe and Na is greater than between K and Na. The dendrogram for six elements of teas (cf. **Figure 7**) separates the same two clusters above in agreement with PCA loadings plot (**Figure 6**). Again, pairs of metals Cu/Zn, Fe/Mn and K/Na split into classes 1 and 2. Copper is closer to Mn than Zn, Fe is closer to Zn than Mn and Na is closer to Fe than K.

The radial tree for six elements of teas (cf. **Figure 8**) separates the same two clusters above in agreement with PCA loadings plot and dendrogram (**Figures 6** and **7**). One more time, pairs of metals Cu/Zn, Fe/Mn and K/Na split into classes 1 and 2. Copper is closer to Mn than Zn, Fe is closer to Zn than Mn, and Na is closer to Fe than K.

Split graph for six elements of teas (cf. **Figure 9**) reveals conflicting relationships between classes. It separates both clusters above in agreement with PCA loadings plot and binary/radial trees (**Figures 6**–**8**). Once more, pairs of metals Cu/Zn, Fe/Mn and K/Na split into classes 1 and 2. Copper is closer to Mn than Zn, Fe is closer to Zn than Mn, and Na is closer to Fe than K.

A PCA was performed for elements. Factor F_1 explains 99.97% variance (0.03% error) and $F_{1/2}$ 100% variance (0% error). In PCA F_2-F_1 score plot for metals (cf. **Figure 10**), Cu and Zn as well as Fe and Na collapse. Two clusters are clearly distinguished: class 1 with three elements ($F_1 >> F_2$, *bottom*) and grouping 2 with three metals ($F_1 << F_2$, *top*). The diagram separates both classes above in qualitative agreement with PCA loadings plot, binary/radial trees and split graph (**Figures 6**–**9**).

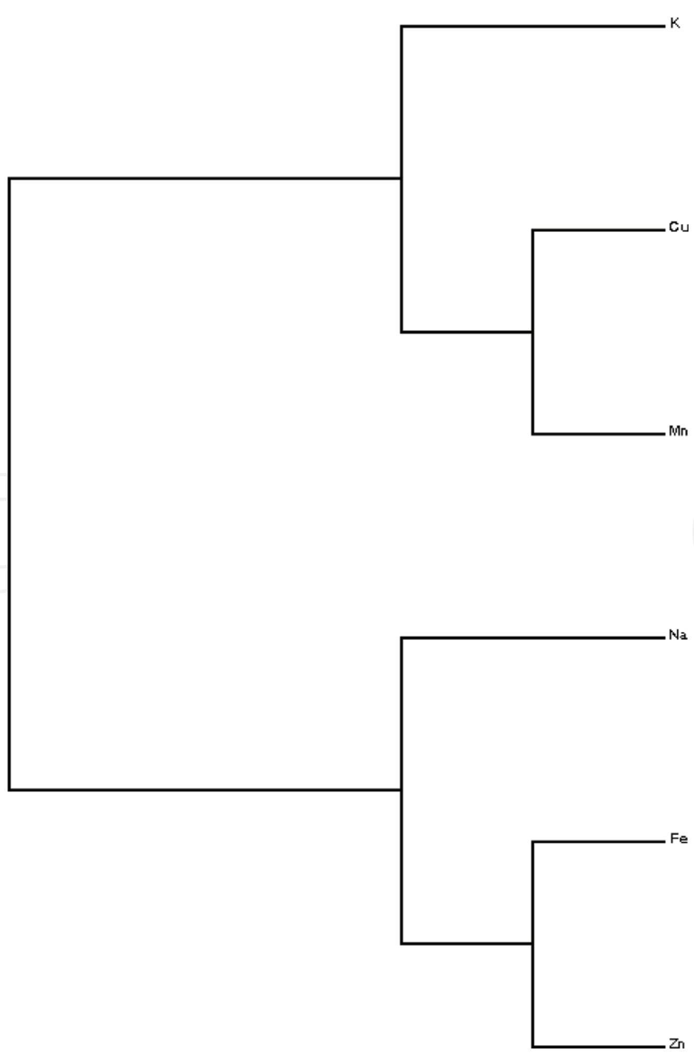

Figure 7.
Dendrogram of elemental analysis for teas.

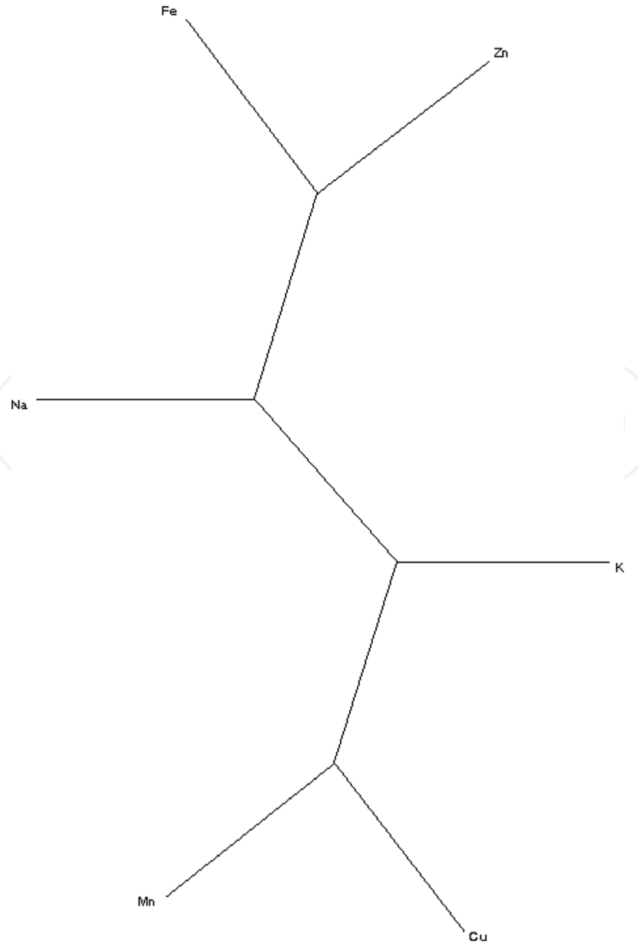

Figure 8.
Radial tree of elemental analysis for teas.

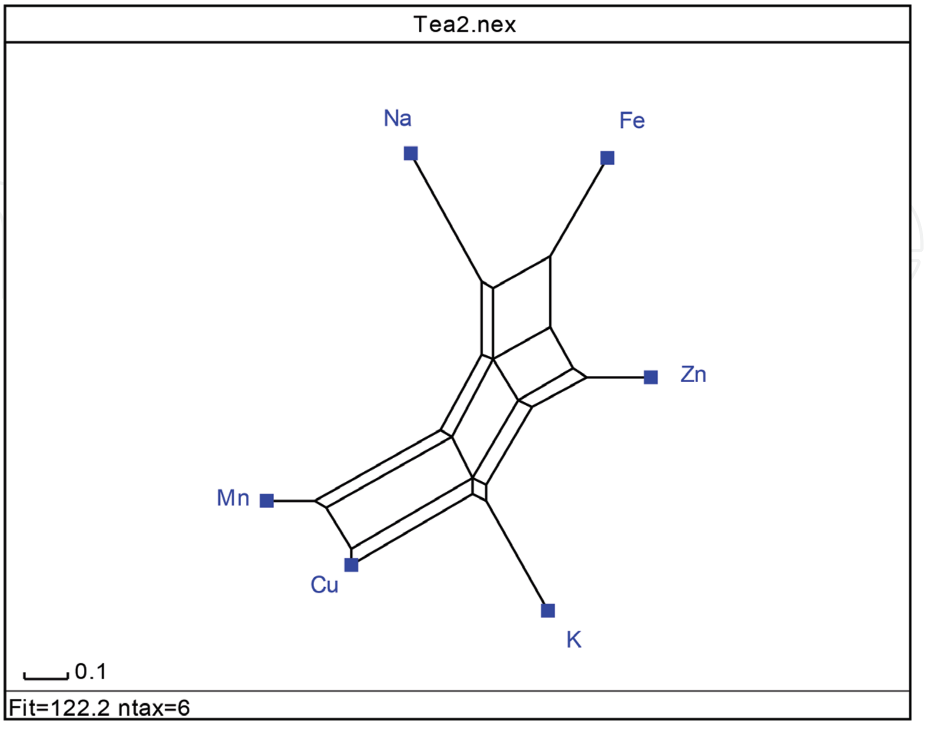

Figure 9.
Split graph of elemental analysis for teas.

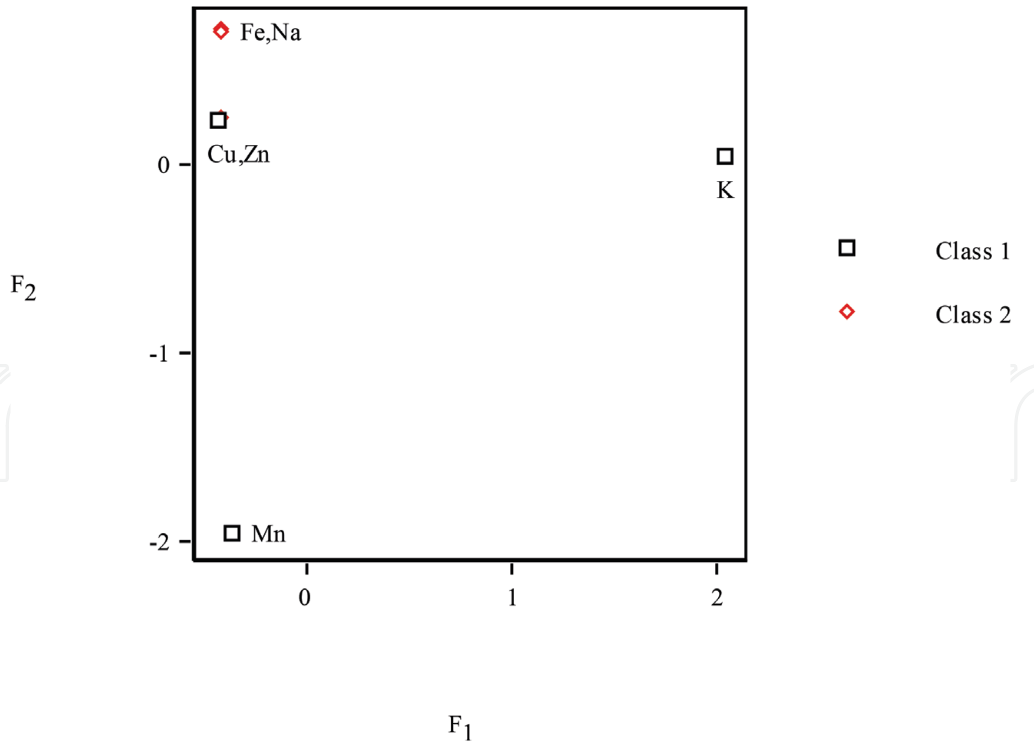

Figure 10.
PCA scores plot of elemental analysis for teas.

Again, pairs of elements Cu/Zn, Fe/Mn and K/Na split into classes 1 and 2. Copper is closer to Mn than Zn, Fe is closer to Zn than Mn, and Na is closer to Fe than K.

4. Discussion

Tea is the second popular beverage. Its chemical components are of interest, especially in relation to health. Flavonoids beneficial effects are vasodilator, antilipemic, antiatherogenic, antithrombotic, anti-inflammatory, apoptotic, antiapoptotic and antioxidant improving health and decreasing CVD. Alkaloid methylxanthines theobromine, theophylline and caff pass via the placental barrier. The GT contains higher amounts of catechins (GTCs) (−)-epigallocatechin 3-*O*-gallate (EGCg) and (−)-epicatechin (EC) than BT. The total contents of Fe, Zn, Cu, Mn, Ni, Na and K in tea leaves and their amounts, available in the corresponding tea infusions, were analysed depending on tea type. Drinking tea impact on the uptake of these metals was examined. The total elemental content of tea leaves differs according to tea type. Tea infusions result a dietary source of essential trace elements, especially Mn, which is the only metal with a dietary amount. The mineral content of the infusions is not related directly to dry tea. The elements in tea leaves were K > Mn > Fe > Na and infusions K > Mn > Na > Fe > Zn > Cu > Ni. Zinc is responsible for enzymatic processes and involved in the working of genetic materials, proteins and immune reactions. It influences maintenance of cell membrane stability and immune system function. It is involved in pathologies (e.g. Alzheimer's disease, epilepsy, ischemia, infantile diarrhoea). Micronutrient Cu is phytotoxic at high concentrations. Its overconsumption is detrimental to health. Change of teas' Cu content was because of different types, grades and producing areas. Its pollution originates from rolling machines and fungicides.

Maceration of GT causes GTC oxidation producing pigmented theaflavins (TFs) and thearubigins (TRs), both 30% dry weight of BT, which affect tea infusion quality.

Authors know that no other similar classification studies grouping teas by their metal content, either computational or experimental.

5. Conclusion

From the present results and discussion, the following conclusions can be drawn.

1. Criteria reduced analysis to a manageable quantity from enormous set of tea metals: they refer to the elemental analysis of tea leaf infusions. Meta-analysis was useful to rise numbers of samples and variety of analysed data. Different behaviours of teas depend on Cu, Fe, K, Mn, Na and Zn. They are considered as a good source of Mn, etc. Two elemental classes are clearly distinguished: Cu-K-Mn and Fe-Na-Zn. With regard to components, heavy metals such as Cu and Zn as well as Fe and Mn and alkalines such as K and Na classed separately. Copper is closer to Mn than Zn, Fe is closer to Zn than Mn, and Na is closer to Fe than K. Heavy metals Fe and Mn as well as K and Na correlate negatively. Teas present adequate elemental contents, good antioxidant capacity and may be used as a functional beverage. They represent plants useful as a natural source for nutraceutical formulations.

2. Principal components analyses of elements and teas cluster analyses allowed classifying them and agreed. Phytochemistry, cytochemistry and understanding of computational methods are essential for tackling associated data mining tasks.

3. More studies are needed contributing more scientific evidence on the benefits above.

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Conflict of interest

The authors declare no conflict of interest.

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