TITLE: Application of multivariate analysis to investigate potential antioxidants in conventional and torrefacto roasted coffee.

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ABSTRACT

In the present work multivariate statistical techniques were applied to the coffee compounds and the overall antioxidant capacity of commercial conventional and torrefacto roasted coffees in order to investigate the main antioxidant compounds in coffee. Statistical analyses showed significant correlations between browned compounds, trigonelline, 5- caffeoylquinic acid and cafeic acid contents with the antioxidant activity measured by both DPPH˙ and redox potential methods. Trigonelline solutions showed an antioxidant capacity close to zero and should not be considered as a potential antioxidant compound. Principal Component Analysis (PCA) was applied to evidence the correlations between antioxidant capacity and coffee chemical compounds. Conventional and torrefacto roasted coffees were separated by PC1 (62.5% of the total variance) characterized by antioxidant capacity and chemical compounds highly correlated with antioxidant capacity. Furthermore, a descriptive chemical characterization of conventional and torrefacto ground roasted coffee has been carried out. Sixty nine volatile compounds were identified and quantified. The correlations suggest a prooxidant capacity that should need further investigations.

KEYWORDS: Coffee - Torrefacto roast – Antioxidant- Correlations- Principal Component Analysis (PCA)

INTRODUCTION

During last decades, epidemiological studies on the relationships between coffee consumption and diseases risks have changed to be focused on the positive impact on health promotion or disease prevention [*1, 2*]. In fact, coffee has been proposed as one of the main contributors of dietary antioxidant intake in the diet in Norway [*3*] and in Spain [*4*]. Furthermore, there is strong evidence that moderate consumption of coffee induces an increase of the antioxidant capacity in the human plasma [*5*].

Coffee is a rich source of phenolics, mainly chlorogenic acids and their degradation products (quinic, caffeic, coumaric and ferulic acids). 5-Caffeoilquinic acid has been demonstrated to be a powerful antioxidant *in vitro* and *ex vivo* [*6*]. However, only a total of 10% of the overall antioxidant capacity of roasted coffee was found to be due to chlorogenic acids [*7*]. Hydroxycinnamic acids are in lower amounts and their standards showed moderate antioxidant capacity [*8*]. Caffeine has also been reported as antioxidant [*9, 10*]. But, although decaffeinated coffees exhibit lower antioxidant capacity than regular coffee, they continue being a high antioxidant beverage [*11, 12*]. Also, another nitrogen compound such as trigonelline has been reported as antioxidant included with other coffee compounds [*13, 6*].

However, due to the coffee roasting process, natural phenolic compounds can be lost while other antioxidant compounds, such as Maillard Reaction Products (MRPs), are developed enhancing overall antioxidant properties [*14, 15*]. Multiple studies suggest that melanoidins are responsible for the strong antioxidant properties exhibited by roasted coffee beverages [*16-20*]. In addition, some heterocyclic volatile compounds developed in roasting process have been suggested as antioxidants compounds [*21-23*].

Most of these studies about the antioxidant capacity of coffee compounds have been carried out using individual coffee compound standards or their combinations in model

systems, or by means of coffee extracts or coffee fractionation without taking into account the whole complex matrix. However, overall antioxidant capacity of coffee could be due to several coffee compounds and their synergic and antagonistic interactions.

In previous works, increases of the antioxidant capacity of coffee by the application of torrefacto roasting process, with sugar addition, were observed [*24*, *25*]. In the present study, multivariate statistical techniques were applied to the coffee compounds and the overall antioxidant capacity of commercial conventional and torrefacto roasted coffees in order to investigate the main antioxidant compounds in coffee avoiding possible artefacts produced by extractions, fractionations or individual compounds. Furthermore, a descriptive chemical characterization of conventional and torrefacto ground roasted coffee has been made.

MATERIALS AND METHODS

Materials. Eleven commercial roasted coffee samples were purchased in a local market: two conventional roasted pure *Coffea arabica* from Colombia (namely as Colombian), three conventional roasted coffee blends arabica/robusta (0 T), two blends arabica/robusta with 30% Torrefacto roasted coffee (30 T), two blends arabica/robusta with 50% Torrefacto roasted coffee (50 T) and two 100% Torrefacto roasted coffees robusta variety (100 T).

Pure reference standards were from the suppliers given in parentheses: pentoxifylline caffeine, trigonelline, 5-caffeoylquinic acid, caffeic acid, ferulic acid and 4-vinylguaiacol, acetaldehyde, dimethylsulfide, propanal, furan, 2-methylpropanal, thiophene, hexanal, 3-penten-2-one, (Aldrich, Saint Quentin Fallavier, France), 2-butanone, 3-methylbutanal, 2,3-butanedione, 2,3-pentanedione, 2-methyl-1-propanol, (Acros organic, Noisy le Grand, France).

General parameters. Water activity (aw) was measured using a Novasina Model 503 water activity-meter. Moisture was determined using the method of AOAC [*26*]. Soluble solids were determined according to official AOAC method [*27*]. Ash content was determined by incineration at 550 °C according to official AOAC method [*28*]. The nitrogen was analyzed using the Kjeldahl method [*29*]. Total fat was determined using the method of AOAC [*30*].

Caffeine and Trigonelline. Extract preparation, cleanup and HPLC analysis have already been described by Maeztu *et al*. [*31*]. The high-performance liquid chromatographic system consisted of a Hewlett-Packard HPLC (model 1100 series Madrid, Spain) equipped with a binary pump and an automated sample injector. A reversed phase Hypersil-ODS (5 μm particle size, 250 x 4.6 mm) column was used. The mobile phase was acetonitrile/water (15:85) in isocratic condition at a constant flow rate

of 2.0 mL min-1. The sample injection volume was 20μL and the column was maintained at 25ºC. Detection was accomplished with a diode-array detector (Hewlett-Packard 1100 series) at wavelength of 280 nm.

5-Caffeoylquinic acid (5-CQA). Extraction of 5-CQA, cleanup and HPLC analysis were carried out according to the method of Bicchi *et al.* [*32*]. The HPLC equipment has been described in caffeine and trigonelline method. The conditions of the gradient solvent system used were 100% citrate-acetic acid buffer solution (pH 3.0) for 2 min, 85:15 buffer/methanol for 8 min, both at a flow rate of 0.8 mL min^{-1}, and 85:15 buffer/methanol for 5 min at a flow rate of 1.2 mL min^{-1}. The wavelength of detection was at 325 nm. The sample injection volume was 100μL and the column was maintained at 25º C.

Hydroxycinnamic acids (caffeic acid and ferulic acid) and 4-vinylguaiacol. The extraction, clean-up and HPLC analysis of these three compounds were performed simultaneously, according to the method developed by Alvarez-Vidaurre *et al.* [*33*]. The HPLC analysis was carried out with the same equipment described in caffeine and trigonelline method. The chromatographic separation was achieved at 25ºC by using a complex gradient solvent system with acetonitrile/ water adjusted to pH 2.5 with a phosphoric acid solution already described by Alvarez-Vidaurre *et al.* [*33*]. The wavelengths of detection were 314 nm for caffeic acid, 325 nm for ferulic acid and 210 nm for 4-vinylguaiacol. The sample injection volume was 100μL.

Browned Compounds (Abs 420nm). 50 μL coffee extract, obtained by solid-liquid extraction 10/100 (g/mL) using deionised water at 100 ºC for 10 min, were diluted up to 2 mL with deionised water. Browned compounds were quantified by measuring the absorbance of sample at 420 nm after exactly 1 min, in a 3 mL capacity glass cuvette (1 cm length) with a spectrophotometer Lambda 25 UV-VIS (Perkin-Elmer Instruments,

Madrid, Spain) connected to a thermostatically controlled chamber (25ºC) and equipped with UV WinLab software (Perkin Elmer). This measurement is employed as convenient index of the development of caramelization and Maillard reactions [*34*].

Volatile Compounds. Volatile compounds extraction by Static Headspace (HS) and Chromatography Mass Spectrometry **(**GC-MS) analysis were carried out with the method described by Sanz *et al.* [*35*]. Volatiles were extracted using a static headspace sampler (Hewlett-Packard model 7694). GC analysis was achieved in a Hewlett Packard gas chromatograph (HP model 6890) equipped with a fused silica capillary column DB-Wax (J&W Scientific, i. d. 0.25 mm, 60 m, film thickness = 0.5 μm). The oven temperature was programmed from 40º C for 6 min to 205º C at 3º C min^{-1}. The injector temperature was 180º C. Helium was used as carrier gas in constant flow mode (1 mL min^{-1}). The volume of the injected sample was 1 μL and the split ratio was 6:1. Mass spectrometry was performed with a Hewlett-Packard mass selective detector (model 5973) coupled to the gas chromatograph. The mass spectrometer operated in the electron impact ionization mode (70 eV). The mass spectrometer scanned mass from m/z 29 to 350. Ion source temperature was set at 230 ºC.

Identification of the Volatile Compounds. Identification of the volatile compounds was based on computer matching of unknown spectra with those of the authentic compounds available in the Wiley 275 K Mass Spectral Database (Palisade Corporation, Mass Spectrometry, Newfield, NY.) and with spectra of the pure reference compounds and, in addition, by comparing their retention indexes with those of standard compounds and data from the literature. Linear retention indexes (RI) of the compounds were calculated using a series of alkanes (C5-C30) injected in the same chromatographic conditions and compared with available literature data.

Quantitative measurements. Identified coffee aroma compounds were quantified by GC-MS. Areas of peaks were measured by calculating of the total ionic current (TIC). Results from volatile analysis are provided in total area counts $x10^{-6}$.

Antioxidant Activity by DPPH˙ assay. The antioxidant activity was measured by using the DPPH˙ (2,2-diphenyl-1-picrylhydrazyl) decolourization assay [*36*]. A $6.1x10^{-5}$M DPPH˙ methanol solution was prepared immediately before use. The DPPH˙ solution was adjusted with methanol to an absorbance of 0.7 (± 0.02) at 515 nm in a 1 cm cuvette at 25ºC (Lambda 25 UV, VIS spectrophotometer, Perkin Elmer Instruments, Madrid, Spain). Trigonelline water solutions at different concentrations (5, 15 and 30g L^{-1}) were diluted 1:50 in water prior to analysis. Samples (20 μL) were added to DPPH˙ solution (1.98 mL). After mixing, the absorbance was measured at 515 nm after exactly 1 min, and then every minute for 18 min, with incubation at 25ºC. Reaction rates were calculated using the equation proposed by Manzocco *et al.* [*37*]:

$1/Abs^3-1/Abs_o^3=-3kt$ where k is the DPPH˙ bleaching rate, Abs_o is the initial absorbance value, and Abs is the absorbance at increasing time, t. The antioxidant activity was expressed as slope obtained from the equation ($-Abs^{-3}$ min^{-1}) per mL of sample.

Statistical analysis. All of the analyses were performed in triplicate. Results are shown as mean ± standard deviation. One-way analysis of variance (ANOVA) was applied to the results. T Tukey was applied as the test *a posteriori* with a level of significance of 95%. Correlations among variables were assessed by means of the Pearson's correlation test. Principal Components Analysis (PCA), based on the Pearson correlation matrix, was applied to the obtained data and to the antioxidant capacity results measured by DPPH and redox potential methods [*25*]. All statistical analyses were performed using the SPSS v.11.0 software package for Windows.

RESULTS AND DISCUSSION

Physicochemical Parameters. Data for the physicochemical parameters of the commercial coffee samples are given in Table 1. Obtained results were in the range legally established by the Spanish Food Legislation [*38, 39*] except for 100 T samples that showed higher soluble solids content than the range legally established for torrefacto roasted coffee (25-40 g/100 g). Actually, soluble solids concentration increased with the higher percentage of torrefacto roasted coffee. The levels of total fat are similar to those reported by scientific literature [40] being approximately 15 g/100 g dry matter (d.m.) for Colombian coffees and lower (≈10 g/100 g d.m.) for arabica/robusta blends or robusta samples. In relation to nitrogenous compounds, Torrefacto coffee (100 T) and torrefacto coffee blends (30 T, 50 T) showed in most cases amounts of caffeine and trigonelline higher than in conventional ones (0 T and Colombian). These results might point out to higher robusta coffee amount in Torrefacto blends [*41*]. Higher amounts of 5-CQA were observed in Colombian samples than in conventional roasted coffee blends (0 T). Hydroxycinnamic acids (caffeic and ferulic acids), partially originated by chlorogenic acids hydrolysis during roasting process, as well as 4-vinylguaiacol (degradation product of ferulic acid), did not show a constant behaviour in relation to torrefacto roast.

Browned compounds include those originated from Maillard reactions, such as melanoidins, and sugar caramelization. Colombian coffees presented higher browned compounds content than conventional roasted coffee blends (0 T). This fact could be partly explained by the higher roasting degree usually applied by roasters to Colombian coffee in order to decrease their high acidity. In fact, Delgado-Andrade *et al.* [*19*] extracted higher melanoidins amount in instant coffee brews when roasting degree increased. On the other hand, as can be observed in Table 1, the greatest production of

browned compounds in coffee blends took place under Torrefacto conditions increasing with the percentage of torrefacto coffee in comparison to conventional roasted coffee blend (0 T). These results could be explained by a higher formation of MRPs, but also to caramelization enhanced by sugar addition in torrefacto roasting process.

Volatile Compounds. Figure 1 shows the chromatographic profiles of the volatile compounds of Colombian (A) and 100% Torrefacto (100 T) (B) ground roasted coffees. At a glance, a clear difference in the gas chromatographic profile is shown. In fact, the total area of volatile compounds was $243x10^{-6}$ for Colombian coffees and $93x10^{-6}$ for 100 T. Table 2 shows the chromatographic areas of the identified compounds in all samples. Sixty nine volatile compounds were identified and quantified including three sulphur compounds, eight aldehydes, six esters, sixteen furans, eight ketones, four alcohols, two thiophenes, six pyrroles, two pyridines, nine pyrazines, two thiazoles, one lactone and two other compounds (one alkene and one acid).

Conventional roasted coffee samples (Colombian and 0 T) showed significantly higher total area of volatiles than torrefacto roasted coffee blends because the most abundant volatile chemical classes (aldehydes, furans, ketones and esters) were significantly higher in conventional roasted coffees. Similar results were observed by Sanz *et al.* [*42*] in ground roasted coffee when a blend of Arabica 80%-Robusta 20% (A80:R20) was compared with 50% Torrefacto. Nevertheless, only esters were higher in a blend of Arabica 20%-Robusta80% (A20:R80) than in 50% torrefacto coffee. These results let us suppose that our conventional roasted coffee blends (0 T) were more similar to A80:R20 than A20:R80, and consequently richer in Arabica coffee variety. The areas of pyrazines, pyridines and pyrroles were the highest in 0 T coffees and the lowest in 100 T. These results seem in contradiction with their possible higher formation in Torrefacto roasting process because the addition of sugar might enhance Maillard

reactions [*43*]. However, the higher total area of the volatiles in conventional ground roasted coffees should be also taken into account. Moreover, the contribution of a particular volatile compound to aroma is related to the balance among different aroma compounds. Thus, comparing the relative percentages of the chemical classes, it can be observed that pyrazines were in significantly higher proportion in torrefacto coffees than in conventional ones (11.1% in 100 T *vs.* 6.6 % in 0 T). Consequently, pyrazines could contribute more to aroma in torrefacto coffees than pyrroles and pyridines which had very low relative percentages (1.9% in both coffees for pyrroles, and 1.6% in 100 T *vs*. 3.3% in 0 T for pyridines).

Statistical Approach to Potential Antioxidants. Correlations were calculated between both antioxidant capacity parameters (DPPH˙ method and redox potential) and chemical content of ground roasted coffee samples (Table 3). Highly significant ($p<0.01$) and excellent ($r>0.75$) correlations between both antioxidant capacity parameters (DPPH˙ and redox potential) and browned compounds content (0.814 and -0.796, respectively) were found. Several studies suggest that brown polymeric melanoidins produced by roasting process are responsible for the strong antioxidant capacity of coffee brews [*16, 19, 20*]. There were significant ($p<0.01$) and very good correlations between trigonelline content and the antioxidant capacity measured by DPPH˙ method (0.809) and redox potential (-0.581) suggesting that trigonelline is likely significant contributor to antioxidant capacity in ground roasted coffee. Other authors include trigonelline as antioxidant, but its efficiency is still unclear [*6, 13*]. Taking into account the obtained high correlations with antioxidant capacity and the unclear results of other works, the DPPH activity of trigonelline water solutions at concentration similar to those obtained in coffee (5g L^{-1}) and higher (15 and 30g L^{-1}) was measured. Figure 2 shows these results. Trigonelline solutions exhibited a dose-dependent antioxidant capacity close to

zero in all concentrations. Consequently, in spite of the highly significant correlation coefficients, trigonelline should not be considered as a potential antioxidant compound. These correlations could be related with a higher amount of robusta coffee in Torrefacto blends previously discussed.

Significant *($p<0.05$)* but moderate correlations between both antioxidant capacity parameters and 5-CQA (0.381 and -0.460 respectively) and caffeic acid (0.485 and -0.430 respectively) were also found. The first agrees with the estimation of only a 10% of the overall antioxidant capacity of coffee is due to chlorogenic acids [*7*] and with the antioxidant and pro-oxidant activity of 5-CQA observed by Fujioka *et al.* [*8*]. The observed correlations for caffeic acid agree with the moderate antioxidant activity of this compound reported in model systems [*8*]. Even though ferulic acid has been described as a natural antioxidant [*44*], in the current work it was not significantly correlated with antioxidant capacity. As regard to 4-vinylguaiacol and contrarily with Fujioka *et al*. [*8*], the obtained results suggest a prooxidant activity of this compound. This opposite effect could be explained by both the higher concentration assayed in the first work [*8*] (3 times higher than in actual brewed coffee), and by the different matrix used (model system *vs*. whole complex matrix).

Although the antioxidant capacity of caffeine has been assessed in a previous work [*9*], the chemopreventive activity of coffee seems to be not related to caffeine because regular and decaffeinated coffee brews induced the glutathione-S-transferasa (GST) in mice [*6, 11*]. The latter agrees with the not significant correlation coefficients found between coffee caffeine and the antioxidant parameters ($DPPH^{\cdot}$ and redox potential) (0.228 and 0.059 respectively). In addition Parras *et al.* [*45*] did not observe caffeine as a contributor to antioxidant capacity in coffee brews.

The most abundant volatiles (aldehydes, furans, ketones and esters) were significantly but negatively correlated with DPPH˙, but not significantly correlated with redox potential. The other volatile chemical classes (alcohols, thiophenes, pyrroles, pyridines, thiazoles and lactones) were significantly correlated with both DPPH˙ (-0.423 for alcohols to -0.742 for pyrroles) and redox potential (0.587 for alcohols to 0.856 for pyridines). These correlations suggest a prooxidant capacity in contradiction with the antioxidant capacity reported by Fuster *et al.* [*21*] and Yanagimoto *et al.* [*22, 23*]. On one hand, the levels of chemicals tested in those studies are considerably higher than levels in coffee. On the other hand, these correlations found could be arbitrary correlation due to the fact that those coffees that present higher antioxidant capacity were the less aromatic imputable to botanical variety and roasting process. Furthermore, it is very difficult to deduce the antioxidant or prooxidant capacity of coffee volatile compounds because although they are also in coffee, they are mainly in the headspace of the coffee and, consequently their real contribution to the protective activity in humans should need further investigations.

Principal components analysis (PCA) was applied to evidence graphically the correlations between antioxidant capacity and coffee chemical compounds previously discussed and the characterization of conventional and torrefacto ground roasted coffees. Four principal components (PC) with eigenvalue greater than 1 were obtained. PC1 and PC2 explained 82.8% of the total variance. Figure 3 shows the bidimensional representation for all the variables and coffee samples defined by the two first principal components. PC1, which explained 62.5% of the total variance, is mainly characterized by antioxidant activity (DPPH˙ and redox potential), browned compounds, trigonelline and volatile compounds, except sulphur ones. Conventional coffee samples are found on the positive values of PC1 (right half graphic), whereas torrefacto coffee samples are

placed on the negative half graphic, being 100 T in the left extreme. In fact, as previously discussed, torrefacto roasted coffees were more antioxidant but less aromatic, and conventional roasted coffees were higher aromatic but less antioxidant. PC2, which explained 20.3% of the total variance, is characterized by 5-CQA, caffeine, ferulic acid and its degradation product 4-vinylguaiacol. However, this PC is less important in order to differentiate coffee samples, only Colombian is placed in the down extreme because of its lower caffeine, 4-vinylguaiacol and ferulic acid level.

To sum up, statistical analyses showed significant correlations between browned compounds, trigonelline, 5-CQA and cafeic acid content with the antioxidant capacity measured by both DPPH˙ and redox potential methods. However, trigonelline solutions showed an antioxidant capacity close to zero and should not be considered as a potential antioxidant compound. This positive correlation could be related with a higher amount of robusta coffee in Torrefacto blends. More investigations must be performed to elucidate the role of volatile compounds in antioxidant capacity of coffee.

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LITERATURE CITED

1. Schilter B, Cavin C, Tritscher A, Constable A Health (2001) Effects and Safety Considerations. In Clarke RJ and Vitzthum O (Eds) Blackwell Science, Oxford, pp 165-183.
2. Dórea JG; da Costa TH (2005) Brit J Nutr 93: 773
3. Svilaas A, Sakhi AK, Andersen LF, Svilaas T, Strom EC, Jacobs DR, *et al.* (2004). J Nutr 134:562
4. Pulido R, Hernández-García M, Saura-Calixto F (2003) Eur. J Clin Nutr 57*:*1275
5. Natella F, Nardini M, Giannetti I, Dattilo C, Scaccini C (2002) J Agric Food Chem 50:6211
6. Daglia M, Racchi M, Papetti A, Lanni C, Govoni S, Gazzani G (2004) J Agric Food Chem 52:1700
7. Somoza V, Lindenmeier M, Wenzel E, Frank O, Erbersdobler HF, Hofmann T (2003) J Agric Food Chem 51:6861
8. Fujioka K, Shibamoto T (2006) J Agric Food Chem 54:6054
9. Devasagayam TPA, Kamat JP, Hari M, Kesavan PC (1996) Biochim Biophys Acta 1282: 63
10. Azam S, Hadi N, Khan NU, Hadi SM (2003) Med Sci Monit 9:325
11. Abraham SK, Singh SP (1999) Food Chem Toxicol 37:733
12. Pellegrini N, Serafini M, Colombi B Del Rio D Salvatores S Bianchi M Brighenti F (2003) J Nutr 133:2812
13. Goodman BA, Glidewell SM, Deighton N, Morrice, AE (1994) Food Chem 51:399
14. Nicoli MC, Anese M, Parpinel MT, Franceschi S, Lerici CR (1997) Cancer Lett 114:71
15. Anese M, Nicoli MC (2003) J Agric Food Chem, 51:942

16. Nicoli MC, Anese M, Manzocco L, Lerici CR (1997) Food Sci Technol 30:292
17. Daglia M, Papetti A, Gregotti C, Bertè F, Gazzani G (2000) J Agric Food Chem 48:1449
18. Borrelli RC, Visconti A, Mennella C, Anese M, Fogliano V (2002) J Agric Food Chem 50:6527
19. Delgado-Andrade C, Morales F J (2005a) J Agric Food Chem 53:1403
20. Delgado-Andrade C, Rufián-Henares JA, Morales FJ (2005b) J Agric Food Chem 53:7832
21. Fuster MD, Mitchell AE, Ochi H, Shibamoto T (2000) J Agric Food Chem 48:5600
22. Yanagimoto K, Lee KG, Ochi H, Shibamoto T (2002) J Agric Food Chem 50:5480
23. Yanagimoto K, Ochi H, Lee KG Shibamoto T (2004) J Agric Food Chem 52:592
24. Andueza S, Cid C, Nicoli MC (2004) Lebensm-Wiss u-Technol 37:893
25. López-Galilea I, Andueza S, di Leonardo I, de Peña MP, Cid C (2006) Food Chem 94:75
26. Association of Official Analytical Chemists (2002a) Moisture in coffee. Official Methods of Analysis, 17th ed. Official Method 950.46; AOAC Gaithersburg MD
27. Association of Official Analytical Chemists (2002b) Solids soluble in Roasted coffee. Official Methods of Analysis, 17th ed. Official Method 973.21. AOAC Gaithersburg MD
28. Association of Official Analytical Chemists (2002c) Roasted coffee. Preparation of Test sample. Official Methods of Analysis, 17th ed. Official Method 920.93. AOAC Gaithersburg MD
29. Kirk RS, Sawyer R (1991) 9th ed. Addison Wesley Longman Ltd.: Essex

30. Association of Official Analytical Chemists (2002d) Petroleum ether extract of Roasted coffee. Official Methods of Analysis 17th ed. Official Method 920.97. AOAC Gaithersburg MD
31. Maeztu L, Andueza S, Ibañez C, de Peña MP, Bello J, Cid C (2001) J Agric Food Chem 49:4743
32. Bicchi CP, Pinello AE, Pellegrino GM, Vanni AC (1995) J Agric Food Chem 43:1549
33. Álvarez-Vidaurre P, Pérez-Martínez M, de Peña MP, Cid C (2005) Proceedings of the 13th Euro Food Chem Hamburg, Germany pp 684
34. Meydav S, Saguy I,. Kopelman IJ (1977) J Agric Food Chem 25:602
35. Sanz C, Ansorena D, Bell J, Cid C (2001) J Agric Food Chem 49:1364
36. Brand-Williams W, Cuvelier ME, Berset C (1995) Food Sci Technol 28:25
37. Manzocco L, Anese M, Nicoli MC (1998) Lebensm-Wiss u-Technol 31:694
38. RD 1231/1988 (1988) *BOE* n° 252, 20 Octubre 1988
39. RD 1372/1997 (1997) *BOE* n° 215, 8 de Septiembre de 1997
40. Illy A, Viani R (2004) In Illy A, Viani R, (ed) Academic Press: London, UK
41. Casal S, Oliveira MBPP, Alves MR, Ferreira MA (2000) J Agric Food Chem 48:3420
42. Sanz C, Maeztu L, Zapelena MJ, Bello J, Cid C (2002) J Sci Food Agric 82:840
43. Reineccius GA (1995) Proceedings of the 16th International Colloquium on the Chemistry of Coffee Paris France ASIC pp 249
44. Miyake T, Hagiwara Y, Shibamoto T (1998) ACS-Symposium Series 701 ACS Washington DC pp 178
45. Parras P, Martínez-Tomé M, Jiménez AM, Murcia MA (2007) Food Chem102:582

Figure captions

Figure 1. HS-GC-MS chromatograms of Colombian (A) and 100% Torrefacto (100 T) (B) ground roasted coffee. For peaks identification see Table 2.

Figure 2. DPPH· antioxidant activity of trigonelline water solutions.

Figure 3. Principal Component Analysis (PCA) of the ground roasted coffees.

Table captions

Table 1. Physicochemical parameters of ground roasted coffees.

Table 2. Chromatographic areas ($x10^{-6}$) of identified volatile compounds in ground roasted coffees [1].

Table 3. Pearson correlation coefficients between coffee compounds and antioxidant capacity (measured by DPPH· and redox potential methods) in ground roasted coffees.

Figure 1. HS-GC-MS chromatograms of Colombian (A) and 100% Torrefacto (100 T) (B) ground roasted coffee. For peaks identification see Table 2.

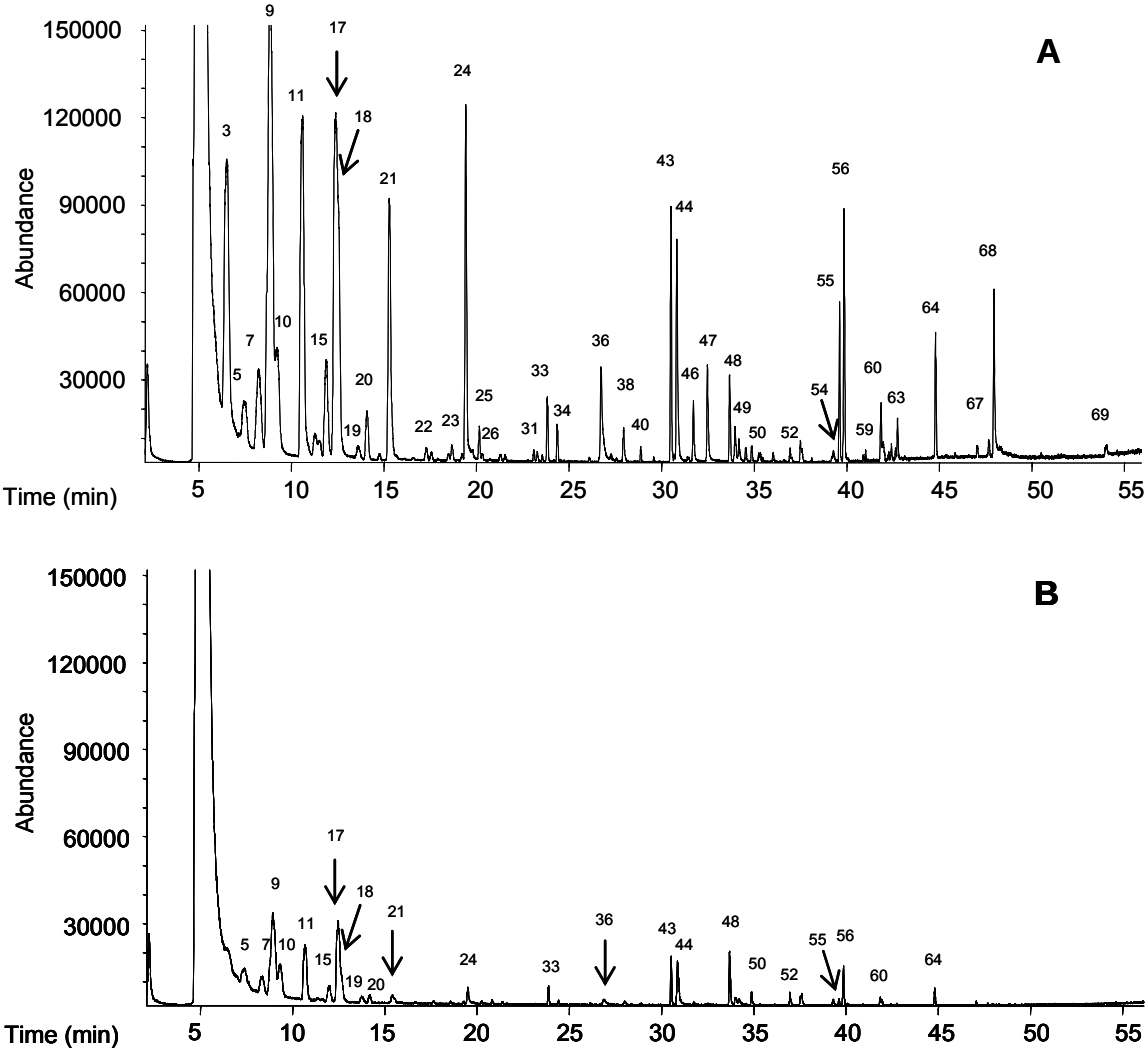
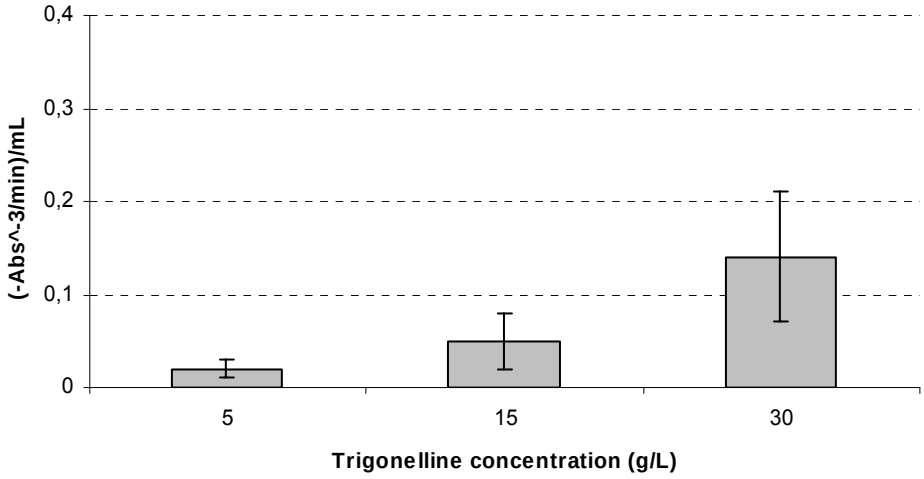


Figure 2. DPPH˙ antioxidant activity of trigonelline water solutions.



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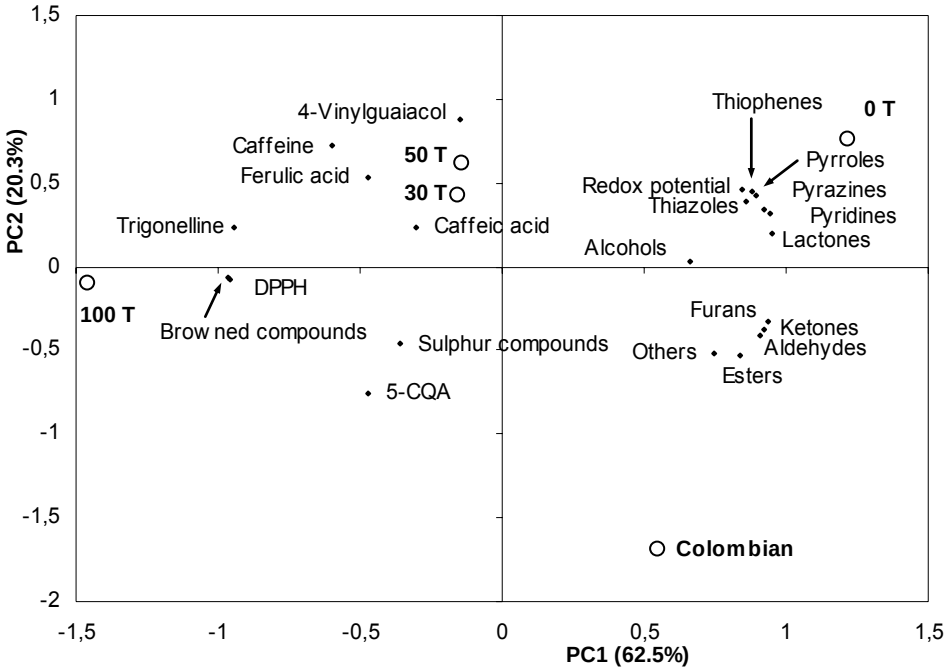

Table 1. Physicochemical parameters of ground roasted coffees.

	Colombian (n=6)	**0 T (n=9)**	**30 T (n=6)**	**50 T (n=6)**	**100 T (n=6)**
Aw	0.07 ± 0.01 ^{a}	0.07 ± 0.01 ^{a}	0.06 ± 0.00 ^{a}	0.07 ± 0.01 ^{a}	0.06 ± 0.00 ^{a}
Moisture (g/100g)	3.23 ± 1.30 ^{bc}	3.88 ± 0.92 ^{bc}	3.06 ± 0.04 ^{b}	4.07 ± 0.16 ^{c}	1.32 ± 0.04 ^{a}
Ash (g/100g)	4.08 ± 0.07 ^{cd}	3.93 ± 0.21 ^{bc}	4.15 ± 0.06 ^{d}	3.88 ± 0.07 ^{b}	3.70 ± 0.01 ^{a}
Soluble Solids (g/100g)	31.33 ± 0.84 ^{a}	32.05 ± 1.78 ^{a}	32.76 ± 0.75 ^{ab}	35.04 ± 1.77 ^{b}	41.95 ± 2.74 ^{c}
Total Fat (g/100g d.m.)	14.56 ± 0.15 ^{c}	10.53 ± 0.60 ^{b}	10.62 ± 0.12 ^{b}	9.12 ± 0.68 ^{a}	9.16 ± 0.23 ^{a}
Nitrogen (g/100g d.m.)	2.44 ± 0.21 ^{a}	2.72 ± 0.14 ^{b}	2.66 ± 0.18 ^{ab}	2.79 ± 0.08 ^{b}	2.64 ± 0.11 ^{ab}
Caffeine (g/100g d.m.)	1.13 ± 0.02 ^{a}	1.64 ± 0.16 ^{b}	1.58 ± 0.16 ^{b}	1.82 ± 0.05 ^{c}	1.95 ± 0.01 ^{d}
Trigonelline (g/100g d.m.)	0.43 ± 0.01 ^{b}	0.38 ± 0.02 ^{a}	0.57 ± 0.08 ^{c}	0.61 ± 0.05 ^{d}	0.68 ± 0.02 ^{e}
5-CQA (g/100g d.m.)	0.43 ± 0.02 ^{c}	0.31 ± 0.02 ^{a}	0.38 ± 0.03 ^{b}	0.29 ± 0.01 ^{a}	0.43 ± 0.01 ^{c}
Caffeic Acid (mg/100g d.m.)	0.55 ± 0.02 ^{ab}	0.39 ± 0.05 ^{a}	0.63 ± 0.06 ^{b}	1.12 ± 0.41 ^{c}	0.58 ± 0.04 ^{b}
Ferulic Acid (mg/100g d.m.)	1.08 ± 0.37 ^{a}	1.60 ± 0.14 ^{bc}	1.93 ± 0.58 ^{c}	1.28 ± 0.12 ^{ab}	1.88 ± 0.37 ^{c}
4-Vinylguaiacol (mg/100g d.m.)	2.82 ± 0.35 ^{a}	6.61 ± 1.74 ^{c}	4.86 ± 0.89 ^{b}	5.76 ± 0.19 ^{bc}	3.06 ± 0.47 ^{a}
Browned Compounds (Abs 420nm)	0.44 ± 0.02 ^{b}	0.32 ± 0.04 ^{a}	0.48 ± 0.03 ^{c}	0.54 ± 0.02 ^{d}	0.62 ± 0.02 ^{e}
Antioxidant capacity †					
DPPH method (-Abs^-3/min)/g	439.45 ± 44.77 ^{b}	344.83 ± 51.30 ^{a}	477.12 ± 28.84 ^{bc}	518.95 ± 31.54 ^{c}	584.86 ± 26.91 ^{d}
Redox potential (mV)	10.75 ± 2.04 ^{b}	28.08 ± 3.37 ^{d}	20.17 ± 5.01 ^{c}	13.93 ± 0.83 ^{bc}	-1.71 ± 11.40 ^{a}

All values are shown as means ± standard deviations. In each row, different letters indicate significant difference ($p < 0.05$) among coffee samples.
d.m.: dry matter. † These results were published in reference 25.

Table 2. Chromatographic areas ($x10^{-6}$) of identified volatile compounds in ground roasted coffees [1].

Peak [2]	KI [3]	Id [4]	Compound	Colombian (n=6)	0 T (n=9)	30 T (n=6)	50 T (n=6)	100 T (n=6)
1	624	B	1,3-Pentadiene	3.10 ± 0.41 ^{c}	1.88 ± 0.74 ^{b}	1.65 ± 1.03 ^{ab}	0.80 ± 0.14 ^{ab}	0.46 ± 0.04 ^{a}
2	635	B	Methanethiol	0.14 ± 0.03 ^{b}	0.02 ± 0.03 ^{a}	n.d.	n.d.	0.16 ± 0.01 ^{b}
3	645	A	Acetaldehyde	8.29 ± 0.21 ^{d}	4.39 ± 0.48 ^{c}	2.73 ± 0.95 ^{b}	2.17 ± 0.37 ^{b}	0.39 ± 0.00 ^{a}
4	671	A	Dimethylsulphide	0.98 ± 0.39 ^{b}	0.37 ± 0.29 ^{a}	n.d.	n.d.	1.59 ± 0.11 ^{c}
5	682	B	Formic acid. methyl ester	21.47 ± 0.77 ^{c}	12.97 ± 2.68 ^{b}	6.09 ± 2.12 ^{a}	5.48 ± 1.11 ^{a}	2.98 ± 0.10 ^{a}
6	712	A	Propanal	7.11 ± 0.39 ^{d}	5.17 ± 0.80 ^{c}	2.43 ± 0.43 ^{b}	2.36 ± 0.30 ^{b}	0.72 ± 0.04 ^{a}
7	716	A	Furan	3.94 ± 0.49 ^{c}	4.13 ± 0.70 ^{c}	2.39 ± 0.78 ^{b}	2.63 ± 0.62 ^{b}	1.14 ± 0.06 ^{a}
8	747	A	2-Methylpropanal	17.39 ± 1.37 ^{c}	16.03 ± 2.40 ^{c}	7.15 ± 1.09 ^{ab}	7.62 ± 1.48 ^{b}	4.34 ± 0.42 ^{a}
9	753	B	2-Propanone	22.83 ± 1.45 ^{c}	24.34 ± 2.38 ^{c}	13.69 ± 1.79 ^{b}	17.13 ± 3.35 ^{b}	4.06 ± 0.08 ^{a}
10	782	B	Acetic acid methyl ester	6.69 ± 0.80 ^{b}	9.35 ± 1.99 ^{c}	4.50 ± 0.44 ^{b}	5.44 ± 0.89 ^{b}	2.12 ± 0.13 ^{a}
11	832	B	2-Methylfuran	28.05 ± 1.21 ^{c}	29.86 ± 4.73 ^{c}	15.00 ± 0.65 ^{b}	18.64 ± 4.94 ^{b}	5.84 ± 0.68 ^{a}
12	839	B	Butanal	0.38 ± 0.04 ^{ab}	0.45 ± 0.13 ^{ab}	0.19 ± 0.12 ^{a}	0.18 ± 0.04 ^{a}	0.69 ± 0.81 ^{b}
13	850	B	Acetic acid ethyl ester	0.63 ± 0.55 ^{a}	n.d.	0.33 ± 0.36 ^{a}	0.34 ± 0.37 ^{a}	n.d.
14	858	B	3-Methylfuran	1.49 ± 0.06 ^{c}	1.52 ± 0.34 ^{c}	1.03 ± 0.39 ^{bc}	0.94 ± 0.18 ^{b}	0.26 ± 0.04 ^{a}
15	866	A	2-Butanone	4.64 ± 0.15 ^{c}	5.11 ± 0.84 ^{c}	3.21 ± 0.75 ^{b}	3.46 ± 0.55 ^{b}	0.95 ± 0.06 ^{a}
16	872	B	Propanoic acid. methyl ester	0.11 ± 0.01 ^{a}	0.14 ± 0.04 ^{a}	0.09 ± 0.04 ^{a}	0.55 ± 0.55 ^{a}	0.25 ± 0.03 ^{a}
17	880	B	2-Methylbutanal	15.54 ± 1.42 ^{c}	15.59 ± 2.48 ^{c}	8.34 ± 1.21 ^{b}	9.85 ± 0.35 ^{b}	4.16 ± 0.43 ^{a}
18	884	A	3-Methylbutanal	21.10 ± 1.25 ^{c}	20.47 ± 3.22 ^{c}	10.72 ± 1.09 ^{b}	10.82 ± 2.54 ^{b}	3.43 ± 0.40 ^{a}
19	913	B	Ethanol	2.13 ± 0.55 ^{ab}	3.32 ± 1.34 ^{b}	0.89 ± 0.05 ^{a}	1.17 ± 0.29 ^{a}	1.07 ± 0.06 ^{a}
20	930	B	2,5-Dimethylfuran	2.12 ± 0.20 ^{bc}	2.32 ± 0.37 ^{c}	1.97 ± 0.37 ^{bc}	1.64 ± 0.24 ^{b}	0.53 ± 0.05 ^{a}
21	962	A	2,3-Butanedione	8.84 ± 1.85 ^{d}	6.44 ± 1.09 ^{c}	3.39 ± 0.51 ^{b}	2.83 ± 0.51 ^{b}	0.68 ± 0.06 ^{a}
22	1021	A	Thiophene	0.38 ± 0.01 ^{b}	0.63 ± 0.12 ^{c}	0.42 ± 0.02 ^{b}	0.45 ± 0.02 ^{b}	0.19 ± 0.02 ^{a}
23	1053	B	3-Hexanone	0.18 ± 0.00 ^{a}	0.25 ± 0.04 ^{b}	0.19 ± 0.01 ^{a}	0.21 ± 0.02 ^{ab}	1.08 ± 0.09 ^{c}
24	1058	A	2,3-Pentanedione	11.68 ± 1.46 ^{d}	7.37 ± 1.03 ^{c}	4.12 ± 0.66 ^{b}	3.10 ± 0.38 ^{b}	1.39 ± 0.13 ^{a}
25	1075	B	2-Vinylfuran	0.47 ± 0.04 ^{d}	0.41 ± 0.05 ^{cd}	0.32 ± 0.08 ^{bc}	0.26 ± 0.03 ^{b}	0.09 ± 0.01 ^{a}
26	1077	B	Dimethyldisulphide	0.29 ± 0.07 ^{ab}	0.74 ± 0.34 ^{c}	0.31 ± 0.10 ^{ab}	0.55 ± 0.08 ^{bc}	0.08 ± 0.01 ^{a}
27	1084	A	Hexanal	n.d.	0.10 ± 0.02 ^{a}	0.11 ± 0.02 ^{a}	0.13 ± 0.02 ^{b}	0.15 ± 0.00 ^{b}
28	1097	B	2-Methylthiophene	0.18 ± 0.01 ^{b}	0.29 ± 0.05 ^{d}	0.22 ± 0.01 ^{bc}	0.24 ± 0.02 ^{c}	0.10 ± 0.01 ^{a}
29	1102	B	2-Methyl-2-butenal	0.20 ± 0.01 ^{b}	0.26 ± 0.03 ^{c}	0.19 ± 0.03 ^{b}	0.18 ± 0.01 ^{b}	0.05 ± 0.01 ^{a}
30	1103	A	2-Methyl-1-propanol	n.d.	0.11 ± 0.17 ^{a}	n.d.	n.d.	n.d.
31	1138	A	3-Penten-2-one	0.18 ± 0.04 ^{c}	0.16 ± 0.03 ^{bc}	0.11 ± 0.04 ^{ab}	0.10 ± 0.02 ^{a}	n.d.
32	1143	B	3,4-Hexanedione	0.31 ± 0.05 ^{c}	0.16 ± 0.05 ^{b}	0.17 ± 0.05 ^{b}	0.13 ± 0.04 ^{b}	0.05 ± 0.01 ^{a}
33	1149	B	1-Methyl-1H-pyrrole	1.64 ± 0.06 ^{b}	2.98 ± 0.77 ^{c}	1.96 ± 0.16 ^{b}	1.96 ± 0.16 ^{b}	0.64 ± 0.06 ^{a}
34	1160	B	2-Vinyl-5-methylfuran	0.68 ± 0.05 ^{c}	0.59 ± 0.08 ^{c}	0.46 ± 0.12 ^{b}	0.41 ± 0.03 ^{b}	0.13 ± 0.01 ^{a}
35	1194	B	1-Ethyl-1H-pyrrole	0.09 ± 0.02 ^{ab}	0.26 ± 0.08 ^{d}	0.16 ± 0.01 ^{bc}	0.19 ± 0.02 ^{cd}	0.05 ± 0.00 ^{a}
36	1203	B	Pyridine	4.00 ± 0.82 ^{b}	7.30 ± 1.56 ^{c}	4.78 ± 0.51 ^{b}	4.01 ± 0.73 ^{b}	0.80 ± 0.08 ^{a}
37	1225	B	2,5-Dimethylpyrrole	0.06 ± 0.01 ^{ab}	0.15 ± 0.04 ^{c}	0.09 ± 0.01 ^{b}	0.08 ± 0.01 ^{b}	0.02 ± 0.00 ^{a}
38	1231	B	Pyrazine	0.96 ± 0.18 ^{b}	1.62 ± 0.42 ^{c}	0.82 ± 0.11 ^{b}	1.02 ± 0.24 ^{b}	0.22 ± 0.01 ^{a}
39	1239	B	2-Methylpyridine	n.d.	0.06 ± 0.04 ^{a}	0.03 ± 0.03 ^{a}	0.05 ± 0.01 ^{a}	n.d.
40	1252	B	Furfurylmethylether	0.25 ± 0.04 ^{b}	0.25 ± 0.03 ^{b}	0.21 ± 0.04 ^{b}	0.19 ± 0.03 ^{b}	0.06 ± 0.01 ^{a}
41	1264	B	3-Methyl-3-buten-1-ol	n.d.	0.02 ± 0.03 ^{a}	0.06 ± 0.06 ^{a}	0.03 ± 0.03 ^{a}	0.60 ± 0.00 ^{b}
42	1270	B	1,3-Thiazole	0.02 ± 0.00 ^{a}	0.04 ± 0.01 ^{b}	0.01 ± 0.01 ^{a}	0.01 ± 0.01 ^{a}	n.d.
43	1283	B	3(2H)-Furanone, dihydro-2-methyl	3.12 ± 0.49 ^{c}	1.60 ± 0.53 ^{ab}	1.85 ± 0.61 ^{b}	1.09 ± 0.12 ^{ab}	0.89 ± 0.04 ^{a}
44	1288	B	2-Methylpyrazine	5.63 ± 0.55 ^{b}	8.60 ± 1.95 ^{c}	5.89 ± 1.04 ^{b}	5.74 ± 1.09 ^{b}	2.99 ± 0.17 ^{a}
45	1304	B	4-Methylthiazole	0.05 ± 0.01 ^{ab}	0.09 ± 0.03 ^{c}	0.05 ± 0.00 ^{ab}	0.06 ± 0.02 ^{bc}	0.03 ± 0.01 ^{a}
46	1323	B	1-Hydroxy-2-propanone	2.45 ± 0.29 ^{b}	1.28 ± 1.01 ^{a}	n.d.	n.d.	n.d.
47	1347	B	2,5-Dimethylpyrazine	0.93 ± 0.24 ^{a}	1.00 ± 0.22 ^{a}	0.88 ± 0.12 ^{a}	0.75 ± 0.10 ^{a}	0.88 ± 0.02 ^{a}
48	1353	B	2,6-Dimethylpyrazine	1.05 ± 0.31 ^{b}	1.28 ± 0.29 ^{b}	0.99 ± 0.14 ^{ab}	0.93 ± 0.15 ^{ab}	0.56 ± 0.05 ^{a}
49	1359	B	2-Ethylpyrazine	0.66 ± 0.16 ^{b}	1.00 ± 0.22 ^{c}	0.69 ± 0.06 ^{b}	0.74 ± 0.12 ^{bc}	0.06 ± 0.01 ^{a}
50	1372	B	2,3-Dimethylpyrazine	0.27 ± 0.07 ^{a}	0.39 ± 0.05 ^{b}	0.36± 0.01 ^{ab}	0.31 ± 0.03 ^{ab}	0.34 ± 0.01 ^{ab}
51	1411	B	2-Ethyl-6-methylpyrazine	0.10 ± 0.02 ^{a}	0.18 ± 0.05 ^{b}	0.11 ± 0.01 ^{a}	0.13 ± 0.03 ^{ab}	0.40 ± 0.01 ^{c}
52	1419	B	2-Ethyl-5-methylpyrazine	0.29 ± 0.05 ^{b}	0.36 ± 0.05 ^{b}	0.34 ± 0.01 ^{b}	0.32 ± 0.02 ^{b}	0.19 ± 0.00 ^{a}
53	1432	B	2-Ethyl-3-methylpyrazine	0.15 ± 0.02 ^{a}	0.21 ± 0.03 ^{b}	0.20 ± 0.01 ^{b}	0.19 ± 0.02 ^{b}	n.d.
54	1480	B	Acetic acid	2.94 ± 0.02 ^{c}	1.70 ± 0.77 ^{b}	0.75 ± 0.05 ^{a}	3.01 ± 0.45 ^{c}	n.d.
55	1484	B	1-Hydroxy-2-propanone acetate	1.91 ± 0.48 ^{c}	1.59 ± 0.35 ^{bc}	1.18 ± 0.41 ^{b}	1.10 ± 0.15 ^{b}	0.24 ± 0.01 ^{a}
56	1490	B	Furfural	6.01 ± 0.18 ^{c}	2.35 ± 0.62 ^{b}	1.65 ± 0.71 ^{ab}	1.99 ± 0.18 ^{b}	1.03 ± 0.01 ^{a}
57	1509	B	2-Ethyl-1-hexanol	n.d.	n.d.	0.03 ± 0.03 ^{a}	n.d.	n.d.
58	1516	B	2-Furfurylmethylsulphide	0.09 ± 0.01 ^{a}	0.16 ± 0.09 ^{a}	0.09 ± 0.01 ^{a}	0.11 ± 0.02 ^{a}	n.d.
59	1519	B	2-Furfurylformate	0.26 ± 0.13 ^{a}	0.20 ± 0.08 ^{a}	0.11 ± 0.12 ^{a}	0.17 ± 0.06 ^{a}	n.d.
60	1536	B	2-Acetylfuran	0.77 ± 0.12 ^{c}	0.47 ± 0.10 ^{b}	0.42 ± 0.13 ^{b}	0.38 ± 0.05 ^{b}	0.15 ± 0.00 ^{a}
61	1542	B	1*H*-Pyrrole	0.43 ± 0.06 ^{b}	0.62 ± 0.19 ^{b}	0.49 ± 0.07 ^{b}	0.51 ± 0.09 ^{b}	0.15 ± 0.01 ^{a}
62	1556	B	1-Hydroxy-2-butanone acetate	0.39 ± 0.10 ^{a}	n.d.	n.d.	n.d.	n.d.
63	1559	B	Furfuryl acetate	0.98 ± 0.20 ^{b}	0.70 ± 0.33 ^{b}	0.91 ± 0.20 ^{b}	0.76 ± 0.25 ^{b}	0.09 ± 0.00 ^{a}

64	1605	B	5-Methylfurfural	2.61 ± 0.29 ^{c}	1.29 ± 0.33 ^{b}	1.02 ± 0.45 ^{b}	0.97 ± 0.03 ^{b}	0.45 ± 0.01 ^{a}
65	1615	B	2-Furfurylfuran	0.07 ± 0.01 ^{a}	0.10 ± 0.03 ^{a}	0.05 ± 0.05 ^{a}	0.10 ± 0.03 ^{a}	n.d.
66	1661	B	2-Formyl-1-methylpyrrole	0.17 ± 0.01 ^{c}	0.15 ± 0.01 ^{bc}	0.15 ± 0.01 ^{bc}	0.13 ± 0.01 ^{b}	0.08 ± 0.00 ^{a}
67	1673	B	γ-Butyrolactone	0.51 ± 0.05 ^{b}	0.72 ± 0.17 ^{c}	0.42 ± 0.06 ^{b}	0.54 ± 0.11 ^{bc}	0.06 ± 0.00 ^{a}
68	1686	B	Furanmethanol	5.16 ± 0.41 ^{c}	5.22 ± 1.66 ^{c}	2.58 ± 0.46 ^{b}	3.51 ± 0.75 ^{bc}	0.10 ± 0.01 ^{a}
69	1833	B	N-Furfurylpyrrole	0.04 ± 0.01 ^{a}	0.05 ± 0.01 ^{a}	0.04 ± 0.00 ^{a}	0.05 ± 0.00 ^{a}	n.d.
			Total	235.55 ± 8.98 ^{c}	218.94 ± 33.71 ^{c}	121.74 ± 19.39 ^{b}	130.92 ± 23.06 ^{b}	50.07 ± 4.27 ^{a}
			Total Sulphur Compounds	1.41 ± 0.42 ^{cd}	1.13 ± 0.56 ^{bc}	0.31 ± 0.31 ^{a}	0.55 ± 0.55 ^{ab}	1.82 ± 0.13 ^{d}
			Total Aldehydes	70.02 ± 3.80 ^{c}	62.46 ± 9.20 ^{c}	31.87 ± 4.74 ^{b}	33.32 ± 4.69 ^{b}	13.91 ± 2.12 ^{a}
			Total Esters	31.12 ± 1.12 ^{d}	24.05 ± 4.21 ^{c}	12.19 ± 3.34 ^{b}	12.90 ± 3.02 ^{b}	5.59 ± 0.26 ^{a}
			Total Furans	56.05 ± 1.66 ^{c}	51.16 ± 8.19 ^{c}	30.06 ± 4.55 ^{b}	33.78 ± 7.14 ^{b}	10.74 ± 0.91 ^{a}
			Total Ketones	51.17 ± 2.06 ^{c}	45.11 ± 5.79 ^{c}	24.87 ± 3.75 ^{b}	26.93 ± 4.72 ^{b}	8.19 ± 0.42 ^{a}
			Total Alcohols	2.13 ± 0.55 ^{ab}	3.46 ± 1.44 ^{b}	0.98 ± 0.14 ^{a}	1.21 ± 0.32 ^{a}	1.67 ± 0.06 ^{a}
			Total Thiophenes	0.56 ± 0.02 ^{b}	0.92 ± 0.17 ^{c}	0.65 ± 0.02 ^{b}	0.69 ± 0.04 ^{b}	0.28 ± 0.03 ^{a}
			Total Pyrroles	2.43 ± 0.07 ^{b}	4.21 ± 0.73 ^{c}	2.89 ± 0.10 ^{b}	2.93 ± 0.28 ^{b}	0.94 ± 0.06 ^{a}
			Total Pyridines	4.00 ± 0.82 ^{b}	7.36 ± 1.59 ^{c}	4.81 ± 0.47 ^{b}	4.06 ± 0.74 ^{b}	0.80 ± 0.08 ^{a}
			Total Pyrazines	10.04 ± 1.58 ^{b}	14.65 ± 3.19 ^{c}	10.25 ± 1.47 ^{b}	10.12 ± 1.77 ^{b}	5.62 ± 0.28 ^{a}
			Total Thiazoles	0.06 ± 0.01 ^{ab}	0.13 ± 0.04 ^{c}	0.05 ± 0.01 ^{ab}	0.07 ± 0.01 ^{b}	0.03 ± 0.01 ^{a}
			Total Lactones	0.51 ± 0.05 ^{b}	0.73 ± 0.17 ^{c}	0.42 ± 0.06 ^{b}	0.54 ± 0.11 ^{bc}	0.06 ± 0.00 ^{a}
			Total Others	6.05 ± 0.87 ^{d}	3.58 ± 0.58 ^{bc}	2.39 ± 1.03 ^{b}	3.81 ± 0.53 ^{c}	0.46 ± 0.04 ^{a}

[1] All values are shown as means ± standard deviations. In each row different letters indicate significant difference ($p < 0.05$) among coffee samples.

[2] Compounds corresponding to chromatographic peaks in Fig. 2

[3] Retention Index determined on HP-Wax capillary column.

[4] Identification proposal is indicated by the following: A, mass spectrum agreed with standards injected in the same conditions; B, tentative identification by comparing mass spectrum with Wiley mass spectral database and retention indexes with literature data.

n.d. not detected

Table 3. Pearson correlation coefficients between coffee compounds and antioxidant capacity (measured by DPPH· and redox potential methods) in ground roasted coffees.

Coffee brew compound	DPPH·	Redox potential
Caffeine	0.228 ^{ns}	0.059 ^{ns}
Trigonelline	0.809 **	-0.581 **
5-CQA	0.381 *	-0.460 *
Caffeic Acid	0.485 **	-0.430 *
Ferulic Acid	-0.131 ^{ns}	0.198 ^{ns}
4-Vinylguaiacol	-0.591 **	0.562 **
Browned Compounds	0.814 **	-0.796 **
Total Sulphur Compounds	-0.238 ^{ns}	-0.148 ^{ns}
Total Aldehydes	-0.641 **	0.338 ^{ns}
Total Esters	-0.529 **	0.217 ^{ns}
Total Furans	-0.637 **	0.355 ^{ns}
Total Ketones	-0.658 **	0.322 ^{ns}
Total Alcohols	-0.423 *	0.587 **
Total Thiophenes	-0.726 **	0.779 **
Total Pyrroles	-0.742 **	0.822 **
Total Pyridines	-0.708 **	0.856 **
Total Pyrazines	-0.566 **	0.808 **
Total Thiazoles	-0.700 **	0.708 **
Total Lactones	-0.684 **	0.648 **
Total Others	-0.250 ^{ns}	-0.092 ^{ns}

The symbols * and ** indicate significance at the 0.05 and 0.01 probability levels, respectively.
^{ns} means not significant.