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DETERMINATION OF GEOGRAPHICAL ORIGIN OF GREEN AND ROASTED COFFEE BASED ON SELECTED CHEMICAL PARAMETERS

Alžbeta Demianová[1], Alica Bobková [1], Lukáš Jurčaga [2], Marek Bobko[2], Ľubomír Belej[1], Július Árvay[3]*

Address(es):
[1]Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Hygiene and Food Safety, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia
[2]Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Technology and Quality of Animal Products, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia
3Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Chemistry, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia

*Corresponding author: xdemianova@uniag.sk

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ABSTRACT

Coffee is one of the most consumed beverages in the world. Its quality depends on many factors, such as, country of origin, altitude, climate, post-harvesting processing and others. This paper is focused on the possibility to determinate origin of American, African, and Asian coffees based on chemical properties of the final beverage, such as total antioxidant capacity (TAC) measured using DPPH radical, content of chlorogenic acids and caffeine determined by HPLC-DAD. Samples of green and roasted coffee (roasting level medium dark Full City ++) were used. In green samples the highest values of TAC and caffeine were measured in American samples (averagely 93.014 % inhibition of DPPH and 0.854 g.100 g^{-1} of caffeine respectively), the highest content of chlorogenic acids showed samples from Africa (averagely 5,037 g.100 g^{-1}). In samples of roasted coffees values of TAC decreased by 7, 47 % in Africa samples, by 18,12 % in American, and 13,73 % in samples from Asia. Roasted African coffees showed on average 1.035 g.100 g^{-1} of caffeine, the highest average was measured in American samples (1.201 g.100 g^{-1}), and lowest Asian samples (1.089 g.100 g^{-1}). Lowest content of CGAs was obtained from African samples (0.595 g.100 g^{-1}), and the higher from American (0.596 g.100 g^{-1}) and African samples (0.6345 g.100 g^{-1}). ANOVA single factor showed significant differences between green samples regarding the TAC and caffeine content. However, content of chlorogenic acids did not show any difference (p-value=0,6809) regarding the geographical origin. Same results were obtained comparing roasted samples.

Keywords: coffee, origin, caffeine, chlorogenic acids, total antioxidant capacity

INTRODUCTION

Coffee trees belong to the genus *Coffea*, Rubiaceae family. Its originally comes from East Africa, more specifically from province Kaffa in Ethiopia. Historical records state that in ancient times the locals knew the extraordinary effects of coffee beans and chewed them to encourage body and soul. According to **Magrach and Ghazoul (2015)**, people in Ethiopia knew and used coffee beans as early as 550 BC.
Yilmaz et al. (2017) also state that coffee comes from Arabic peninsula, where they call the fruit of the coffee tree "kahva". But its origin of coffee cannot be precisely determined because there are several inconsistent written records.
Nowadays, coffee is ranked as one of the most traded commodity and furthermore consumed beverage in the world. Due to specific growth requirements, coffee growing is relatively strictly limited by the Tropics of Cancer and Capricorn **(Lashermes and Coombes, 2018).** The coffee tree requires a tropical or subtropical climate for its growth. The soil composition, the amount of precipitation, as well as the altitude are highly important factors affecting final properties for growing coffee beans. Despite these attributes, growing area is still relatively wide, consisting of almost a hundred countries. However, 5 basic areas of cultivation are recognized: South America, Central America, Africa, Asia, and Oceania **(Hoffmann, 2018).** There are several varieties of coffee tree and therefore the ideal growing temperature cannot be precisely defined. In general, however, these plants require a stable temperature, with no significant differences between day and night or the seasons. Significant temperature fluctuations may cause beans degradation. Continuous exposure to temperatures above 30 ° C can seriously damage the plant, causing stunting, yellowing of the leaves, or even the formation of tumours **(Farrah, 2019).**
Regarding the world market, consumers mostly seeks mostly Arabica coffee (*Coffea arabica)*. Arabica is native to the southwestern highlands of Ethiopia, south-eastern Sudan, and northern Kenya. It is currently grown mostly in Central and South America, Africa and Oceania. The most well-known producers in Central and South America are Colombia, Brazil, Costa Rica, Jamaica, Mexico, Peru, Ecuador, Cuba, Panama, Venezuela and Guatemala. *Coffea arabica* represents the majority of their production. Moreover, African plantations are situated in Ethiopia, Kenya, Uganda, Angola, Zaire, Togo, Madagascar, Tanzania. The most important producers in the Pacific region are Indonesia, Java, Sumatra, Papua New Guinea, Vietnam, the Philippines, Laos, Sri Lanka and India. However, *Coffea canephora* (Robusta) is usually grown here **(Veselá, 2016; Phan et al. 2012).**
Given the popularity of Arabica variety, it is very important to know its chemical properties, and whether or to what extend are these properties affected by the geographical origin. However, it is essential to state that it is biological material, that can contain more then 800 chemical compounds. Their content can vary based on the geographical origin of specific beans. From the consumer's point of view, carbohydrates, lipids, nitrogen compounds, the alkaloid caffeine, polyphenol compounds and others are extremely important (**Farah, 2012).** According to **Bobková et al. (2020)**, coffee is considered to be a rich source of substances that are characterized by the potent ability of scavenging free radicals; therefore they are referred to as antioxidants. One of them is caffeine that is consumed on daily basis from coffee beverages. **Cappaletti et al. (2015)** claimed that caffeine intake is generally associated with increased attention since is an antagonist of adenosine receptors. During its half-life, (2-6 hours after intake), it can increase the body's performance and improve mood. However, high doses can manifest with negative effects e.g. anxiety, tachycardia and insomnia. However, with long-term consumption, most of these acute effects may disappear as metabolic adaptation occurs in the body.
Furthermore, coffee its well known for the content of chlorogenic acids (CGAs) that are considered to be antioxidants **(Moon et al., 2009).** More than 30 CGA isomers are available in coffee beans, including the caffeoylquinic components,

dicofeoylquinic acid, feruloylquinic acid, p-coumaroylquinic acid, and others **(Farah and Lima, 2019).**
This research was aimed to evaluate whether chemical parameters such as total antioxidant capacity, caffeine and chlorogenic acids content can be used for the determination of coffee origin.

MATERIAL AND METHODS

Extract preparation

Green coffee samples were obtained from Barzzuz s.r.o., a company that is focused on importing and processing of coffee from various well-known producers. Our monitored group of 15 samples were divided into three sub-groups based on the declared country of origin. Group one contained five samples from America, group two five samples from Asia, and group three similarly contained five samples from Africa. Subsequently, all of our analyses were done for both green and roasted samples. Samples were roasted at the medium roasting level, Full City ++, using traditional technology in a drum roasting machine with gas heating (Toper TKSMX 10, Turkey; a batch of 1 sample of green coffee was 500 g) under air or nitrogen at 232 °C. The roasting time was in the range between 500 and 840 s. After roasting, coffee beans were cooled in the same atmosphere as was used during roasting.
Green coffee beans are characterized by their structure, so we froze them at -80 °C for 30 minutes before the analysis due to easier and better homogenization. We weighed 10 g of roasted coffee using Kern PLJ 600-3-CM (Germany). The coffee sample was then homogenized using an IKA A10 basic (IKA Works, Wilmington, USA) for 30 s, to the required grinding thickness. The homogenized samples were then sieved through a sieve with a mesh diameter of 1 mm. Particle size analysis showed that a sample particle size of 0.23 to 0.5 mm is optimal for both roasted and green coffee use, as a size is commonly used to prepare espresso. For final extract preparation, 7 g of homogenized sample was weighted using Kern 120-5DM (Germany) in glass beakers with a volume of 150 mL and added 120 mL of demineralized water (95 °C), extraction time was 5 min with occasional stirring. Final extracts were filtered through Sartorius filter paper (type Grade-390-quantitative, Germany) and the obtained stock solution was used to determine the TAC using DPPH method according Brandt-Williams, caffeine and chlorogenic acid content by HPLC.

Determination of Total Antioxidant Capacity (TAC) using DPPH radical method according Brandt-Williams

The total antioxidant capacity (TAC) was determined as free radical scavenging assay using DPPH (2,2-diphenyl1-picrylhydrazyl) according to Brand-Williams et al. method with minor modifications **Bobková et al. (2020).** The amount of 0.025 mg of DPPH was weighed properly, then dissolved with methanol and the volumetric flask with the stock solution was filled up to 100 mL. The stock solution was diluted with methanol (1:9) to obtain absorbance of approximately 0.7. The diluted 3.9 mL DPPH solution was put into the glass cuvettes and the initial DPPH absorbance (A_0) was measured at a wavelength of 515.6 nm. Then 100 µL of sample extract was pipetted into cuvette and the mixture was stirred with a glass stick. Absorbance (A_t) was measured after 10 minutes at 515.6 nm (T80 UV/VIS Spectrometer). The scavenging capacity was calculated using the following equation:

$$\%\ inhibition\ of\ DPPH = \frac{(A_o - A_s) - (A_t - A_s)}{(A_0 - A_s)} . 100$$

Determination of caffeine content and chlorogenic acids (CGAs) using HPLC

Preparation of coffee extracts for HPLC analysis

Prepared coffee extracts for HPLC analysis were additionally filtered using 25 mm PVDF syringe filters, pore size 0.45 µmm (Agilent Technologies, Germany - Agilent 1260 Infinity equipped with DAD detector (1260 DAD VL+), and subsequently placed in HPLC vials.

Reagents used for HPLC analysis

Caffeine standard (HPLC standard with a purity of 98% from Sigma - Aldrich GmbH, Steinheim, Germany), and standards of three chlorogenic acids (chlorogenic acid: 5-Caffeoylquinic acid, phyproof ® Reference Substance, purity ≥95.0% for HPLC; neochlorogenic acid: 5-O-(trans-3,4-Dihydroxycinnamoyl)-D-quinic acid, phyproof ® Reference Substance, purity≥95.0% for HPLC; and cryptochlorogenic acid: 4-O-(3,4-Dihydroxycinnamoyl)-D-quinic acid, phyproof ® Reference Substance, purity ≥98.0% for HPLC, all produced by PhytoLab GmbH & Co. KG, Vestenbergsgreuth, Germany) purchased from Lambda Life, ltd., Slovakia. HPLC acetonitrile (Chromasolv Gradient, purity ≥ 99.8%, Sigma-Aldrich GmbH, Steinheim, Germany), phosphoric acid (ACS, purity ≥ 96%, Sigma-Aldrich GmbH, Steinheim, Germany), and deionized water ddH_2O (18.2 MΩ cm^{-1}, 25 °C) were used for standards and mobile phase preparation.

HPLC determination of caffeine and chlorogenic acids

Agilent Infinity 1260 (Agilent Technologies GmbH, Waldbronn, Germany) was used for the analysis. The separation itself was performed on a C-18 Poroshell 120 column (150 mm x 3 mm x 2.7 µm) (Agilent Technologies, Waldbronn, Germany). The mobile phases were as follows: acetonitrile (A) and 0.1% H_3PO_4 in ddH_2O (v/v) (B), the separation being carried out by gradient elution according to the following program: 0-1 min. isocratic elution (20% A + 80% B), 1-5 min. linear gradient elution (25% A + 75% B), 5-15 min. (30% A + 70% B), 15-25 min. (40% A + 60% B). The equilibration time was 3 min. before the next injection. Flow was mobile phase was 1 $mL.min^{-1}$, the injection was 10 µL and the separation temperature was set at 30 °C, the samples were stored at 4 °C prior the analysis. The detection wavelengths were set as follows: 276 nm (caffeine) and 320 nm (chlorogenic acids), with a total of data collected in the wavelength range 210-400 nm. According to the UV spectrum and retention times content of chlorogenic acids is expressed as sum of abovementioned acids.

Statistical Analysis

For the summarizing and describing of our results descriptive statistic was used. Parameters such as minimum, maximum, arithmetic means, and standard deviation were used for interpreting the results. For the determination of significant differences between selected geographical origin, regarding the TAC, caffeine and content of chlorogenic acids, ANOVA single factor was used separately for both green and roasted coffee. This statistical analysis was performed using Microsoft Office Excel 2016 for Windows. Moreover, for an appropriate graphical representation of results, Quadratic Discrimination Analysis (QDA) was performed using XLSTAT Excel for Windows.

RESULTS AND DISCUSSION

To evaluate the total antioxidant capacity of green and roasted coffees, we, similarly to **Patay et al. (2016**), used the method by Brand-Williams (1995) using the DPPH radical, which consists in the reaction of antioxidants with the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol dilution. It is well known that the antioxidant activity of green coffee is related to the content of chlorogenic, ferulic, caffeic and n-coumaric acids contained in it, and their content depends mainly on the type of coffee (*C. arabica*, *C canephora*) and also of origin **(Stalmach et al., 2006).**
Among geographical group of green samples from America, Africa and Asia, the highest values were measured in samples from America, ranged from 91.89 % to 93.96% inhibition of DPPH. Lower values were obtained from coffee samples from Africa, ranging from 81.62 % to 91.52%. The lowest values were measured in Asian coffees, and ranged from 83.81% to 91.63%. Moreover, using ANOVA single factor, and by further comparing the values of total antioxidant capacity of green coffee samples, we proved statistically significant differences between places of coffee origin. Based on results showed in Table 1, is clear that there are statistically significant differences between samples (marked as upper index a and b in Table 1) from Africa and America and America and Asia regarding the TAC. Nevertheless, there is no significant difference between green samples from Asia and Africa regarding the total antioxidant capacity (Table 1).
Chlorogenic acids are the main polyphenolic compound with antioxidant properties **(Monteiro, 2005; Mullen, 2013),** According to **Doo** and **Maskarinec (2014),** chlorogenic acids are found in amounts from 70 to 350 mg per cup (approx. 140 mL) and can be the cause of the beneficial effects of coffee. Green coffee bean can contain up to 5 chlorogenic acid derivatives, caffeoylquinic acid (CQA), dicafeoylquinic acid (diCQA), feruoylquinic acid (FQA), pcoumaroylquinic acid (CoQA) and caffeeoferluoylquinic acid (CFQA). **Narita and Inouye (2011)** reported that green coffee beans of *Coffea arabica* contain approximately from 3.5 to 7.5% of chlorogenic acids and their derivatives. **Farah and Lima (2019)** added that beverages prepared from various cultivars of *Coffea arabica* can contain 4-6 g CGA. 100 g^{-1}. Which is in accordance with our measured values. Highest values of chlorogenic acids were measured in samples from Africa, ranged from 3.89 to 5.79 of g CGA. 100 g^{-1}, on the other hand, the lowest were found in samples from Asia (from 4.507 to 4.913 g CGA. 100 g^{-1}). However, based on our results is clear that there is no significant difference between samples from Asia, America and Asia regarding the content of chlorogenic acids.
Another measured parameter in green samples was caffeine content. According to **Belitz et al (2009**), caffeine (1,3,7-trimethylxanthine) is due to its physiological effect the best-known nitrogen compound of coffee. The average caffeine content in beans of *Coffea arabica* is 0.8 - 1.4%, which is roughly 1.0 – 1.5 $g.100g^{-1}$, depending of Arabica variety. Highest amount of caffeine was recorded in samples from America (0,761 to 0,890 g.100 g^{-1}). In African coffee, values ranged from 0,7617 to 0,8923 g.100 g^{-1} of caffeine. On the other hand, Asian coffees showed averagely lowest values, ranging from 0,7264 to 0,7969 g.100 g^{-1}. Based on results shown in Table 1, we observed a significant difference

between samples from Africa and America regarding the caffeine content, however, we are not able to differentiate between samples from Asia and Africa just based on this parameter.

Table 1 ANOVA single factor for total antioxidant capacity, content of chlorogenic acids and caffeine in samples of green coffee

Total antioxidant capacity				
Groups	*Count*	*Average*	*SD*	*Variance*
Africa[b]	5	86.402 %	3.446	14.843
America[a]	5	93.014 %	0.913	1.425
Asia[b]	5	85.952 %	2.879	10.362
p-value				**0.0042**
Chlorogenic acids content				
Groups	*Count*	*Average*	*SD*	*Variance*
Africa	5	5.037 g CGA. 100 g^{-1}	0.773	0.748
America	5	4.817 g CGA. 100 g^{-1}	0.424	0.224
Asia	5	4.718 g CGA. 100 g^{-1}	0.157	0.031
p-value				**0.6809**
Caffeine content				
Groups	*Count*	*Average*	*SD*	*Variance*
Africa[b]	5	0.806 g.100 g^{-1}	0.047	0,002859
America[a]	5	0.854 g.100 g^{-1}	0.024	0,000724
Asia[b]	5	0.771 g.100 g^{-1}	0.029	0,001088
p-value				**0,01986**

Notes: a,b = groups within a column with different superscripts differ significantly at P≤0.05, one-way ANOVA.

In samples of roasted coffees values of TAC decreased by 7, 47 % in Africa samples, by 18,12 % in American, and 13,73 % in samples from Asia. By comparing values of total antioxidant capacity of roasted coffee samples we proved statistically significant differences between places of coffee origin, comparing samples from Africa and Asia, and the biggest difference of variances is clearly visible between these two groups. On the contrary though, by comparing Africa vs America and America vs Asia, we can see differences in their variance. However, statistical analysis shows that those differences are not significant, but p-value of both mutual comparisons are approaching the set α of 0,05 of used test. These results are a hint that TAC content can be used as a tool to separate coffee samples by their geographical origin (Table 2).

ANOVA test of chlorogenic acids showed that we accept null hypothesis, there is no difference among groups of samples.

Average values of CGA is relatively even among all groups with very small standard deviation of whole groups, and very small variance within and among geographical groups. Similarly, as in green samples, this parameter did not show a significant difference among groups (Table 2).

In all our samples of coffee roasted at roasting level Full City ++ (232 °C), we found a slight increase in the level of extractable caffeine compared to green coffee samples. These findings are in accordance with **Farah (2012)** so that the caffeine content in roasted coffees can ranged from 1.1 to 1.3 g.100 g^{-1}, and the author also noted a slight increase in the caffeine content compared to green samples. Possible, although minimal changes in caffeine content is justified by the fact that caffeine does not change significantly during roasting, but a slight decrease in its content may be due to sublimation (overheating) and vice versa, its increase may be observed due to decline of other components.

Content of caffeine seems to be the best factor for separating groups based on geographical origin in samples of roasted coffee given p-value among groups is 0.0083 which is highly significant. Based on comparations between pairs we are able to separate Africa and America samples, America and Asia samples. However, Africa and Asia samples showed p-value over 0.05, so we, again, accepting null hypothesis (Table 2).

Table 2 ANOVA single factor for Total Antioxidant Capacity, content of chlorogenic acids and caffeine in roasted coffee samples

Total Antioxidant Capacity				
Groups	*Count*	*Average*	*SD*	*Variance*
Africa[a]	5	78.946 %	2.751	9.462
America[a,b]	5	74.906 %	2.288	6.541
Asia[b]	5	72.224 %	1.215	1.845
p-value				**0.0032**
Content of chlorogenic acids				
Groups	*Count*	*Average*	*SD*	*Variance*
Africa	5	0.5954 g CGA. 100 g^{-1}	0.0993	0.0123
America	5	0.5964 g CGA. 100 g^{-1}	0.1106	0.0153
Asia	5	0.6346 g CGA. 100 g^{-1}	0.1509	0.0285
p-value				**0.8761**
Caffeine content				
Groups	*Count*	*Average*	*SD*	*Variance*
Africa[b]	5	1.0352 g.100 g^{-1}	0.0535	0.0036
America[a]	5	1.2012 g.100 g^{-1}	0.0811	0.0082
Asia[b]	5	1.0896 g.100 g^{-1}	0.0475	0.0028
p-value				**0.0083**

Notes: a,b = groups within a column with different superscripts differ significantly at P≤0.05, one-way ANOVA.

For multi-component analysis QDA was used. Based on Figure 1it is obvious that selected parameters (variables) are almost equally suitable and affect the possible determination of coffee origin. Wilks' Lambda test (Rao's approximation) showed significant differences among groups (p-value=0.026). Confusion matrix for the cross-validation results showed that we were able to determine the origin of green American coffee with accuracy of 100 % based on total antioxidant activity, caffeine and chlorogenic acids content, nevertheless, regarding the other two geographical groups, more parameters, or samples respectively, would be needed to achieve higher percentage accuracy.

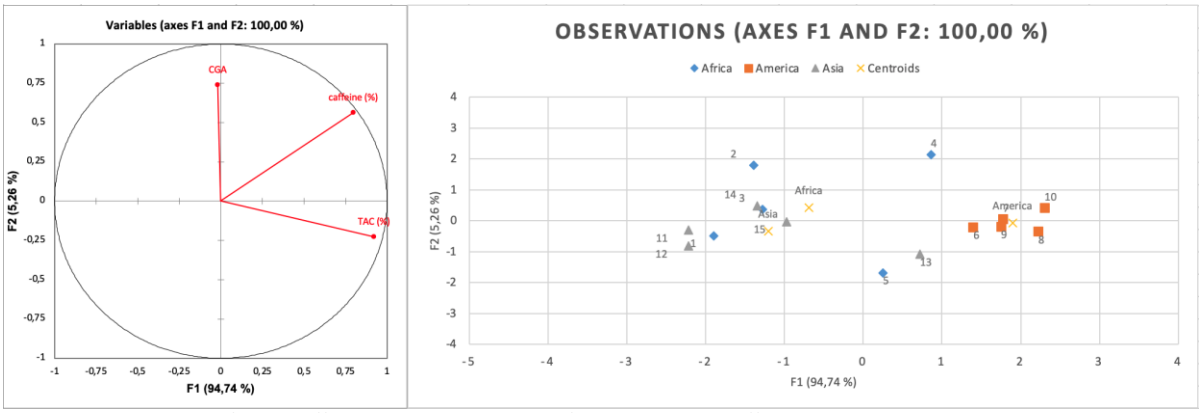

Figure 1 Determination of green coffee origin regarding values of TAC, GCAs and caffeine content using Multi-component QDA analysis

Same statistic was done for roasted samples. On the basis of Figure 2 is obvious that TAC and caffeine content appears to be more suitable parameters and for determination of geographical origin of roasted coffee more than content of chlorogenic acids. This may be caused by the fact that CGAs are thermally unstable, therefore are degraded during roasting to produce products that contribute to the formation of coffee aroma, the final taste of the drink and according to **Jeszka-Skowron et al. (2016)** their levels in the coffee beverage also depend on the degree of roasting. Coffee roasted to dark roasting level can containing less than 10% of their original content. On the contrary, caffeine is more stable and even though some of antioxidants are degraded during roasting, this decrease is compensated by formatting of products of Maillard reaction, which show antioxidant properties too. Wilks' Lambda test (Rao's approximation) showed significant differences among groups (p-value=0.002).

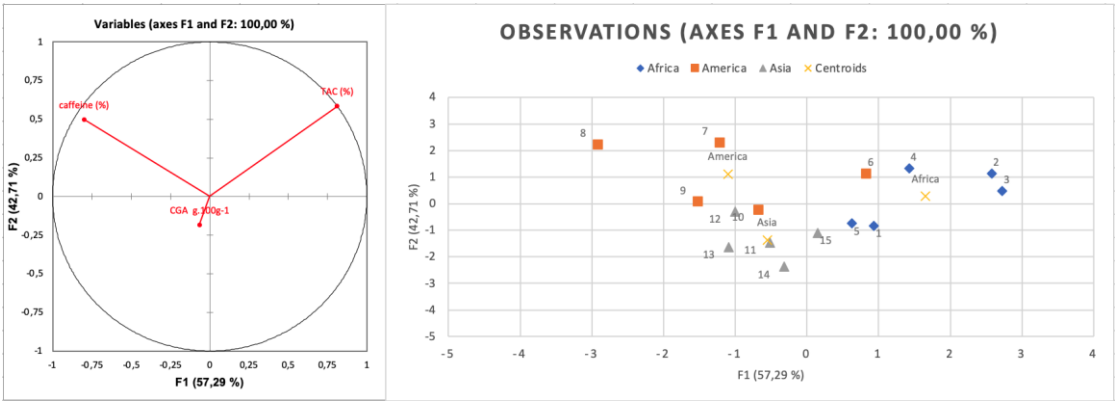

Figure 2 Determination of roasted coffee origin regarding values of TAC, GCAs and caffeine content using Multi-component QDA analysis

CONCLUSION

This research suggested that certain chemical parameters may be used for the determination of geographical origin of both green and roasted coffee samples. Using Multi-component QDA analysis was observed that in green coffee samples TAC, and both caffeine and chlorogenic acids contend almost equally affected the determination among selected groups, on the other hand regarding the roasted samples, content of chlorogenic acids may be a less effective parameter. It is essential to add that even though we have been able to determine significant difference between American, African and Asian coffees, further research that would cover more samples or chemical parameters such as total polyphenol content or content of certain volatile substances, respectively, is needed to achieve higher accuracy in determination of geographical origin of coffee.

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