Master en Seguridad y Biotecnología Alimentarias

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"Antioxidant Activity in Decaffeinated Beverages: Instant Coffee and Black Tea"

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1. Resumen

Introducción: Café y té son dos de las fuentes más importantes de antioxidantes entre los productos naturales. Sin embargo ciertos consumidores no quieren verse afectados por la cafeína ya que ésta produce nerviosismo, excitación e insomnio. Durante la descafeinización de ambos productos, té y café, pueden producirse alteraciones químicas y físicas. El objetivo de este estudio fue determinar si estos cambios también afectaban a su actividad antioxidante (AA).

Materiales y métodos: Se estudió la AA de 6 cafés solubles, 5 tés de fermentación completa y sus respectivos productos descafeinados. Para el análisis se usaron los métodos de ABTS, DPPH y FRAP. Además se estudió el contenido en catequinas (TCC) y polifenoles (TPP) así como el color de la muestra (absorbancia a 365 nm). Se efectuó un estudio de las correlaciones entre los métodos así como un análisis factorial de los resultados.

Resultados y discusión: *Estudio univariante:* Ninguna bebida mostró diferencias significativas en todas las medidas. Dos tés y un café no descafeinados mostraron mayor actividad que su descafeinados equivalentes en 4 de los 5 análisis. Estas diferencias no pueden atribuirse a la presencia de cafeína ya que no producen ninguna reacción en ninguno de los métodos seguidos. Por otra parte, uno de los tés descafeinados presentó valores más altos que su equivalente no descafeinado en 4 de los 5 análisis. En general no se encontró una clara diferencia en las propiedades antioxidantes entre las bebidas no descafeinadas y las descafeinadas.

A pesar de haber encontrado 7 veces más TCC en té que en café, el té presentó 25% menos TPP que el café. Esto indica que el café tiene otros compuestos fenólicos de naturaleza no catequínica (en su mayoría los ácidos clorogénico y cafeico). Éstos compuestos contribuyen de manera importante a la AA del café lo cual explica que presentará valores más altos en FRAP y DPPH que el té.

Correlaciones: Las correlaciones más fuertes fueron detectadas entre las muestras de té. TCC y TPP estuvieron fuertemente correlacionados con la medida del color, DPPH y FRAP ($p<0,0001$) lo que indica que catequinas y polifenoles son los principales responsables de la AA y el color del té. En el caso del café las correlaciones fueron más débiles. Contrariamente a lo que sugiere la bibliografía, TPP no tuvo relación significativa ni con FRAP ni con DPPH. Este hecho se puede explicar por la presencia de otros antioxidantes, como las melanoidinas, con gran poder antioxidante, incluso mayor que los compuestos de naturaleza fenólica

Análisis factorial: Cuando se utilizaron todos los resultados sólo se detectó diferencias marcadas entre el grupo de cafés y de té. Al analizar los resultados obtenidos del café y del té por separado las muestras se distribuyeron en función de la marca con la que se comercializan y no en función de si habían sido o no descafeinadas. Por tanto, el análisis factorial no mostró agrupaciones de los productos descafeinados y los que no habían sido descafeinados. De estos resultados se desprende que otros factores asociados a la marca (por ejemplo materia prima, grado de tostado o fermentación) son los que realmente influyen en la AA del producto final.

Conclusión: La descafeinización de té y café no afecta significativamente a sus propiedades antioxidantes. Por tanto, no existe pérdida del efecto antioxidante para aquellas personas que prefieren consumir café o té descafeinado en vez de sus equivalentes sin descafeinar.

2. Introduction

Coffee and tea are considered as two of the most consumed drinks in the United Kingdom. According to the last European coffee report, 3 Kg coffee per person was consumed during 2010; which means that more than 60 million of cup of coffee are consumed per day in UK. Due to its ease of use, instant coffee is the most consumed among different coffee types (75 % of the coffee is drunk as instant coffee). As tea is concerned, United Kingdom has the second highest tea consumption rate in the world (2,1 kg of tea per person). More than 165 million of cups of tea are drunk per day, according to the estimations of UK Tea Council.

Demand of decaffeinated coffee and tea is increasing and their intake represents around 15 % of the coffee market. Consumers are aware of caffeine effect on the human state and health and that could be the cause of the increase in the demand of decaffeinated products. It is well known that sleep is affected by consumption of caffeine. Moreover, nervousness, agitation, and anxiety are caused by consumption of non-decaffeinated coffee and tea (Nehlig et al., 1992). In addition to that, caffeine has been positively associated with several types of cancer (Al-Hachim, 1989), coronary disease (Wei et al., 1995), reproductive health problems and abortion (Astill et al., 2001). However these impacts are not clear yet and further research is required.

Caffeine antioxidant activity (AA) has not been totally clarified yet. Although its capability to inhibit the lipid peroxidation has been reported by Brezová et al. (2009), its contribution to AA in coffee and tea does not seem to be as important as the contribution of phenolic compounds and melanoidins. Moreover caffeine can also be a proxidant, depending on the material to which caffeine reacts. In those cases, caffeine leads to oxidations and decreases on AA.

Commercial decaffeination techniques may be classified in three groups. The first one consists of procedures in which the soluble compounds of the material are directly extracted by a chemical solvent such as methylene chloride. After that, steam treatment is applied in order to remove the solvent from the beans or leaves. In the second group ("water decaffeination"), beans and leaves are soaked in hot water and caffeine from that water is removed by the use of activated carbon filter. In these methods, dry process at high temperature is required, thus chemical reactions are likely to happen. The third group includes the techniques in which caffeine is extracted by supercritical fluidal (CO_2). In this process high temperatures are not involved as CO_2 becomes gas after extraction at atmospheric pressure.

Decaffeination process causes several changes of the physical and chemical characteristics. Some antioxidant compounds may be removed with the caffeine, and others may be modified by the heat treatment applied in the decaffeination process. To avoid losses of the flavour and aroma from coffee roasting, decaffeination process is conducted on green coffee beans. Thus, changes produced by decaffeination process may influence on the following steps of coffee production (roasting, extraction and drying). Those processes are crucial as in them, AA and characteristics of the final product is developed. In the case of tea, decaffeination of green leaves produce unusual taste. Because of that, decaffeination process is carried out after tea leaves have already been through the manufacturing process.

The aim of this study was to determine whether there is a remarkable difference on the AA between regular beverages and their decaffeinated equivalents.

3. Experimental

3.1. Chemicals and equipment

2,2′-azinobis-(3-ethyl benzothiazoline-6-sulphonic acid) (ABTS), potassium persulphate ($K_2S_2O_8$) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), were purchased from Calbiochem (San Diego, U.S.A.) and Acros (New,Jersey, U.S.A.), respectively. 2,4,6-tris(2-pyridyl)-S-triazine (TPTZ) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich (St.Lois, MO, USA). Sodium carbonate (Na_2CO_3), sodium acetate (CH_3COONa), acetic acid (CH_3COOH) and ferric (III) chloride ($FeCl_3$) were procured from Panreac (Barcelona, Spain). Folin-Ciocalteu (FC) reagent, hydrochloric acid (HCl) and vanillin were provided by Merck (Darmstadt, Germany). All reagents used were of analytical grade. All dissolutions were stored at fridge temperature away from light in tinted (amber) glass reagent bottles.

Distilled water to prepare the dissolutions was made in the laboratory by distillation. The Milli-Q water required for Ferric Reducing Antioxidant Power (FRAP) assay was purified and deionized by Milli-Pore Integral 3. Methanol (high-performance liquid chromatography grade) used to dissolve DPPH radical, was from Lab-Scan (Dublin, Ireland).

Two precision balances were used: Radwag PS750/C/1 with readability of 1 mg and Sartorius basic when readability of 0,1 mg was required (measurement of DPPH powder). Spectrometric ABTS and colour measurements were recorder on Cecil 1020 spectrophotometer, and the absorbance readings for catechins, TPP, DPPH and FRAP assays were made on Beckman DU 650 spectrophotometer.

3.2. Samples

Six soluble coffees, five black teas and their respective decaffeinated products from the same brand were purchased at Morrison Supermarkets and Sainsbury's Supermarkets (Table 1).

Coffee samples were prepared by pouring 250 ml of boiling tap water on 2 grams of instant coffee. To brew tea samples, one tea bag was added to 250 ml of boiling tap water for 10 minutes. After that, the tea bag was stirred three times before being removed from the infusion. Samples were allowed to cool until they reached room temperature. All the samples were analysed on the same day or the day after they were brewed. The extractions were stored at fridge temperature away from light.

Three and two extractions were made for each tea and coffee respectively (analytical replicate); being each extraction analysed twice (instrumental replicate). The results are expressed by g of coffee powder or tea leaf (dividing by the weight of sample used)

Table 1. Samples studied: name and some origin and manufacturing information declared by correspondent companies.

Code	Tea sample	Decaffeination process
YT	Yorkshire Tea	Methylene Chloride
TW	Twinings Earl Grey Tea	Carbon dioxide
TL	Tetley	Dichloromethane *
PG	PG tips	Not declared
TY	Typhoo Tea	Methylene Chloride

* Tetley declared that the tea blend used in the decaffeinated product is different to that used to make no-decaffeinated ones.

Code	Coffee sample	Characteristics
CR	Carte Noire	Arabica Freeze dry
NA	Nescafé Alta Rica	Arabica Freeze dry
SG	Sainsbury´s Gold Roast	No bean specified Freeze dry
KE	Kenco Smooth	No bean specified Freeze dry
NO	Nescafé Original	No bean specified Spray dry
MR	Morrisons Full Roast	No bean specified Spray dry

3.3. Parameters analysed.

a. Colour (Rivero-Pérez et al., 2002)

A spectrophotometer Cecil 1020 was used to evaluate sample colour. According to bibliography, 365 nm, 420 nm and 440 nm absorbances were measured with a quarz cell of 1mm of pathway. Samples were previously dilluted at 5:100 for coffee and 3:100 for tea

b. Total Polyphenols (TPP) (Singleton and Rossi, 1965)

That method is based on the capacity of phenolic compounds to reduce molybdenum (VI) to molybdenum (V). In this method, the sample is mixed with FC reagent, which is a mixture of phosphomolybdate and phosphotungstate. A chain of reactions leads to formation of blue compound (absorbance at 750 nm) which is directly correlated with the quantity of total polyphenols.

Total polyphenol contents of coffee and tea were determined using FC reagent. 0,5 ml of the samples, previously diluted (4:25 and 1:4 for tea and coffee respectively) were mixed with 0,5 ml of FC reagent and 10 ml of Na_2CO_3 solution, making a final volume of 25 ml with distilled water. The absorbance was measured at 750 nm after one hour of reaction time. Results were expressed as mg gallic acid by gram of sample using the calibration curve previously calculated with gallic acid.

c. Total Catechins Content (TCC) (Swain and Hillis, 1959)

Vanillin-HCl is a colorimetric method used to determine total catechins content. This assay is a condensation reaction between vanillin and catechins in acid medium (HCl). Chromophores with red colour are produced by that reaction and their concentration is measured by reading absorbance at 500 nm.

Tea samples were diluted (4:25) with distilled water and one ml of that dilution was added to a test tube with 2 ml of vanillin reagent and 7 ml of HCl (26%). Coffee extracts were analysed without prior dilution. Absorbance at 500 nm was measured after 25 minutes of reaction time. Calibration regression curve was made with solutions of well-known concentrations of catechin.

d. ABTS method (Re et al., 1999)

This method is based on reading the decolouration (absorbance at 734 nm) after the reduction of ABTS* radical by antioxidant compounds (Figure 1). ABTS radicals are produced by a previous reaction between ABTS and $K_2S_2O_8$.

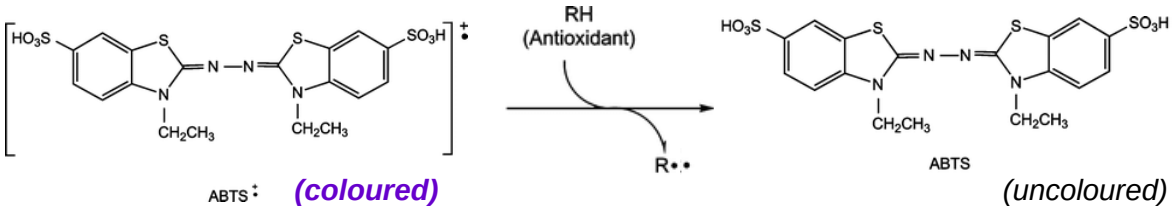

Figure 1. Reaction between ABTS radical and antioxidant

ABTS radical cation was produced by reacting 0.0962 g ABTS powder with 0.0165 g potassium persulphate in 100 cm^3 distilled water. The solution was wrapped in aluminium foil and stored in the dark for at least 16 hours before being used. The ABTS radical solution was diluted with phosphate buffered saline (PBS) (pH 7.4) to obtain an absorbance ranging from 0.6 to 0.8 (Rivero-Pérez et al., 2007)

Samples were diluted with distilled water (5:100 for coffee and 3:100 for tea). Then 40 µl of sample were mixed with 2 ml of ABTS* reagent directly in the cuvette. which were shaken manually. After 10 minutes absorbance at 734 nm were measured and compared with control sample (40 µl of PBS plus 2 ml of ABTS reagent). The same procedure was followed with ethanol solutions of well-known concentrations of Trolox. The difference in absorbance was related to concentration of Trolox by a standard curve.

e. DPPH method (Brand-Williams et al., 1995)

This method measures the decrease of DPPH radical due to its reaction with antioxidant compounds from the sample (Figure 2). AA is correlated with losses of absorbance at 515 nm.

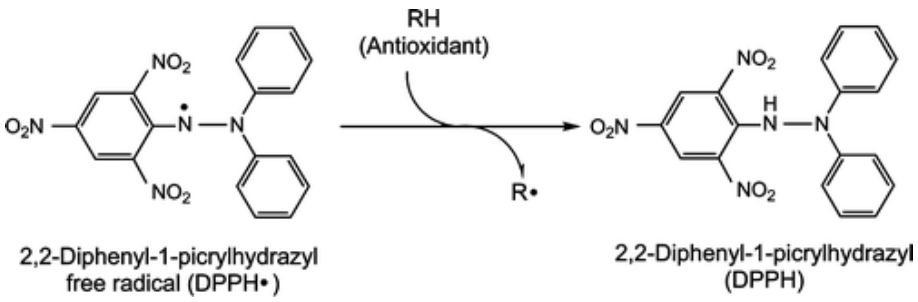

Figure 2. Reaction between antioxidant and DPPH radical

Sample was previously diluted at 4:25 in the case of tea and at 1:10 in the case of coffee. DPPH reagent was prepared at the necessary concentration to obtain an absorbance of 0,7±0,1 at 517 nm. Twenty µl of sample was added to 980 µl of DPPH reagent and incubated during 30 minutes in case of tea and 90 minutes in case of coffee. These times were found after studying the reaction time to reach stable value of absorbance. A blank sample was made by mixing 980 µl methanol and 20 µl of diluted drink. The difference of absorbance between blank and sample is directly proportional to the AA of samples. Results were expressed as mg of Trolox by gram of sample using the respective calibration curve made from well-known concentrations of solutions of Trolox.

f. FRAP method (Benzie and Strain, 1996)

FRAP method measures the ability of a sample to reduce a ferric-tripyridyltriazine (Fe (III)-TPTZ) complex (Figure 3). The formation of the ferrous (Fe (II)) form leads to the development of an intense blue colour which can be quantified spectrophotometrically at 593 nm.

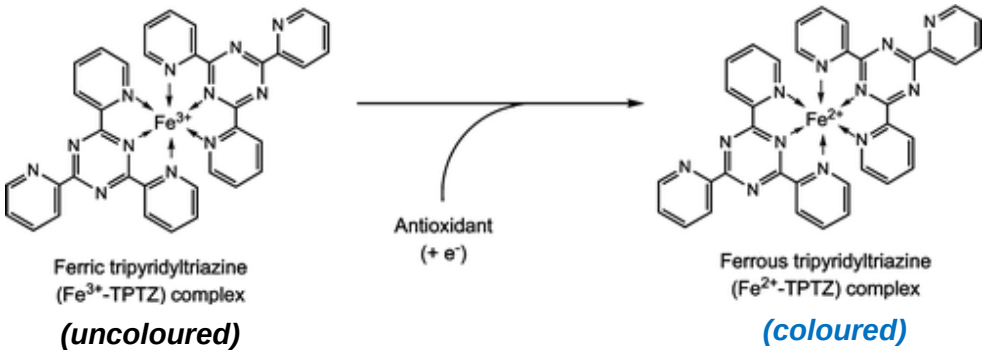

Figure 3. Reduction reaction of Fe (III)-TPTZ complex

FRAP reagent was prepared from 25 ml of 300 mM sodium acetate buffer solution at pH 3.6, 2,5 ml of TPTZ (10 mM), 2,5 ml of ferric chloride (20 mM) and 3 ml of miliQ water. Nine hundreds seventy µl of that FRAP reagent were mixed with 30 µl of sample (diluted in water at 1:25) and incubated at 37ºC for 30 minutes. Then, absorbance at 593 nm were measured and compared with the absorbance of a control (FRAP reagent). The concentrations were calculated according to the calibration curve, which was obtained by using solutions of well-known concentrations of $FeSO_4$. The results were expressed as millimoles of Fe (II) by gram of sample.

3.4. Statistical analysis

Coefficient of regression and analysis of residues were considered to estimate the quality of each standard curve. Equality of variance was tested by Cochran´s and Bartlett´s tests and factor effects were checked by ANOVA test.

Pearson product correlations between each pair of methods were calculated to identify the significant correlations at a confidence level of 95%.

Factor analysis was conducted extracting those factors that had eigenvalues greater than or equal to 1,0. A varimax rotation was performed in order to simplify the explanation of the factors.

Statistical analyses were carried out using the Statgraphic Computer System program version Centurión XVI.I.

4. Results and discussion

4.1. Univariante analysis

It is hard to define what is considered as antioxidant in food matrices. Physiologically speaking, an antioxidant could be defined as any substance which is capable to inhibit oxidative processes (Pokorný, 2007). However, there is an antioxidant/prooxidant balance and no compound acts always as antioxidant. In addition to that, foodstuff may be really complex matrices, because of that several analyses are required to know the AA of samples.

FRAP, DPPH and ABTS methods were used in this study to assay the AA of coffee and tea. Furthermore, Total Polyphenols and Catechins levels were measured due to they are some of the antioxidants present in the studied beverages.

a. Colour

Data from the three wavelengths were similar and higher values were those from 365 nm, showing a predominant yellow tonality with low influence of red colours. The results did not show any surprising results. Coffees were darker than teas and no relevant differences were detected between decaffeinated and regular products (Figure 4)

b. Total Polyphenols (TPP)

Polyphenols of coffee and tea have been studied as they contribute to the astringent taste of those drinks (Ding et al., 1992, De Maria et al., 1995). Attention has now turned to their health benefit and the mechanisms by which polyphenols may help to protect us against chronic diseases have been described by several studies (Kuriyama et al., 2006, Nichenametla et al., 2006, Van Dam, 2008)

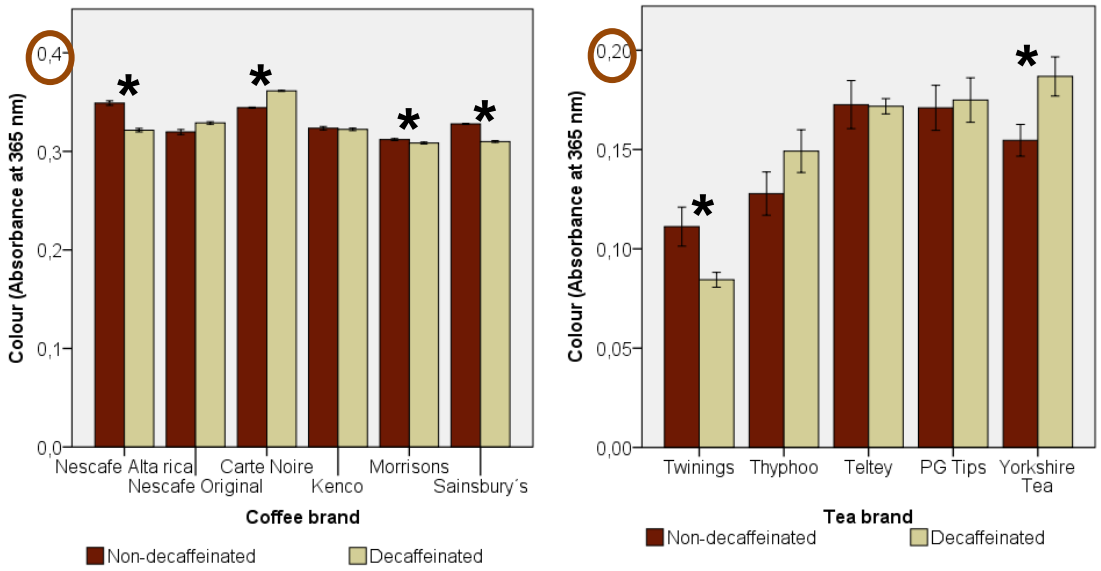

Figure 4. Colour measured by reading absorbance at 365 nm. The results were corrected by the weight of the sample and by the dilution. Bars show mean values, asterisks present significant differences and whiskers indicate standard deviation values. n = 3. Note that the scale of each graph is not the same.

Data of TPP showed that soluble coffees are richer on polyphenols than teas (Figure 5). The mean value of coffees was 114,1 ± 5,6 mg gallic acid/g of sample while teas showed less than 75% of this value (83,2 ± 16,0 mg gallic acid/g of sample). Variability among teas was greater than among coffees as the TPP ranged between 123,4 and 103,3 mg gallic acid/g of coffee and between 98,9 and 42,4 mg gallic acid /g of tea leaf. Those ranges of TPP are similar to those found in bibliography (Table 2).

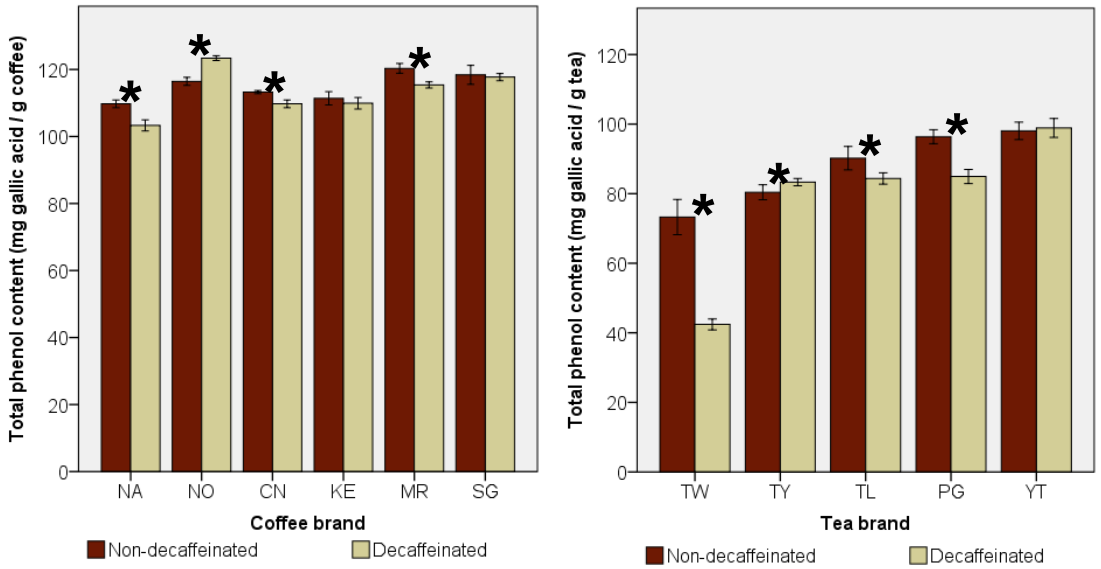

Figure 5. Total polyphenols content expressed as mg of gallic acid by gram of studied samples. Bars show mean values, asterisks present significant differences and whiskers indicate standard deviation values. n_{coffee} = 2 x 2 ; n_{tea} = 3 x 2.

Table 2. Range of TPP produced in studies conducted in similar conditions

Reference	Material	Range (mg gallic acid/g)
(Sánchez-González et al., 2005)	Freeze dried coffee (regular)	121
	Freeze dried coffee (decaffeinated)	121
(Brezová et al., 2009)	Instant coffee	114-140
(Alves et al., 2010)	Espresso coffee	145-109
	Decaffeinated espresso coffee	124-85
(Chu et al., 2011)	Freeze dried coffee (regular)	155
	Freeze dried coffee (decaffeinated by CO_2)	180
(Venditti et al., 2010)	Black tea	130
(Shio et al., 2011)	Black tea	65

Respect the comparative studied between decaffeinated and caffeinated products, results showed that three coffees and three teas had statistically higher TPP content than their correspondent regular samples. Whereas, decaffeinated samples from one tea and one coffee reported higher TPP content than their regular samples. So, no significant differences between means of regular and decaffeinated products were detected. These results agree with those published by Sánchez-González (2005) and Chu et al. (2011), which did not found significant differences in TPP levels of regular and decaffeinated instant coffees. However Alves et al. (2010) suggested that decaffeination process has influence on TPP levels, as they detected greater amounts of TPP in regular coffee than in decaffeinated ones. Considering all these data it seems possible to assert that decaffeination process is not directly correlated with losses of phenolic contents.

c. Total Catechins Content (TCC)

All the samples showed a relevant amount of catechins measured by the Vanillin-HCL assay (Figure 6).

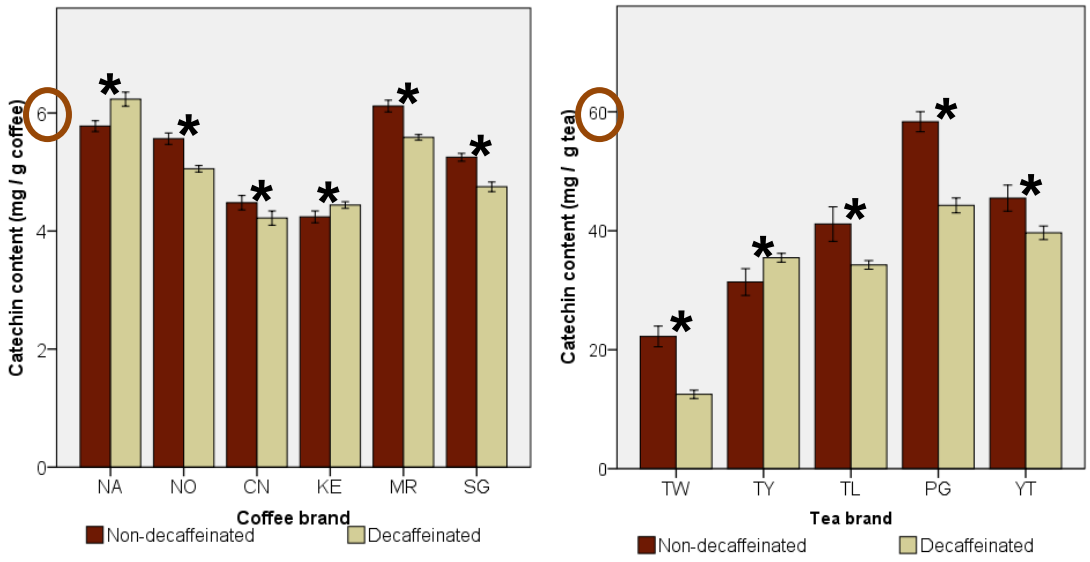

Figure 6. Total catechins content expressed as mg of catechin by gram of studied samples. Bars show mean values, asterisks present significant differences and whiskers indicate standard deviation values. $n_{coffee} = 2 \times 2$; $n_{tea} = 3 \times 2$. Note that the scale of each graph is not the same.

The TCC range was from 12,5 to 53,3 mg/g of tea leaf and from 4,22 to 6,24 mg/g of coffee. Although TPP of teas was lower than that of coffees; teas reported a mean value of catechins (36,5±12,3) 7-fold higher than that reported by coffees (5,1±0,7). This is could be explained by the composition of each beverage. Tea is especially rich in catechins, and they represent up to 15 % of the total polyphenols (Astill et al., 2001). On the other hand, the main phenolic compounds in coffee belong to the family of chlorogenic and no one of the four primary catechins have been detected in coffee (Arts et al., 2000).

The difference between decaffeinated and regular samples was significant in all of the 5 teas and 6 coffees assayed. Liang et al., (2007) reported differences between levels of TCC of regular tea and decaffeinated one. Levels of TCC were quantified by HPLC, and data showed decaffeination removed at least a 5% of the TCC. Perva-Uzunalic et al. (2006) reported losses of 17% by hot water decaffeination. The authors suggested prior moistening and ulterior drying were the main cause of these losses. The present study showed an intense decrease (around 44%) in the case of Twinings products, whiles decaffeinated Thyphoo showed higher content of TCC than its regular equivalent. Obtained data seem to do possible to assert that, in general, decaffeination has a significant influence on TCC levels of teas

d. ABTS method

Phenolic compounds have been reported to consume ABTS*. Rice-Evans et al., (1996) noted the catechins reactions, while theaflavins or thearubigins reactions were indicated by Miller et al.,(1996). Coffee has also polyphenols which are scavengers of ABTS radical such as caffeic, ferulic and chlorogenic acids (Gómez-Ruiz et al., 2007). Non-phenolic compounds of coffee which are produced by roasting process (melanoidns) also reacts to ABTS radical (Greco et al., 2004).

In this work all of the samples showed activity against ABTS radical (Figure 7).

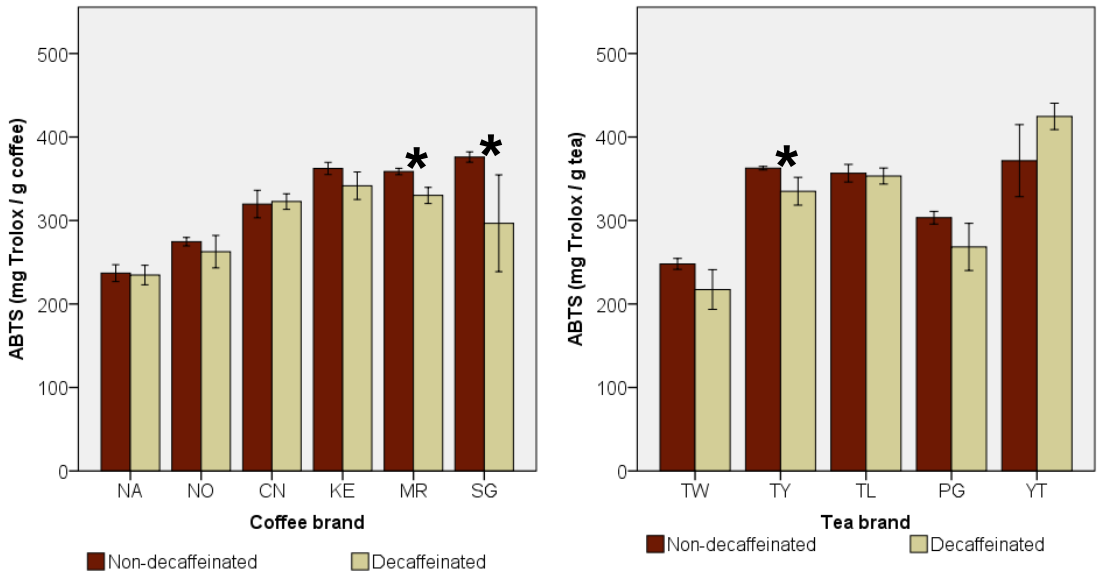

Figure 7. Antioxidant activity measured by ABTS assay expressed as mg of Trolox by gram of studied samples. Bars show mean values, asterisks present significant differences and whiskers indicate standard deviation values. n = 3

The mean ABTS value of coffee was 312±48 mg Trolox / g of sample whiles teas showed a higher value (324±63). ABTS values ranged between 234 and 375 mg Trolox / g of coffee and between 217 and 425 mg Trolox / g of tea leaf. Those ranges are similar to those found in bibliography (Table 3).

Table 3. Results of ABTS assay that can be found in bibliography.

Reference	Material	ABTS (mg Trolox / g sample)
(Pellegrini et al., 2003)	Coffee soluble	334
	Coffee espresso decaffeinated	277
(Sánchez-González et al., 2005)	Freeze dried coffee (regular)	252,3
	Freeze dried coffee (decaffeinated)	252,0
(Brezová et al., 2009)	Coffee soluble	303-421
(Pellegrini et al., 2003)	Black tea	463
(Venditti et al., 2010)	Black tea	300
(Shio et al., 2011)	Black tea	125

There were statistical differences in one tea and 2 coffees as their regular products reported higher ABTS value than their decaffeinated equivalent. ANOVA test did not detect the factor “decaffeinated” as significant in any product. Those results are in agreement with those obtained by Sanchéz-Gonzalez et al. (2005) and by Parras et al. (2007). In the studies, there were generally no differences between the AA of regular coffee and that of decaffeinated coffee. However Pellegrini et al. (2003) suggested that the absence of caffeine may lead to a decrease of ABTS value in decaffeinated coffee.

The suggestion of Pellegrini about caffeine and ABTS was checked in this work. ABTS method was followed with a dilution of caffeine at 10 g/l. That solution did not consume ABTS radical, which is in agreement with results of Berzova et al. (2009). Therefore it can be said that caffeine is inert to ABTS radical, and it cannot be the responsible of AA difference.

These results about ABTS can be summarized as the slight decrease on activity against ABTS in decaffeinated is not important enough to be considered.

e. DPPH method

Although the compounds named in the previous section (catechin, theaflavins , thearubigins ,caffeic acid, ferulic acid, chlorogenic acid and melanoidns) are also able to consume DPPH radical (Nanjo et al., 1996, Rice-Evans et al., 1996, López-Galilea et al., 2008, Maurya and Devasagayam, 2010); the response to DPPH radical does not have to be the same than the response to ABTS assay (Rivero-Pérez et al., 2007)

All the samples studied, regardless of brand, were effective scavengers of DPPH radical (Figure 8). In contrast with ABTS assay, DPPH assay showed significant differences between coffee and tea. The mean DPPH value of coffee was 131,3 ± 10,1 mg Trolox/g of sample, whiles teas reported more than 95 % of this value (124,8 ± 16,5). This fact could be explained by considering that the same antioxidant may react in different ways with different radicals, but also having in mind the global composition of both types of products.

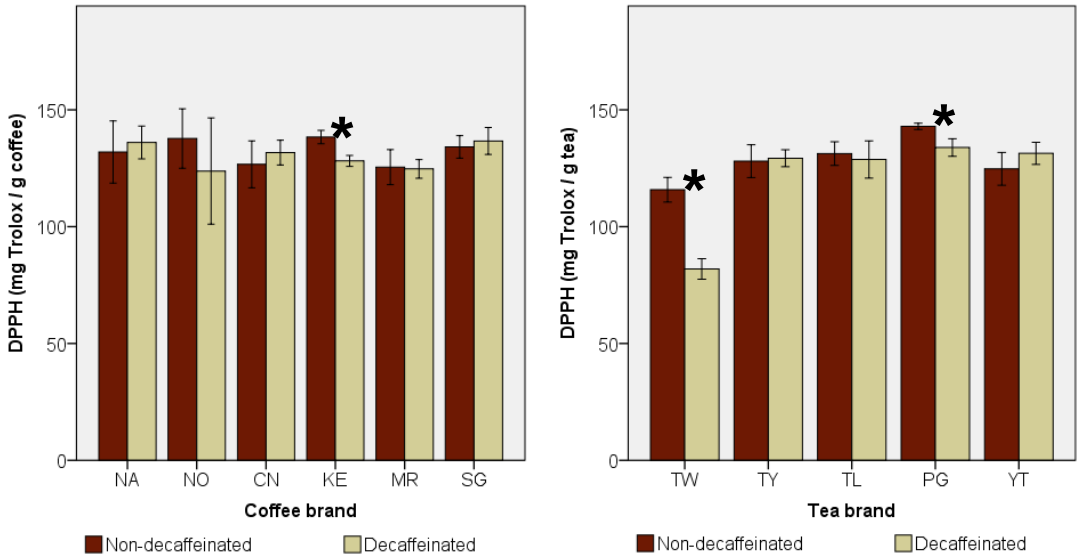

Figure 8. Antioxidant activity measured by DPPH assay expressed as mg of Trolox by gram of studied samples. Bars show mean values, asterisks present significant differences and whiskers indicate standard deviation values. $n_{coffee} = 2 \times 2$; $n_{tea} = 3 \times 2$.

Variability among teas was greater than among coffees as the range was from 123,7 to 137,7 mg Trolox / g of coffee and from 81,9 to 142 mg Trolox / g of tea leaf. Those ranges are different than those reported by Brezová et al. (2009) and by Muthuiah et al. (2009) (Table 4). That could be explained by differences on the conditions in which DPPH assays were conducted.

Table 4. Results of DPPH assay from other studies.

Reference	Material	DPPH*
(Brezová et al., 2009)	Instant coffee	319-390 mg Trolox / g
(Alves et al., 2010)	Espresso coffee	33-40%
	Decaffeinated espresso coffee	26-36%
(Buscemi et al., 2010)	Caffeinated coffee	IP 50 = 1,13 ± 0,02 µl
	Decaffeinated coffee	IP 50 = 1,30 ± 0,03 µl
(Du Toit et al., 2001)	TW Early Gray	IP 50 = 500 mg/l
	TW Early Gray decaffeinated	IP 50 = 750 mg/l
(Muthuiah et al., 2009)	Black tea	27,7 - 31,8 mg Trolox / g

*Note that different units are used as they cannot be transformed. IP50: Amount of sample required to reach 50% of decrease in the absorbance

Three regular products exhibited higher AA than their decaffeinated equivalents. The greatest difference was observed in Twinings tea as the decaffeinated sample reported 30% less AA than their regular equivalent. That decrease was similar to that reported by the study of Du Toit (2001) in the same brand. Although differences were observed in three products, ANOVA test did not detect significant differences between means of regular and decaffeinated samples. Alves et al. (2010) and Buscemi et al. (2010) observed similar coffee results.

In conclusion, there may be a slight loss of antioxidant substances responsible of scavenge DPPH activity during decaffeination process, but those differences are not strong enough to be taken into consideration.

f. FRAP method

All the 22 samples showed reductive capacity against Fe (III) (Figure 9). The mean FRAP value of coffees was 112,9 ± 8,5 mg Fe (II)/g of sample while teas showed less than 80% of this value (85,6 ± 10,0) (p-value<0,0001). FRAP values ranged between 98,6 and 123,8 mg Fe (II)/g of coffee and between 62,2 and 98,4 mg Fe (II)/g of tea leaf. Those ranges are similar to others given by bibliography (Table 5). Ryan and Sutherland (2011) conducted FRAP assay with 4 out of the 5 regular teas analysed in our study. Although FRAP values of our study are higher than those of Ryan and Sutherland; the order was the same: Typhoo<Twinings <Teltey<PG tips. The quantitative differences could be due to the analysis conditions but also in the way in which samples were brewed.

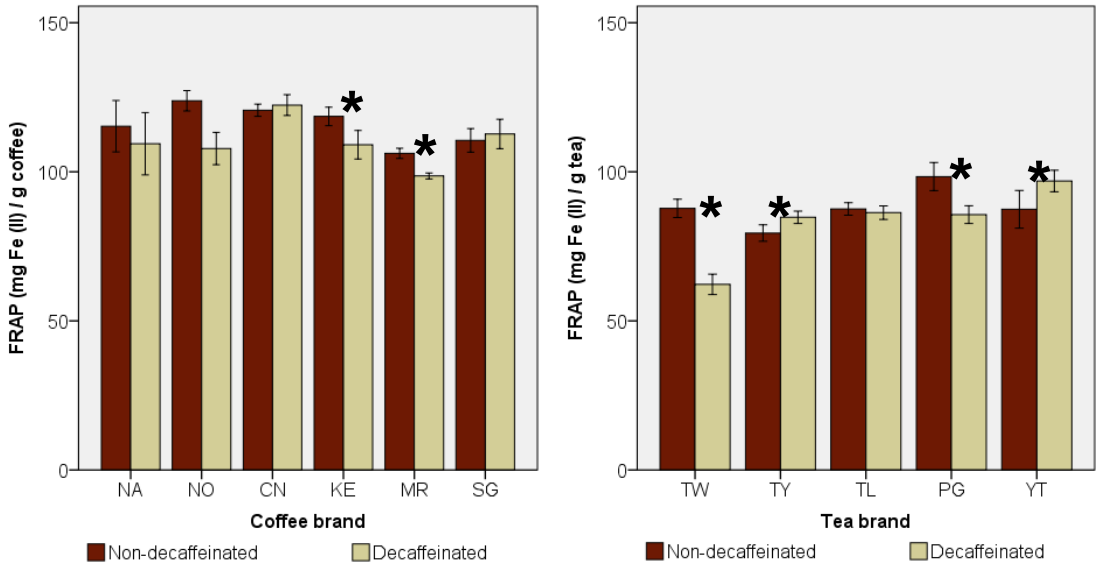

Figure 9. Antioxidant activity measured by FRAP assay expessed as mg of Fe (II) by gram of studied samples. Bars show mean values, asterisks present significant differences and whiskers indicate standard deviation values. n_{coffee} = 2 x 2; n_{tea} = 3 x 2.

Table 5. Results of FRAP assay produced by previous studies from bibliography

Reference	Material	FRAP (mg Fe (II)/g sample
(Pellegrini et al., 2003)	Espresso	144,5
	Espresso decaffeinated	103,9
	Soluble	121,3
(Moreira et al., 2005)	Soluble coffee	100-290
(Benzie and Szeto, 1999)	Black tea	9-46
(Pellegrini et al., 2003)	Black tea (5 min 250 ml)	70,5
(Ryan and Sutherland, 2011)	PG tips (4 min 200 ml)	48,0
	Teltey (4 min 200 ml)	44,4
	Twinings (4 min 200 ml)	43,5
	Typhoo(4 min 200 ml)	40,8

The FRAP value reported by regular coffee was 115,8±7,3 mg Fe (II)/g of sample while that reported by decaffeinated coffee was 95% of this value (110,1±8,8). ANOVA test showed that the difference was significant at the 95,0% confidence level. That factor was not significant when the ANOVA test was conducted with tea results. Two coffees and 2 teas shown statistically differences as their regular products yielded higher FRAP values than their decaffeinated equivalents. Two decaffeinated tea reported higher values than their regular equivalents.

Sánchez-Gonzalez et al. (2005) assayed the iron reducing activity of decaffeinated coffee and regular coffee brewed by three different methods and they did not found any significant differences. As in ABTS assay, Pellegrini et al. (2003) found significant lower FRAP values in decaffeinated coffee. They explained that decrease by the absence of caffeine but caffeine actually does not contribute to FRAP activity (Moreira et al., 2005) (this fact was also corroborated in this work). Significant decrease (5,9%-19,3%) was also reported by Moreira et al. (2005) but no relevant explanation was cited.

In spite of these results, the relation between decaffeination process and reduction power measured by FRAP is not evident and more studies will be necessary to clarify this fact.

To sum up, Regular Twinings and PG tips had higher values than their decaffeinated equivalents and that difference was significant in all the methods except in ABTS. The same case was observed in Morrisons coffee as the difference was significant in TCC, TPP, FRAP and ABTS but not in DPPH method. In contrast, decaffeinated Typhoo exhibited higher values than their regular equivalents in TCC, TPP, FRAP and DPPH. Thirty eight out of 55 (69%) analysis reported higher values in non-decaffeinated product, being significant in 25 out of those cases. In 17 out of 55 (31%) cases decaffeinated sample yielded higher values than regular being significant only 7 times. Consequently, neither the results of the present study nor results from bibliography (Tables 2,3,4,5) showed any clear trend in the difference between caffeinated and decaffeinated coffees and black teas.

4.2. Analysis of correlation between methods

In order to study the relation between results of different analytical methods, correlations were calculated among them. This statistical analysis was conducted in three ways: using all the results, using just the results from coffee analysis and using just the results from tea analysis.

a. Results of coffee and tea analysis together

The analysis showed strong correlations between colour and TCC, TPP and FRAP so as between FRAP and TPP. Similar correlations have been described for other beverages as wines (Rivero-Pérez et al., 2007) (Table 6).

Table 6. Correlation coefficient evaluated by linear regression of results from coffee and tea samples. P-values are written in bold letters. Only correlations whose p-values are lower than 0,05 are presented.

	TPP	DPPH	FRAP	ABTS	Colour
TCC	-0,41		-0,5407		-0,7682
	<0,0001		**<0,0001**		**<0,0001**
TPP		0,6003	0,8626	0,3038	0,8582
		<0,0001	**<0,0001**	**0,0014**	**<0,0001**
DPPH			0,5254	0,2164	0,3499
			<0,0001	**0,0245**	**0,0002**
FRAP					0,8833
					<0,0001

n= 12 coffees x 2 preparations x 2 analysis + 10 teas x 3 preparations x 2 analysis

Surprisingly, the correlation between colour and TCC was negative, which would mean that the higher TCC, the fewer the colour. When that correlation was plotted (Figure 10), it could be observed two different groups clearly differentiated (coffee and teas). In order to do a better study of the correlations, they were analysed by products.

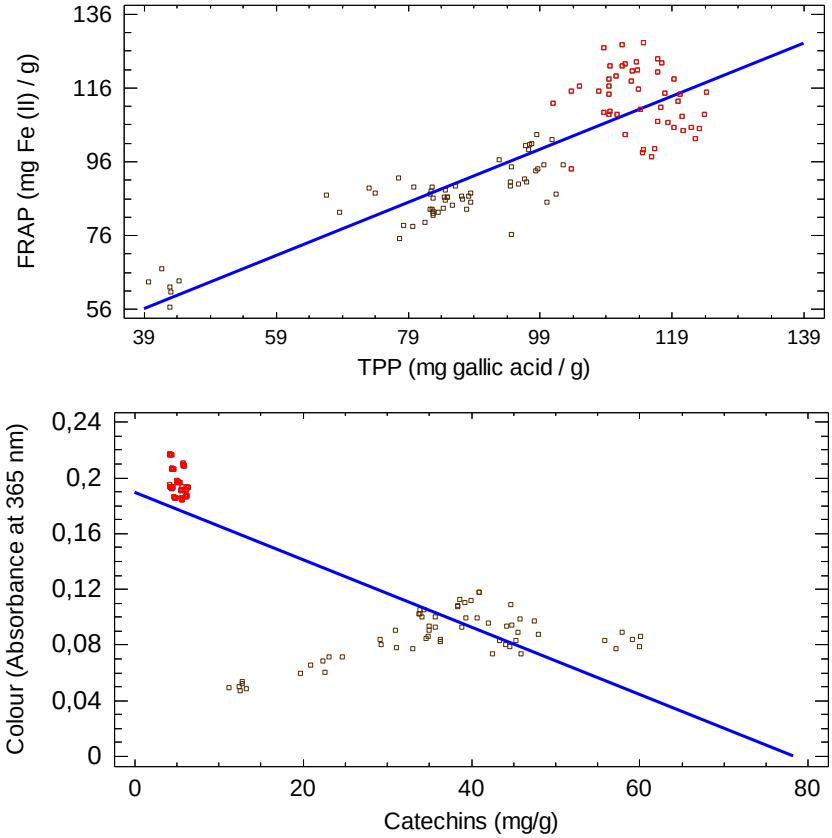

Figure 10. Distribution of studied coffees (red points) and teas (brown points) on the described spaces: TPP vs FRAP and Catechins vs colour.

b. Results of coffee analysis

Results showed significant correlation among data (Table 7) but these were no so strong than the correlations previously showed (Table 6).

Table 7. Correlation coefficient evaluated by linear regression of results from coffee samples. P-values are written in bold letters. Only correlations whose p-values are lower than 0,05 are presented.

	FRAP	ABTS	Colour
TCC	-0,3516	-0,4383	-0,3693
	0,0143	**0,0018**	**0,0098**
TPP		0,3223	-0,3363
		0,0255	**0,0194**
FRAP			0,5084
			0,0002

n = 12 coffees x 2 preparations x 2 analysis

TPP were statistically related to ABTS results but the relation was not strong enough to be taken into consideration. The correlation between TPP and FRAP and DPPH results were not statistically significant. The low correlation among phenols and AA suggested that other compounds are responsible of coffee AA. Those compounds are probably melanoidins, which are responsible also of brown colour, scavenge activity against DPPH radical (López-Galilea et al., 2008) and the reductive power (FRAP value) (Cioroi et al., 2005). These results agree with other work cited previously (Rivero-Pérez et al., 2007) in which is indicated that phenols and AA are not always positively correlated. However, several published studies found correlation between TPP and ABTS (Naranjo et al., 2011), DPPH (Naranjo et al., 2011, Ramalakshmi et al., 2011) and FRAP (Sánchez-González et al., 2005). Brezová et al. (2009) also found those correlations in ground coffee but not in instant coffee. During extraction and drying process, phenolic compounds may lose its antioxidant activity. Then, melanoidins have the greatest influence on AA instead of phenolic compounds and that may explain the absence of relation between TPP and AA. However, the effect of dry process is confusing as Vignali et al. (2011) found strong correlation between TPP and DPPH and ABTS in instant coffee. In consequence, no definitive conclusion can be drawn from these results.

TCC was not related to TPP and negatively related to FRAP, ABTS and colour. As it was previously indicated the major phenolic compounds in coffee are caffeic acid, chlorogenic acid and ferulic acid (Gómez-Ruiz et al., 2007) and coffee is not specially rich in catechins.

The negative correlation between TPP and colour (absorbance at 365 nm) could be also explained attending melanoidins contents of coffees. During roasting process melanoidins are formed incorporating phenols to their structure. The stronger the roasting process, the higher the amount of melanoidns produced and the higher the amount of polyphenols degraded. As much as phenols are incorporated, melanoidins are darker (Pérez-Magariño et al., 2000). So, coffees whose levels of phenols remained are low, they are darker than others coffees with higher levels of polyphenols. The results obtained and commented are consistent with those of Vignoli et al. (2011) who found a negative correlation between melanoidns and 5-*O*-Caffeoylquinic acid.

c. Results of tea analysis

The correlations presented in this case (Table 8) were all significant except to TCC and ABTS. The strongest correlation was found between TPP and TCC as it was expected due to catechins have an important contribution to phenolic content of teas.

Results indicated that AA assays and colour are better correlated with TPP than with TCC. Thus, there are phenolic compounds, different to catechins, which have also important influence on AA assays and colour. During fermentation, the tea composition changes because catechins from green tea are degraded and other compounds are formed (mainly thearubigns and theaflavins). Those compounds have an important contribution to AA and colour of the final product (black tea).

Table 8. Correlation coefficient evaluated by linear regression of results from tea samples. P-values are written in bold letters. Only correlations whose p-values are lower than 0,05 are presented.

	TPP	DPPH	FRAP	ABTS	Colour
TCC	0,8647	0,8267	0,7652		0,5656
	<0,0001	**<0,0001**	**<0,0001**		**<0,0001**
TPP		0,8636	0,8617	0,5306	0,8082
		<0,0001	**<0,0001**	**<0,0001**	**<0,0001**
DPPH			0,8032	0,3025	0,664
			<0,0001	**0,0188**	**<0,0001**
FRAP				0,2877	0,6419
				0,0258	**<0,0001**
ABTS					0,7487
					<0,0001

n = 10 teas x 3 preparations x 2 analysis

TPP were strongly correlated to AA assay as DPPH, FRAP and ABTS, being that correlation positive. Those results suggested that polyphenol compounds are responsible of the scavenge activity against DPPH and ABTS radical and reduction power of tea beverages. Previous studies have shown this capacity of tea polyphenols (Table 9).

Table 9. Correlations between Total polyphenols and antioxidant assays reported by several studies

References	Method	Material	R^2
(Fu et al., 2011)	FRAP	Tea and herbal infusions	0,7929*
	ABTS		0.8043*
(Jayasekera et al., 2011)	FRAP	Green tea leaves and their corresponding black tea	0.25*
	DPPH		0.01
(Shio et al., 2011)	DPPH	Tea at different fermentation points	0.914*
	FRAP		0.953*

*R^2 values with asterisks are considered as significant.

DPPH, FRAP and ABTS were statistically correlated to each other, which may be due to the same compounds (catechins, theaflavins, thearubigns) are the responsible of the AA measured by those methods. In this case, DPPH, FRAP and ABTS gave similar information about the samples as they were strongly correlated. These results agree with those found by previous studies (Fu et al., 2011, Shio et al., 2011)

It is worth reminding that the correlations found in this study are only valid in the small group of black tea and instant coffee analysed. The composition of other sorts of tea (unfermented, oolong) or coffee (ground, green) is different and different antioxidant compounds may be presented. Further experimental investigations with a greater range of products should be needed to estimate the real relation between assays.

4.3. Multifactorial analysis

A factor analysis was conducted to detect multiple association of variables and to check natural grouping of samples. That analysis calculated a small number of factors, which were lineal combination of the studied variables and which can explain as much variance as possible. Three sets of results were tested: all the results, the results from coffee analysis and the results from tea analysis.

a. Results of coffee and tea analysis together

In this case 2 factors with eigenvalues higher than one were extracted. They accounted for the 80,4% of the total variability. Factor 1 which was mainly correlated to colour, TCC, FRAP and TPP (Figure 11); detected two different groups: coffee and tea (Figure 12).

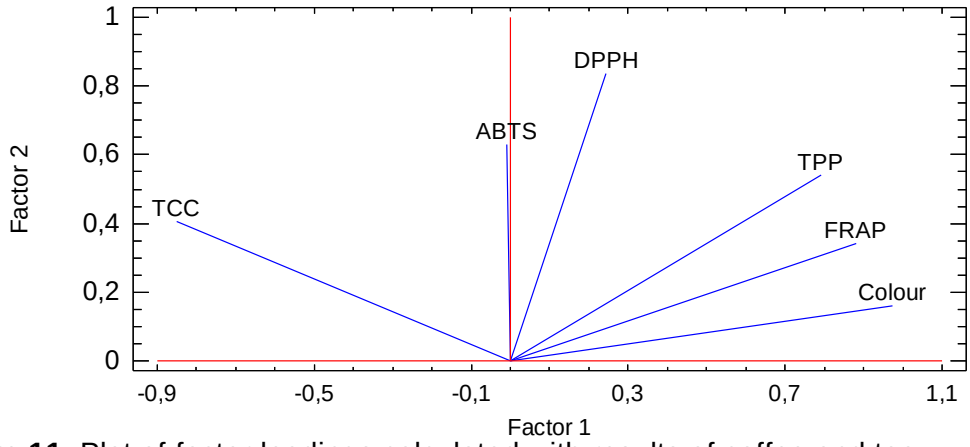

Figure 11. Plot of factor loadings calculated with results of coffee and tea

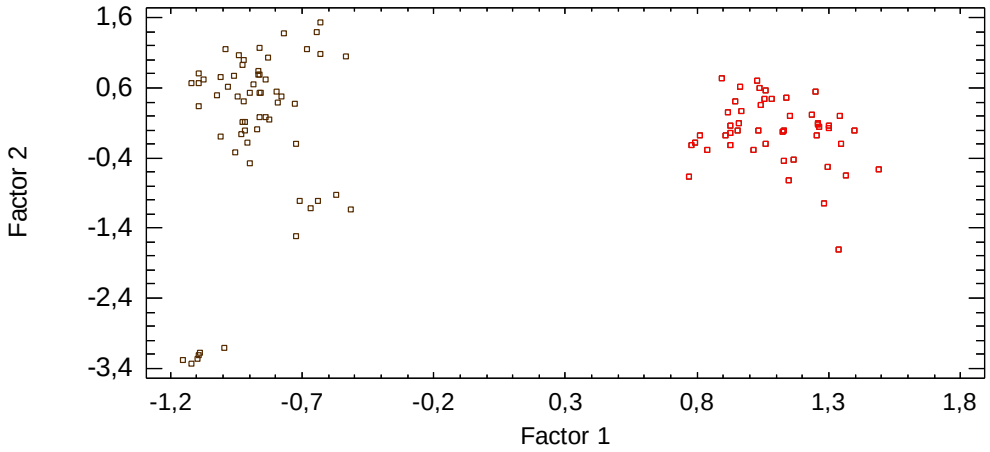

Figure 12. Factor analysis of coffee (red points) and tea (brown points) samples

According to previous comments, great differences were observed between coffees (great scores) and teas (low scores). The load of catechins was negative as tea beverages reported 7-fold higher TCC than coffees. Colour, FRAP and TPP presented positive factor as coffee had significantly higher values than tea in those assays. There were no significant differences between coffee and tea in ABTS assay which has no influence on factor 1.

DPPH and ABTS had the greatest influence on Factor 2 (Figure 11). This factor detected significantly different samples which were teas from Twinings (regular and decaffeinated). Those teas reported low values of ABTS and DPPH.

Consequently with results of this factor analysis is possible to assert that samples were grouped by the sort of drinks and no association between decaffeinated samples was observed (Figure 12). Then, it seems that decaffeination process did not influence the studied characteristics of these products.

b. Results of coffee analysis

In this section, factor analysis was carried out only with coffee results in order to do a better analysis of the results. In this case three factors reported a eigenvalue higher than 1,0 and they accounted for the 76,5% of the total variability.

Factor 1 was able to distinguish Morrisons (low score) and Carte Noire (high score) from the others samples (Figure 13). FRAP and colour reported positive effect, whereas catechins and TPP had negative effect on factor 1 (Figure 14). Factor 2 was mainly correlated to colour (positively) and catechins (negatively). That factor was able to identify as different Nescafe Alta Rica as it reported high TCC and low colour. Although factor 3 had a eigenvalue higher than 1,0, it did not give any additional information.

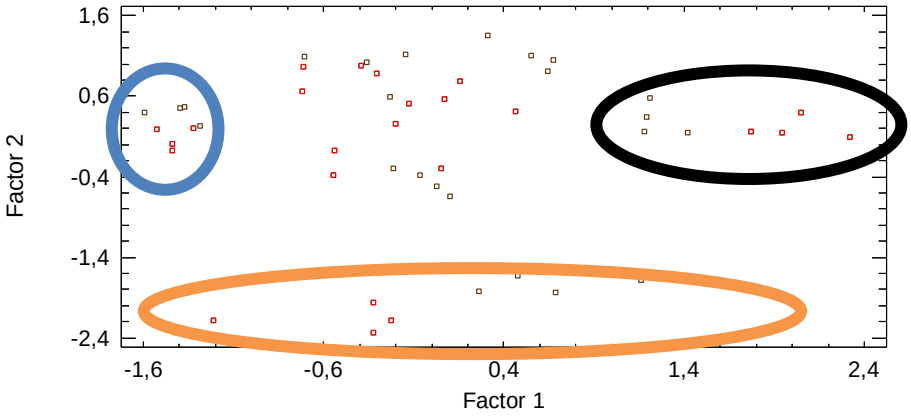

Figure 13. Factor analysis of decaffeinated coffee (red points) and regular coffee (brown points). Morrisons samples in blue circle. Carte Noire samples in black circle. Nescafe Alta Rica samples in orange circle

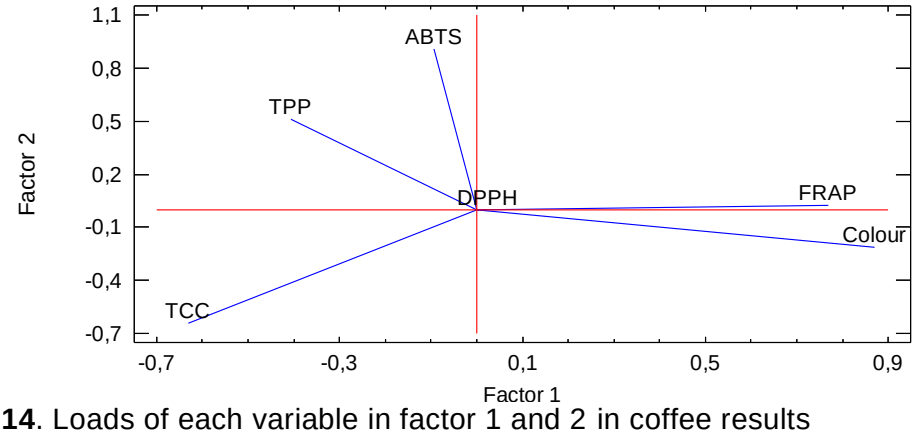

Figure 14. Loads of each variable in factor 1 and 2 in coffee results

It was checked that there was no trend among coffees made by 100% pure Arabica and no associations of coffees processed by spray-dry or freeze-dry were detected. Samples were distributed according to their brand and not according to their caffeine content. Although there may be changes during coffee decaffeination process, those modifications were not strong enough to be observed.

The observed results about brands could be explained considering that coffee is a complex matrix and the AA of the final product depends on several factors such as blend, raw material and roasting. Those factors are related to each coffee brand.

AA of coffee depends on the balance between two groups of antioxidants: phenolic compounds (mainly chlorogenic acids) and melanoidns. Chlorogenic acids are found in green beans and partially degraded by roasting process. On the other hand, melanoidns are formed by Maillard reaction during roasting process. (Anese and Nicoli, 2003, Moreira et al., 2005, Cämmerer and Kroh, 2006). Several studies (table 10) concluded that AA increases after roasting because of melanoidns production. Other studies suggested that AA decreases after roasting due to chlorogenic acid degradation. The roasting conditions to yield the highest AA possible should be calculated for each specific product.

Table 10. Studies that assayed the effect of roasting process on AA, method used and final conclusion

Reference	Material	Method	Conclusion
(Nicoli et al., 1997)	Ground coffee	DPPH ABTS	Medium roasted coffee shows the highest AA
(Borrelli et al., 2002)	Ground coffee	ABTS	The stronger roasting conditions, the fewer AA
(Anese and Nicoli, 2003)	Ready-to drink brews	DPPH Redox potential	The stronger roasting conditions, the higher AA
(Moreira et al., 2005)	Ground and soluble coffee	FRAP	The stronger roasting conditions, the fewer AA
(Cämmerer and Kroh, 2006)	Ground coffee	ABTS	The stronger roasting conditions, the fewer AA
(Alves et al., 2010)	Arabica ground coffee	DPPH	The stronger roasting conditions, the higher AA

c. Results of tea analysis

The factorial analysis of tea results gave two factors with eigenvalues higher than 1,0. They accounted for the 90,5% of the total variability. Factor 2 was able to differentiate two brands (PG tips and Twinings) as their scores were lower than the scores of the others brands (Figure 15) especially in ABTS and colour, which were the main variables for this factor (Figure 16).

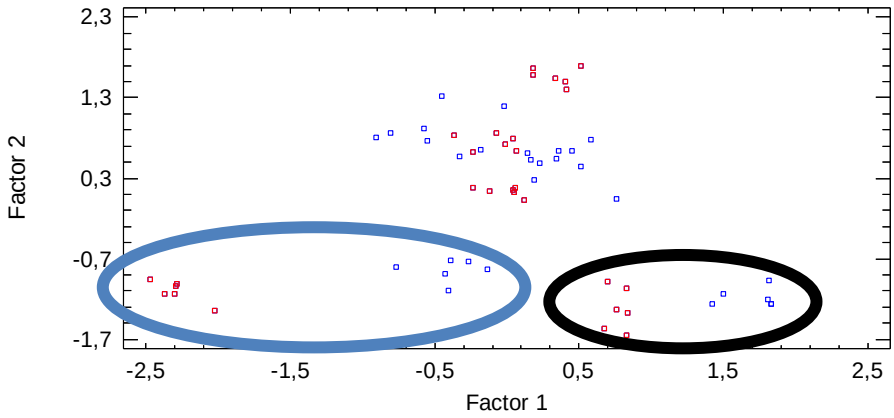

Figure 15. Factor analysis of decaffeinated tea (red points) and regular tea (blue points). Twnings samples in blue circle. PG tips samples in black circle

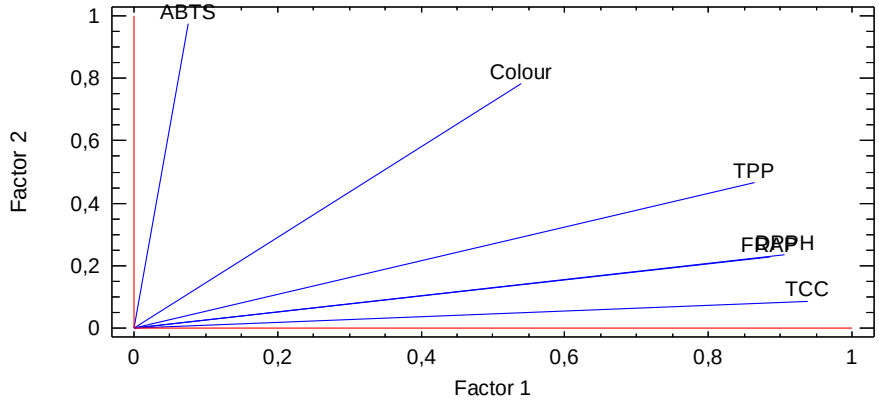

Figure 16. Loads of each variable in factor 1 and 2 in tea results

Factor 1 was mainly correlated to TCC, DPPH, FRAP and TPP. This factor showed differences between PG tips (high values) and Twinings (low values). As in the case of coffees, samples were associated according to their brand and not according to their caffeine content. The influence of decaffeination process on AA was not detected by factor analysis. The differences that were really detected are those due to other factors associated to the brand.

Several factors such as harvest conditions or grade of fermentation were shown to have influence on AA of tea samples. Thomas et al. (2009) and Jayaganesh and Venkatesan (2010) shown that the use of the adequate fertilizer increases TCC and TPP of the final infusion. Furthermore, the levels of those compounds may be reduced in diseased plant leaves (Premkumar et al., 2008)

During tea fermentation, there is a balance between antioxidants degraded (catechins) and antioxidants formed (mainly theaflavins and thearubigins) (Pellegrini et al., 2003). Different grades of fermentation may lead to different AA in the same sample. Generally, decreases of AA have been related to fermentation process (Benzie and Szeto, 1999, Pellegrini et al., 2003). Thus, the formation of theaflavins and thearubigins cannot balance the degradation of catechins.

5. Conclusion

Decaffeinated coffee and teas are a good source of antioxidants as all of them showed AA against DPPH, FRAP and ABTS radical. No clear difference between decaffeinated and regular beverages was detected on AA. There are other factors associated to the brand which determine the antioxidant profile of the final product. Then, decaffeinated beverages are an appropriate choice for those who cannot consume products with high caffeine content.

To study better the influence of the decaffeination procedure on AA, an analysis of a higher number of decaffeinated samples, preferably from the same original batch of caffeinated green beans or leaves, would be necessary.

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