COMPARISON OF DIFFERENT METHODS OF ANTIOXIDANT ACTIVITY EVALUATION OF GREEN AND ROAST *C. ARABICA* AND *C. ROBUSTA* COFFEE BEANS

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Coffee beans contain a large amount of antioxidants, which are subjected to various changes during roasting. In this study, antioxidant potential of raw and roasted to different degree (light, medium, dark) *C. arabica* and *C. robusta* coffee beans was evaluated by the four antioxidant assay methods, TPC, FRAP, TEAC, and DPPH·.

The obtained results revealed significant differences between the coffee types, roasting degree, and antioxidant activity assessment methods. FRAP and TPC appeared to be the most appropriate methods for revealing the differences in antioxidant potential of different coffee types and the effects of roasting. The results obtained by these methods were in good correlation. ABTS and DPPH· methods are not enough sensitive for the determination of roasting degrees.

In general, based on statistical data evaluation, antioxidant activity is more dependent on the coffee type than on the degree of roasting, however, the selection of analytical method may also be significant.

Keywords: coffee, antioxidant capacity, TEAC, DPPH·, FRAP, TPC

Coffee is one of the most popular drinks all over the world. For instance, comparing with other frequently consumed beverages, filtered and espresso coffee demonstrate higher antioxidant activity due to their content of phenolic compounds, in some cases exceeding that of red wine or green tea (Richelle et al., 2001; Vignoli et al., 2011). Several phenolic compounds naturally present in plant crops, same as in green coffee beans, are consequently responsible for the antioxidant activity in roast coffee, and are beneficial for human health (Brindzová et al., 2009; Alves et al., 2010).

There are many in vitro methods for the assessment of antioxidant activity of foods, plant origin preparations, and other substances; the majority of them are based on single electron (SET) and hydrogen atom (HAT) transfer reactions (Huang et al., 2005). Although the principle of each group of the antioxidant assay methods is similar, their sensitivity depends on various factors, such as media pH, the presence of lipophilic and/or hydrophilic compounds and others. Consequently, it is strongly advisable to apply more than one method for the evaluation of antioxidant properties, particularly when such phytochemically complex matrices as coffee beans and its roasting products are studied.

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Taking these aspects into account, this study was aimed at comparing four analytical in vitro methods for the evaluation of antioxidant activity of two main commercial types of coffee in relation to three roasting intensities (light, medium, and dark). It is expected that such approach may provide more systematic evaluation of both different types of coffee beans and different processing procedures.

1. Materials and methods

1.1. Coffee samples

Two important coffee brands, Brazilian Arabica Sul de Minas NY 2 and Indian Robusta AA Monsooned Malabar, were selected. The beans were purchased from the JSC "Klingai" (Kaunas, Lithuania). Representative samples of both coffee batches consisting of 280 g were used for roasting and all analyses. The roasting was performed in a New I-Roast 2 Home Coffee Roaster (USA) using 70 g of the beans at 200 °C and different time length corresponding to three basic levels of roasting, light, medium, and dark, which were defined by the colour measurement of coffee beans (CIElab system using a colorimeter Colour-guide sphere spex - Byk Gardner, Germany) (Somporn et al., 2011). Raw coffee beans were ground in an ultra centrifugal grinder ZM 200 (Retsch, Haan, Germany).

1.1.1. Preparation of coffee extract. One gram of ground coffee was placed in a test tube and diluted with 40 ml of 50% methanol, followed by the method by Montreau (1972). All antioxidant assays were repeated three times (Somporn et al., 2011).

1.2. Total phenolic content by Folin-Ciocalteu reagent (TPC)

For determination of total phenolic content by Folin-Ciocalteu reagent (TPC), a method according Medina (2011) was used. The absorbance was measured in a FLUOstar Omega spectrophotometer (BMG Labtech, Offenburg, Germany) at 765 nm. All measurements were repeated three times and TPC was expressed as a mean in mg of gallic acid equivalents (mg GAE/g of sample).

1.3. Ferric reducing-antioxidant power (FRAP) assay

Ferric Reducing-Antioxidant Power (FRAP) assay manual was used according to Benzie and Strain (1996) and Huang and co-workers (2005). This method is based on reduction of Fe^{3+}, TPTZ (2,4,6-tripyridyl-*s*-triazine) complex to the ferrous Fe^{2+} form at low pH. This reduction is followed by the measurement of absorption change at 593 nm. All measurements were repeated three times. FRAP values were expressed in µmol Fe^{2+}/g of the sample.

1.4. Trolox equivalent antioxidant capacity (TEAC) determination by ABTS assay

The method is based on the ability of antioxidant molecules to scavenge the radical species with long halftime such as $ABTS^{\cdot+}$ (2,2'-azinobis) (3-ethylbenzothiazoline-6-sulphonic acid), 734 mm being used for the specification. All measurements were repeated three times. TEAC was expressed in Trolox equivalents (µmol Trolox/g of sample) (Huang et al., 2005; Ložiené et al., 2007).

1.5. DPPH˙ assay

This test is based on the ability of DPPH˙ (2,2–diphenyl-1-picrylhydrazyl) to accept an electron or in some cases hydrogen atom from a molecule (HUANG et al., 2005; MISHRA et al., 2012). Antioxidant activity of coffee bean extract was assessed by DPPH˙ method according to BRAND-WILLIAMS and co-workers (1995) with the minor modifications. The absorbance was measured after 30 min at 515 nm by FLUOstar Omega. All measurements were repeated three times. Antioxidant activity was expressed in mg Trolox equivalent/g of sample.

The results may also be expressed as inhibition percentage according to ALVES and co-workers (2010).

$$\text{Scavenging effect (\%)} = \frac{(\text{absorbance}_{t=0min} - \text{absorbance}_{t=30min})}{(\text{absorbance}_{t=0min})} \times 100$$

1.6. Statistical data evaluation

Remote values were tested by Q-test. Single-factor ANOVA was used for monitoring the relations of the results of the individual analytical methods on three degrees of roasting for each coffee type, separately. Paired *t*-test was used for monitoring the differences between the individual types of roast coffees (*C. arabica* and *C. robusta*). The pairs were created in relation to the roasting level. Green coffees were assessed by a separate *t*-test with non-homogeneous variance. Correlation analysis was used to analyze the relation of the results of the individual methods. The significance level of all tests was specified to be P=0.05.

2. Results and discussion

This study assessed antioxidant potential of both coffee types by using four different methods, which gave different antioxidant activity values (Table 1).

2.1. Total phenolic content by Folin-Ciocalteu reagent (TPC)

The values measured with Folin-Ciocalteu reagent almost in all previously published studies are considered as a TPC; however, it should be noted that original method stemmed from reagents used for tyrosine analysis, in which oxidation of phenols by a molybdate tungstate reagent yields a coloured product with absorption maximum at 745–750 nm. Consequently the basic mechanism is an oxidation/reduction reaction that can be considered as another antioxidant activity evaluation method (PRIOR et al., 2005). These values in our study are expressed in milligrams of gallic acid (GAE) per gram of coffee beans. Statistically significant correlation between the TPC values and the level of roasting was found (Table 1): in general, the TPC gradually decreased during roasting. However, in the case of *C. arabica,* the TPC value was higher by 9% (light roasting), which decreased by 16 and 30% (medium and dark roasting). In the case of *C. robusta,* this trend was even more pronounced, the decrease was by 18% (light roasting) and by 35 and 47% (medium and dark roasting) compared to green coffee.

In general, our results are in agreement with the ones previously reported by SOMPORN and co-workers (2011), who found that TPC gradually decreases during roasting by *C. arabica*. It is also worth noting that BALASUNDRAM and co-workers (2006) reported 52.5–57.0 mg GAE/g in dry matter of different ground coffee types (the level of roasting not specified); these values are very close to the TPC determined for green *C. robusta* beans in our study (Table 1).

Table 1. Antioxidant activity values of green and roasted coffee beans measured by different methods (mean±SD)

Assays	Roasting degree	Arabica	Robusta
Total phenolic content (mg GAE/g of sample)	Green	34.8±4.2[A]	57.6±4.9[A]
	Light	38.1±2.6[aB]	47.4±5.2[bB]
	Medium	29.1±2.7[aB]	37.5±2.1[bB]
	Dark	24.3±0.8[aB]	30.5±4.6[bB]
FRAP (μmol Fe^{2+}/g of sample)	Green	553±73[A]	692±31[A]
	Light	541±39[a]	571±85[b]
	Medium	461±28[a]	447±38[b]
	Dark	386±15[a]	351±33[b]
ABTS (μmol Trolox/g of sample)	Green	327±21[A]	547±73[A]
	Light	108±8[B]	452±64[B]
	Medium	112±20[B]	500±36[B]
	Dark	109±13[B]	508±42[B]
DPPH˙ (μmol Trolox/g of sample)	Green	83±18[A]	116±15[A]
	Light	112±17[a]	133±24
	Medium	113±20[a]	111±10
	Dark	131±24[a]	125±27
DPPH (% inhibition)	Green	33.6±8.8[A]	50.2±7.4[A]
	Light	60.4±2.1[aB]	63.2±2.5[B]
	Medium	60.4±2.5[aB]	61.2±1.0[B]
	Dark	62.6±3.0[aB]	62.5±3.3[B]

[a,b,A,B]: statistically significant difference of mean (P=0.05) for each method; [a]: dependence on the roasting level by Arabica coffee samples (ANOVA); [b]: dependence on the roasting level by Robusta coffee samples (ANOVA); [A]: comparing between green Arabica versus green Robusta coffee (*t*-test); [B]: comparing between roasted Arabica versus roasted Robusta coffee (paired *t*-test)

2.2. Ferric reducing-antioxidant power (FRAP) assay

A significantly higher FRAP value (by 25%) was determined for green *C. robusta* than green *C. arabica* (Table 1). According to VIGNOLI and co-workers (2011), the higher antioxidant activity of *C. robusta* can be explained with the higher caffeine content.

In FRAP assay both studied coffee types demonstrated significant dependence (P=0.05) of antioxidant activity on the roasting level: FRAP gradually decreased by continuing roasting procedure (Table 1). On the other hand, no significant difference was measured between the values for the individual roasting levels of *C. arabica* and of *C. robusta* using a paired *t*-test.

In the case of roasted *C. robusta,* the drop of antioxidant activity comparing green beans was 49%, in the case of comparing roasted and green *C. arabica* beans the decrease was 30%. Consequently, our results indicate that the concentration of ferric ions reducing antioxidant substances in coffee should gradually decrease during the roasting of beans. Other authors reported that FRAP values for coffee drink and dry soluble substances of *C. robusta* were by 30.4–70.2% higher than those for *C. arabica*, which is in agreement with our findings (MOREIRA et al., 2005).

2.3. Trolox equivalent antioxidant capacity (TEAC) measured by ABTS assay

Roasting of *C. arabica* beans resulted in a triple reduction of their TEAC by light roasting degree (Table 1). There were no significant differences in TEAC values between light, medium, and dark degrees in case of *C. arabica* roasting. TEAC values of roasted *C. robusta* were also lower than TEAC of green beans, however the differences were not so remarkable compared to *C. arabica*.

Consequently, roasted *C. robusta* possessed approximately 5 times higher TEAC values than roasted *C. arabica*. Twice higher antioxidant activity of roasted *C. robusta* than that of roasted *C. arabica* was reported by RICHELLE and co-workers (2001). DEL CASTILLO and co-workers (2002) observed that antioxidant activity ABTS of *C. arabica* coffee increased during roasting lightly and at medium roasted levels, however further roasting did not increase antioxidant activity. However, the same authors noted that ABTS may yield different results when the applied wavelength is changed. For example, at 420 nm the values of antioxidant activity increased with the degree of roasting.

2.4. DPPH˙ assay

Green *C. robusta* coffee possessed significantly higher DPPH˙ scavenging capacity than green *C. arabica*; however, after roasting, the differences between *C. robusta* and *C. arabica* were not significant (Table 1). Significant differences were found between green beans of *C. arabica* and *C. robusta* in all used methods (*C. robusta* always possessed stronger antioxidant activity than *C. arabica*), however, after roasting, DPPH˙ assay gave different results compared to other methods.

Application of DPPH˙ assay is limited by certain disadvantages. In addition to the different reaction in the hydrogen atom transfer mechanism usually taking place between antioxidants and peroxyl radicals, DPPH˙ is a nitrogen radical possessing long halftime and no similarity to the highly reactive and transient peroxyl radicals contributing to the oxidation of lipids. Many antioxidants quickly reacting with peroxide radicals may react slowly or be inert to DPPH˙. As a consequence of this, the antioxidant activity may not be judged correctly. In addition, the reaction kinetics between DPPH˙ and antioxidants is not linear (HUANG et al., 2005). Therefore, it is better to express antioxidant activity by an effective concentration EC_{50} that is required to scavenge 50% of the radicals present in the reaction or at least by DPPH˙ inhibition percentage. Reaction of DPPH˙ with phenolic antioxidant may be reversible as it was demonstrated for eugenol; therefore false lower antioxidant activity values may be measured for the samples containing eugenol and other phenols with similar *o*-methoxyphenol structures (BONDET et al., 1997). This may serve as one of the reasons explaining 5 times higher antioxidant activity of *C. robusta* in ABTS assay than in DPPH˙ method (Table 1). Although the resulting values expressed as percentage of inhibition corresponding to the results in µmol Trolox/g sample units, statistical data handling yields slightly different results

(Table 1). The differences between both types of roast coffee in μmol Trolox/g sample units are insignificant, while significant results are produced by expression as percentage of inhibition. This may be related to the above-mentioned opinions (HUANG et al., 2005).

2.5. Summary and comparison of above mentioned methods

From our values in Table 1, the greatest mutual correlation is shown between the results obtained by TPC and FRAP methods, with the correlation coefficient in the case of *C. arabica* being 0.959 and in the case of *C. robusta* even higher, 0.999. The other methods gave different results, especially in the case of roasted coffees, when low correlation between them and FRAP and TPC was found. Except for ABTS method, the differences in antioxidant activity between roasted *C. arabica* and roasted *C. robusta* decrease with the increase of roasting level (Table 1). In the case of FRAP and DPPH˙**,** these differences are already statistically insignificant.

3. Conclusions

The most informative methods for the evaluation of the effect of the level of roasting on the changes of antioxidant activity of coffee beans were FRAP and TPC; both methods clearly demonstrated gradually decreasing antioxidant activity values that were in high correlation.

In the case of green coffees, independently of the analytical method used, antioxidant activity of *C. robusta* was always significantly higher than antioxidant activity of *C. arabica*, which indicates that all applied assays may reveal the differences in antioxidant potential between different types of raw coffee beans. However, these differences may be significantly influenced by roasting, e.g. as in case of TPC in *C. arabica*, which significantly increased the values.

Conclusion of our results is that the relation between antioxidant activity and roasting level appears to differ for different coffee types, roasting conditions, extraction procedure, and methodology of antioxidant activity evaluation. What needs to be emphasized is that different methods of evaluation of antioxidant activity of coffee infusion yield different results, probably because the simultaneously present antioxidants in coffee extracts show different mechanisms of action.

It may be concluded that antioxidant activity is statistically significantly more dependent on the coffee type than on the roasting level, the difference between roasted *C. arabica* and roasted *C. robusta* mostly decreases, and also the particular analytical method used is decisive, too.

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