



## Determination of the mineral composition of Caigua (*Cyclanthera pedata*) and evaluation using multivariate analysis



Adriana Caires Oliveira<sup>a,b</sup>, Vitor Silva dos Santos<sup>c</sup>, Debora Correia dos Santos<sup>a,b</sup>, Rosemary Duarte Sales Carvalho<sup>d</sup>, Anderson Santos Souza<sup>a,b</sup>, Sergio Luis Costa Ferreira<sup>a,b,\*</sup>

<sup>a</sup> Universidade Federal da Bahia, Instituto de Química, Grupo de Pesquisa em Química e Quimiometria, CEP 40170-290 Salvador, BA, Brazil

<sup>b</sup> Instituto Nacional de Ciência e Tecnologia, INCT de Energia e Ambiente, Universidade Federal da Bahia, 40170-290 Salvador, BA, Brazil

<sup>c</sup> Centro Universitário Jorge Amado, 41745-130 Salvador, BA, Brazil

<sup>d</sup> Faculdade de Farmácia, Universidade Federal da Bahia, 41745-130 Salvador, BA, Brazil

### ARTICLE INFO

#### Article history:

Received 16 March 2013

Received in revised form 25 November 2013

Accepted 4 December 2013

Available online 11 December 2013

#### Keywords:

Caigua

Mineral composition

PCA

HCA

### ABSTRACT

Caigua (in Brazil “*maxixe do reino*”) is a fruit that is generally consumed either cooked or even raw as salad. This fruit has been used as a food and also in folk medicine. In this work, the mineral composition of Caigua was determined for the first time. Twenty-nine samples from five farms located in the south-western region of Bahia, Brazil were acquired and analyzed using inductively coupled plasma optical emission spectrometry. The elements determined in this fruit included calcium, magnesium, sodium, potassium, phosphorus, manganese, iron, zinc, copper and vanadium. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were applied to evaluate the obtained results. The average concentrations of the determined elements (expressed as mg 100 g<sup>-1</sup>) were as follows: 0.91 for sodium, 152 for potassium, 19.4 for phosphorus, 11.9 for calcium, 8.4 for magnesium, 0.074 for manganese, 0.21 for iron, 0.013 for copper, 0.13 for zinc and 0.015 for vanadium.

© 2013 Elsevier Ltd. All rights reserved.

### 1. Introduction

In Brazil, Caigua is known as “*maxixe do reino*”, and it is an herbaceous vine that is traditionally distributed from Colombia to Bolivia and is generally consumed either cooked or even raw as salad. Caigua (species *Cyclanthera pedata* (L.) Schrader) belongs to the Cucurbitaceae family. The consumption of Caigua as a food is still very limited. However, some studies have investigated the use of the fruit of this plant in medicine. Gonzales, Gonez, and Villena, 1995 investigated the use of oral doses of dehydrated natural Caigua on serum cholesterol levels of post-menopausal women. The results showed a reduction in cholesterol levels with a daily dosage of 1800 mg of micro-pulverized Caigua. Hypercholesterol was reduced from 75% to 12.5%. The levels of LDL cholesterol decreased while the levels of HDL cholesterol increased. De Tommasi, De Simone, Speranza, & Pizza (1996) isolated and characterized six new cucurbitacin glycosides from the seeds of Caigua. Another study analyzed the methanolic extract of the fruits of Caigua. The authors isolated and elucidated structures of nine triterpenoid saponins, among them, six new natural compounds were characterized. A

comparative study of the seeds and fruits was also performed (De Tommasi, De Simone, Speranza, & Pizza, 1999). Montoro, Carbone, De Simone, Pizza, and De Tommasi (2001) used chloroform and methanol for the extraction of six organic compounds from Caigua fruits. These authors elucidated the structures and determined the antioxidant activities of four new flavonoid glycosides. Carbone, Montoro, de Tommasi, and Pizza (2004) employed high performance liquid chromatography (HPLC) followed by detection with electrospray ionization mass spectrometry for the separation and quantification of flavonoid glycosides present in the methanolic extracts from Caigua fruits. Montoro, Carbone, and Pizza (2005) also used HPLC coupled with electrospray MS for the separation and determination of flavonoid metabolites from the leaves and fruits of Caigua. Two new minor components of this fruit were isolated and their structures were characterized. A previous study investigated the phenolic profiles of several plants, as well as their antioxidant activity and *in vitro* inhibitory potential against key enzymes relevant to hyperglycemia and hypertension. Caigua significantly inhibited hypertension (Ranilla, Kwon, Apostolidis, & Shetty, 2010).

Despite all these results, the mineral composition of Caigua is completely unknown. In food research, the amount of analytical data obtained is generally large and the interpretation of these results can become complicated. This amount of data depends directly on the number of samples analyzed and the number of

\* Corresponding author at: Instituto Nacional de Ciência e Tecnologia, INCT de Energia e Ambiente, 13 Universidade Federal da Bahia, 40170-290 Salvador, BA, Brazil. Tel.: +55 7132836831; fax: +55 7132355166.

E-mail address: [slcf@ufba.br](mailto:slcf@ufba.br) (S.L.C. Ferreira).

**Table 1**  
Evaluation of accuracy using the CRM trace elements in spinach leaves.

Element	Certified value	Achieved value
Ca (%)	1.527 ± 0.041	1.456 ± 0.093
Cu (mg kg <sup>-1</sup> )	12.2 ± 0.6	12.9 ± 0.7
K (%)	2.903 ± 0.052	2.856 ± 0.126
Mg (%)	0.89 <sup>a</sup>	0.86 ± 0.09
Mn (mg kg <sup>-1</sup> )	75.9 ± 1.9	70.1 ± 6.5
Na (%)	1.818 ± 0.043	1.912 ± 0.107
P (%)	0.518 ± 0.011	0.467 ± 0.085
V (mg kg <sup>-1</sup> )	0.57 ± 0.03	0.52 ± 0.08
Zn (mg kg <sup>-1</sup> )	82 ± 3	90 ± 11

<sup>a</sup> Value provided.

parameters that are determined, so multivariate analysis techniques have often been employed to facilitate understanding of the results. The principal component analysis (PCA) and hierarchical cluster analysis (HCA) techniques are very commonly used in food analyses (Gonzalez, Gonzalez, Cancho, & Simal, 2011; Karadas & Kara, 2012; Rotondi, Beghe, Fabbri, & Ganino, 2011; Sarbu et al., 2012). PCA reduces the number of original variables to a few latent variables called principal components (PC). The first component, PC1, is determined in the direction of greatest data variance. PC2 is defined to be orthogonal and represents the maximum variance not explained by PC1. The remaining PCs are obtained in the same way in decreasing order of variance. This technique simplifies the graphical representation of the data (Beebe, Pell, & Seasholtz, 1998).

HCA is used to classify samples into groups, considering the values of a set of variables. This classification is established according to the similarity of the samples, and the groups are represented on two-dimensional diagrams called dendrograms.

Plant foods provide minerals that are vital for human health and many studies have been performed with the objective of quantifying these substances in plant matrices. The aim of this study was to determine, for the first time, the mineral composition of Caigua fruits.

**Table 2**  
Determination of the mineral composition of Caigua (mg kg<sup>-1</sup>).

Sample	Ca	Cu	Fe	K	Mg	Mn	Na	P	V	Zn
L1	50.4	0.186	2.04	1755	71.7	0.429	15.00	228	0.169	1.22
L2	40.9	0.186	2.17	1683	69.4	0.456	15.50	229	0.164	1.43
L3	40.6	0.138	1.63	1601	62.0	0.351	13.89	184	0.152	1.12
L4	230.2	0.059	1.46	808	153.6	0.729	11.69	90	0.130	0.91
L5	204.3	0.109	1.33	738	149.4	0.647	12.78	90	0.150	0.81
L6	141.6	0.066	1.67	745	104.6	0.540	10.98	84	0.129	0.97
J1	107.9	0.059	1.78	1431	111.6	0.216	14.55	46	0.130	1.13
J2	101.3	0.026	1.23	1470	77.7	0.178	12.06	45	0.144	1.42
J3	67.6	0.015	1.43	1338	53.7	0.178	12.22	48	0.168	1.33
J4	143.2	0.074	1.67	1104	58.3	0.547	12.29	200	0.142	1.68
J5	136.0	0.093	1.60	1127	50.1	0.552	12.38	205	0.159	1.56
J6	128.0	0.077	2.09	1108	48.7	0.545	11.38	207	0.129	1.47
D1	94.3	0.022	1.38	1321	49.4	0.589	13.78	115	0.148	0.60
D2	93.1	0.083	1.65	1452	52.7	1.011	11.46	175	0.147	0.81
D3	73.0	0.033	1.30	1265	42.2	0.713	11.44	134	0.136	0.72
D4	197.7	0.047	1.75	2162	129.2	0.565	8.44	159	0.154	0.82
D5	144.5	0.096	1.84	1975	121.1	0.512	6.20	199	0.144	0.96
P1	215.8	0.097	1.96	1974	143.2	0.634	6.11	191	0.138	0.84
P2	71.6	0.132	2.29	2037	70.2	0.316	8.06	168	0.137	1.12
P3	90.9	0.066	2.43	2183	72.7	0.251	5.91	135	0.144	0.84
P4	83.6	0.062	1.51	2067	69.5	0.272	6.24	146	0.158	0.94
P5	106.2	0.115	2.73	1196	57.4	0.558	2.98	138	0.127	1.24
P6	117.2	0.089	3.01	1146	60.0	0.547	3.32	132	0.147	1.23
R1	118.6	0.315	2.68	2146	94.0	1.262	5.54	395	0.162	1.82
R2	110.9	0.351	2.80	1699	95.1	1.174	4.71	347	0.155	1.92
R3	111.2	0.314	2.53	1643	94.4	1.118	3.36	311	0.133	1.74
R4	132.3	0.296	3.92	1617	102.8	2.302	1.75	444	0.133	2.01
R5	140.4	0.232	3.81	1591	95.5	2.260	3.08	402	0.162	1.85
R6	151.4	0.238	3.57	1669	85.7	2.042	6.48	378	0.152	2.23

## 2. Experimental

### 2.1. Reagents

All chemical reagents used in the experiments were of analytical grade and obtained from Merck (Darmstadt, Germany). Ultrapure water with a specific resistivity of 18 MΩ cm was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). All containers and glassware were maintained in a 10% v/v nitric acid solution for at least 12 h for decontamination before use. Standard stock solutions with a concentration of 1000 mg L<sup>-1</sup> for all elements were used to prepare working standard solutions by dilution using a 1% (v/v) nitric acid solution. For sample digestion, concentrated nitric acid and hydrogen peroxide were used.

### 2.2. Instrumentation

The determination of the elements was performed using a Varian Vista PRO Inductively Coupled Plasma Optical Emission Spectrometer (Mulgrave, Australia) with axial viewing and a charge coupled device (CCD) detector. A Sturman–Master chamber and a V-groove nebulizer were also utilized. The instrumental conditions were as follows: power (1.3 kW), plasma gas flow (15.0 L min<sup>-1</sup>), auxiliary gas flow (1.5 L min<sup>-1</sup>) and nebulizer gas flow (0.8 L min<sup>-1</sup>). The elements and the emission lines used for quantification were as follows: Ca II (317.933 nm), Cu I (324.754 nm), Fe II (238.204 nm), K II (766.491 nm), Mg II (285.213 nm), Mn II (259.372 nm), Na I (589.592 nm), P I (213.613 nm), V II (292.401 nm) and Zn II (202.548 nm).

### 2.3. Sample collection and storage

*C. pedata* (L.) Schrad fruits were obtained from five commercial farms, located in the southwestern region of Bahia, Brazil.

In the laboratory, these fruits were refrigerated until their analysis to prevent the proliferation of fungi and bacteria. Each sample analyzed was composed of three fruits, which were randomly chosen. The fruits were cleaned with deionized water and then separated into peels, seeds and pulps using a plastic knife. The pulps were selected and the husks and seeds were discarded.

The pulps were grated using a plastic grater and were then packed into small plastic pots after being homogenized by mechanical agitation (manual) using plastic knife.

#### 2.4. Sample digestion

Samples were digested using a digester block and a reflux system equipped with a cold finger (Ferreira, Silva, de Santana, Junior, Matos, & dos Santos, 2013). Approximately 2 g of each sample were placed into glass tubes. Concentrated nitric acid (4 ml) and 30% (v/v) hydrogen peroxide (3 ml) were added to each vial. The samples were digested for 2 h at 110 °C. Subsequently, the digested samples were transferred to a 25 ml volumetric flask and ultrapure water was used to make up the volume.

#### 2.5. Accuracy evaluation

During the validation step, an accuracy test was performed using a certified reference material (Trace Elements in Spinach Leaves (NIST 1570a) furnished by the National Institute of Standards and Technology (Gaithersburg, MD, USA)). Digestion of this vegetable was performed using the same procedure employed for the Caigua samples. The analytical results were in agreement with the certified values (expressed as fresh weight), as shown in Table 1.

Iron content was not certified in the NIST 1570a CRM. Thus, the accuracy of the analytical method used for determination of iron was evaluated by analysis of the certified reference material of 1568a rice flour also furnished by NIST, which has certified value for iron of  $7.5 \pm 0.4 \text{ mg kg}^{-1}$ . The method has satisfactory accuracy considering that the iron content obtained was  $7.4 \pm 0.9 \text{ mg kg}^{-1}$ .

### 3. Results and discussion

#### 3.1. Determination of mineral content in Caigua

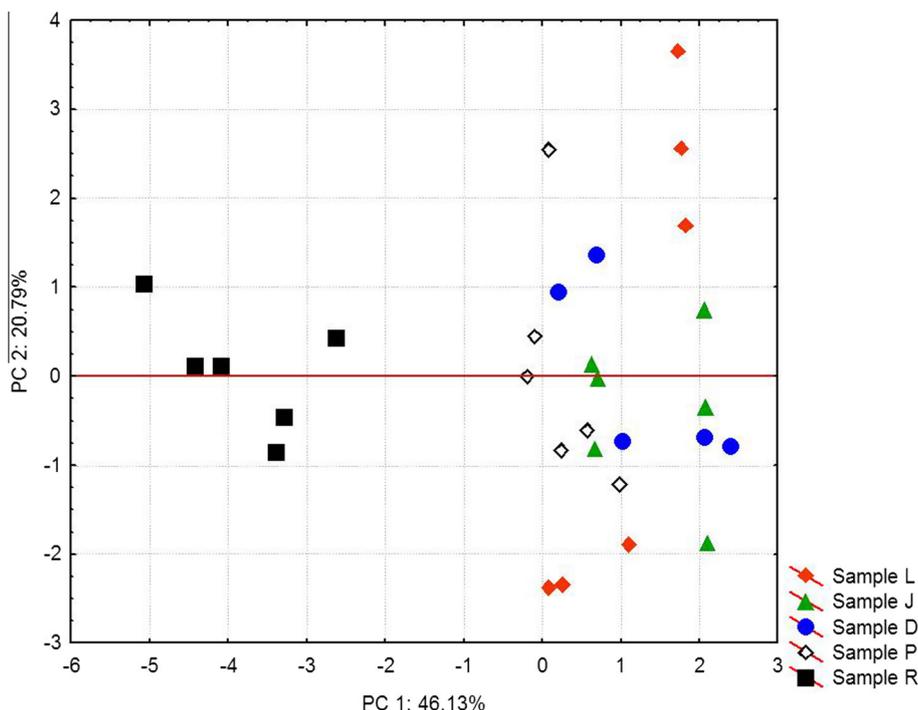
Twenty-nine samples from five farms located in the southwestern region of Bahia, Brazil were prepared and analyzed. Inductively coupled plasma optical emission spectrometry was used to determine calcium, magnesium, sodium, potassium, phosphorus, manganese, iron, zinc, copper and vanadium. The results obtained are shown in Table 2 with concentrations expressed as mg of analyte per kg of sample.

#### 3.2. Multivariate analysis of the results obtained

The results for the 10 elements in the 29 samples analyzed were evaluated by PCA. A (10 × 29) data matrix was constructed using the elements as columns and the samples as rows (Table 2). The data were auto-scaled because the element concentrations were of different orders of magnitude. The loadings of the original variables on the first three principal components and the variances explained by each principal component are shown in Table 3. The

**Table 3**  
Loading of the variables for the first three principal components.

Element	PC1	PC2	PC3
Ca	-0.0954	0.9117	-0.1187
Cu	-0.8929	-0.1002	-0.0165
Fe	-0.9110	0.0070	0.1202
K	-0.3595	-0.3324	-0.7738
Mg	-0.1374	0.7955	-0.4461
Mn	-0.8680	0.1852	0.1821
Na	0.7150	-0.2463	0.1169
P	-0.9483	-0.1117	-0.0112
V	-0.1396	-0.5965	-0.4228
Zn	-0.8031	-0.1760	0.3127
Total variance (%)	46.13	20.79	11.50
Cumulative variance (%)	46.13	66.92	78.42



**Fig. 1.** Plot of the first principal component (PC1) versus the second principal component (PC2) for the twenty-nine Caigua samples.

data were modeled considering the first three components because they described 78.42% of the total variance.

The dominant variables for the first principal component (PC1) were phosphorus and the micronutrients copper, iron, sodium, zinc and manganese because these variables represented 46.13% of the total variance. All six elements contributed to the variability shown in the samples and were positively correlated, except sodium. The second principal component (PC2) accounted for 21% of the total variance, with calcium and magnesium as the dominant variables.

The score plot of the first two components is shown in Fig. 1.

Fig. 1 clearly shows a systematic separation between the analyzed samples. The six samples collected from R farm are separated from the others. These samples had low scores for PC1, but they had the highest concentrations for the following elements: phosphorus, copper, iron, zinc and manganese. Table 3 shows that these elements had negative loadings in PC1. In the samples from the other four farms, there were no clusters and the element concentrations varied randomly for the same farm.

The data from Table 2 were also evaluated by HCA. These data were also auto-scaled using the single linkage method, and the Euclidean distances were used to calculate sample interpoint

distances and similarities. The dendrogram obtained is shown in Fig. 2. The results correlated well with other results from the PCA. Again, samples from R farm were shown to have mineral compositions different from samples collected from the other farms.

### 3.3. Evaluation of the mineral composition of Caigua

Considering the results obtained by PCA and HCA, the mineral composition of Caigua was determined from the analysis of samples collected from the five farms. The average and concentration ranges (expressed as mg of analyte per 100 g of sample) for calcium, copper, iron, potassium, magnesium, manganese, sodium, phosphorus, vanadium and zinc are shown in Table 4.

Table 4 shows also the mineral contents of five others cucurbits: pumpkin, cucumber, chayote, watermelon and melon, considering data reported by United States Department of Agriculture (USDA, 2013). In this, it can be seen that caigua fruits have mineral content similar with other cucurbits, where, all these have relatively high concentrations of potassium and low sodium contents.

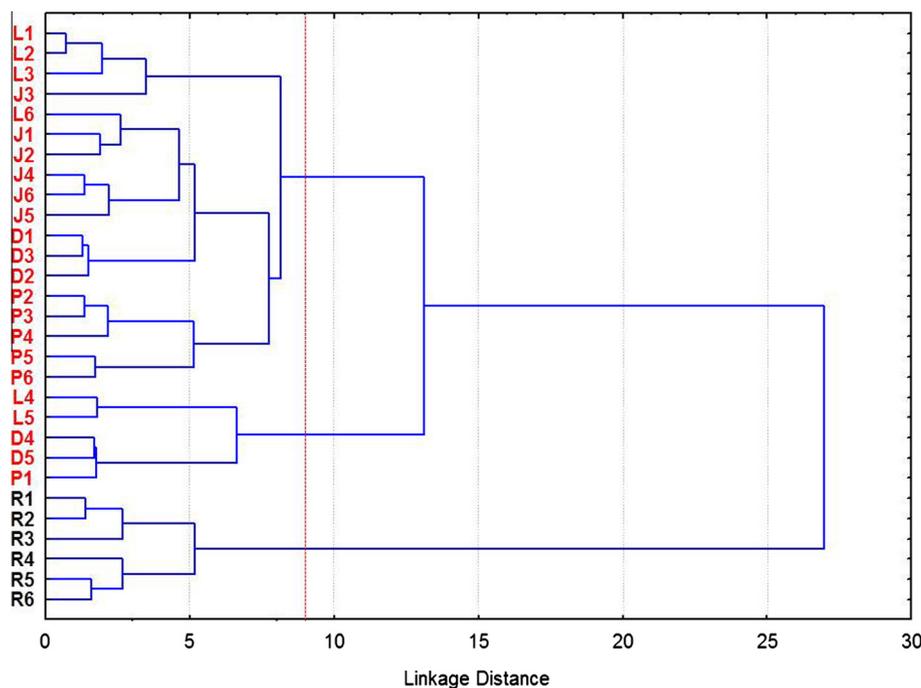


Fig. 2. Dendrogram for Caigua samples showing single linkage with Euclidean distances.

Table 4  
Mineral content in caigua and others cucurbits (mg 100 g<sup>-1</sup>).

Element	Caigua <sup>a</sup>	Caigua <sup>b</sup>	Pumpkin <sup>c</sup>	Cucumber <sup>c</sup>	Chayote <sup>c</sup>	Watermelon <sup>c</sup>	Melon <sup>c</sup>
Ca	4.1–23.0	11.9	21	14	17	7	6
Cu	0.002–0.035	0.013	0.127	0.071	0.123	0.042	0.024
Fe	0.12–0.39	0.21	0.8	0.22	0.34	0.24	0.17
K	74–218	152	340	136	125	112	228
Mg	4.2–15.4	8.4	12	12	12	10	10
Mn	0.018–0.230	0.074	0.125	0.073	0.189	0.038	0.027
Na	0.18–1.55	0.91	1	2	2	1	18
P	4.5–44.4	19.4	44	21	18	11	11
Zn	0.06–0.22	0.13	0.32	0.17	0.74	0.1	0.09
V	0.013–0.017	0.015	–	–	–	–	–

<sup>a</sup> Concentration ranges.

<sup>b</sup> Average concentrations.

<sup>c</sup> Data obtained in <http://ndb.nal.usda.gov/ndb/search/list>.

#### 4. Conclusions

In this paper, the mineral composition of Caigua was determined for the first time. The results demonstrated that Caigua had a high content of potassium, calcium, phosphorus and magnesium. The sodium content was very low.

Caigua can be strongly recommended as nutritional supplement considering its high content of potassium, calcium, phosphorus and magnesium.

PCA demonstrated that phosphorus, sodium, copper, iron, zinc and manganese were the elements contributing to the variation presented by the samples. The results from the HCA were compatible with the results from the PCA.

Caigua has mineral composition similar to watermelon, cucumber, chayote, pumpkin and melon, which also are cucurbits.

#### Acknowledgements

The authors are grateful to the Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for providing grants, fellowships and other financial support.

#### References

- Beebe, K. R., Pell, R. J., & Seasholtz, M. B. (1998). *Chemometrics: A practical guide*. New York: John Wiley & Sons Ltd.
- Carbone, V., Montoro, P., de Tommasi, N., & Pizza, C. (2004). Analysis of flavonoids from *Cyclanthera pedata* fruits by liquid chromatography/electrospray mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*, *34*, 295–304.
- De Tommasi, N., De Simone, F., Speranza, G., & Pizza, C. (1996). Studies on the constituents of *Cyclanthera pedata* (Caigua) seeds: Isolation and characterization of six new cucurbitacin glycosides. *Journal of Agricultural and Food Chemistry*, *44*, 2020–2025.
- De Tommasi, N., De Simone, F., Speranza, G., & Pizza, C. (1999). Studies on the constituents of *Cyclanthera pedata* fruits: Isolation and structure elucidation of new triterpenoid saponins. *Journal of Agricultural and Food Chemistry*, *47*, 4512–4519.
- Ferreira, S. L. C., Silva, L. O. B., de Santana, F. A., Junior, M. M. S., Matos, G. D., & dos Santos, W. N. L. (2013). A review of reflux systems using cold finger for sample preparation in the determination of volatile elements. *Microchemical Journal*, *106*, 307–310.
- Gonzales, G. F., Gonez, C., & Villena, A. (1995). Serum-lipid and lipoprotein levels in postmenopausal women – Short-course effect of Caigua. *Menopause – The Journal of the North American Menopause Society*, *2*, 225–234.
- Gonzalez, A. M., Gonzalez, C. B., Cancho, B. G., & Simal, J. G. (2011). Relationships between *Godello* white wine sensory properties and its aromatic fingerprinting obtained by GC–MS. *Food Chemistry*, *129*, 890–898.
- Karadas, C., & Kara, D. (2012). Chemometric approach to evaluate trace metal concentrations in some spices and herbs. *Food Chemistry*, *130*, 196–202.
- Montoro, P., Carbone, V., De Simone, F., Pizza, C., & De Tommasi, N. (2001). Studies on the constituents of *Cyclanthera pedata*: Isolation and structure elucidation of new flavonoid glycosides and their antioxidant activity. *Journal of Agricultural and Food Chemistry*, *49*, 5156–5160.
- Montoro, P., Carbone, V., & Pizza, C. (2005). Flavonoids from the leaves of *Cyclanthera pedata*: Two new malonyl derivatives. *Phytochemical Analysis*, *16*, 210–216.
- Ranilla, L. G., Kwon, Y. I., Apostolidis, E., & Shetty, K. (2010). Phenolic compounds, antioxidant activity and in vitro inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America. *Bioresource Technology*, *101*, 4676–4689.
- Rotondi, A., Beghe, D., Fabbri, A., & Ganino, T. (2011). Olive oil traceability by means of chemical and sensory analyses: A comparison with SSR biomolecular profiles. *Food Chemistry*, *129*, 1825–1831.
- Sarbu, C., Nascu-Briciu, R. D., Kot-Wasik, A., Gorinstein, S., Wasik, A., & Namiesnik, J. (2012). Classification and fingerprinting of kiwi and pomelo fruits by multivariate analysis of chromatographic and spectroscopic data. *Food Chemistry*, *130*, 994–1002.
- USDA (United States Department of Agriculture – Agricultural Research Service), <<http://ndb.nal.usda.gov/ndb/search/list>> Accessed 22.10.13.