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RESEARCH ARTICLE

Optimization and Evaluation of phenolic compounds and their antioxidant activity from coffee beans.

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Abstract

Solvent extraction for estimation of polyphenols from green and roasted coffee beans (*Coffea arabica*) with methanol was optimized using response surface methodology, central composite design. Graphical optimization was carried out to find out experimental conditions that yield maximum polyphenol extraction and antioxidant activity. Results showed that significant factor affecting polyphenol extraction from green coffee beans was methanol concentration and time of extraction in roasted coffee beans. Average maximum polyphenol content extractable was 55 mg Gallic Acid Equivalence / g for green and 72 mg Gallic Acid Equivalence/ g for roasted coffee beans. Roasted beans were found to have higher polyphenolic content than green coffee beans and a positive nonlinear relationship was observed between polyphenol content and radical scavenging activity in both the cases. Also, extraction kinetics with respect to time was studied at their respective optimized conditions and stability of antioxidant compounds was compared. Initially, similar extraction pattern was observed in both types after which they traced different paths. Extraction of polyphenols from green beans showed continuously increasing polyphenol content and remained constant after attaining peak. Whereas, roasted coffee beans attained peak extraction within a few hours after which it drifted to a falling course showing formation of less stable and volatile phenolic compounds during roasting. Thus conditions for ideal spectrometric quantification of polyphenols using methanol as solvent was optimized for coffee beans.

INTRODUCTION

Natural polyphenols are a subject of continuous interest among scientist around the world and are being studied from different sources for quantitative and qualitative purposes. One of the major dietary sources of antioxidants is coffee, the antioxidant activity of which is attributed to their inherent phenolics such as Chlorogenic acid (CGA), caffeic acid, ferrulic acid, p-coumaric acid and maillard reaction products formed during roasting.

Estimation of polyphenols in coffee beans is done to evaluate the quality of coffee beans (Belay and Gholap, 2009) and that of the final product (Farah et al., 2006) and also for producing antioxidant rich conserves that serve nutritional interventions (Glei et al., 2006; Mussatto et al., 2011; Naidu et al., 2008; Nissen et al., 2004). Chlorogenic acids (CGA) form the main phenolic compounds in raw coffee beans (Farah et al., 2005). According to Farah et al. (2006) green (or raw) coffee contains 5–12 g/100 g of CGA. Roasting is required to get the desired blend of flavor and aroma. Coffee beans are roasted to different degrees between 200- 300 °C, during which complex

chemical and structural changes occur. The high heat sensitivity of CGAs causes a considerable reduction in total polyphenol content (TPC). However, maillard reaction products and their derivatives posses antioxidant capacity and compensates for the loss incurred during roasting. Nevertheless, the antioxidant composition, stability and activity of the polyphenolic compounds present in raw and roasted coffee beans vary by large.

Characterization of phenolic compounds in food have been reviewed by many (Jin and Mumper, 2010; Naczk and Shahidi, 2004; Rong, 2010; Routray and Orsat, 2012; Stalikas, 2007*)*. Despite emergence of new techniques, solvent extraction being simple and economical remains the most preferred. Previous studies on solvent extraction of polyphenols from coffee beans have focused on quantifying and analyzing the antioxidant ability of the isolated polyphenols with respect to different processing and extraction conditions. Some of them include Arellano-González et al. (2011) (fermented and non-fermented coffee pulp), Cämmerer and Kroh, (2006) (coffee brews), Kreicbergs et al. (2011) (coffee powder), Mussatto et al. (2011) (spent coffee grounds), and Pérez-Hernández et al. (2012) (Green coffee beans). Estimated values in these studies fall between a broad ranges of 18- 335 mg GAE/g. This high level of variance is due to the different extraction techniques used that vary between high resolution and less sophisticated methods of analysis. Efficient extraction is influenced by the solvent, solvent concentration, time and temperature of extraction, particle size, pH and solid-solvent ratio (Chirinos et al., 2007; Escribano-Bailón and Santos-Buelga, 2003; Pinelo et al., 2007). Optimization is the key to reproducible and comparable results as the extraction is affected by several extrinsic and intrinsic factors. Only a few have restored to optimizing extraction variables in estimating polyphenols from coffee. Naidu et al., 2008 optimized conditions for extraction of phenols from green coffee beans to produce an antioxidant rich conserve that can be used as a food additive. Mussatto et al., (2011) optimized extraction of polyphenols from spent coffee grounds.

Compilation of databank using reliable quantitative measurements of foods is a highly important objective in the food industry. This study was aimed to optimize extraction parameters using response surface methodology for green and roasted coffee beans by solvent extraction technique using methanol. Standardization of this simple and widely used method will aid in obtaining reliable results in laboratories that lack sophisticated analytical tools. The conditions for extraction and quantification of maximum polyphenols present in both green and roasted coffee beans were established. The difference in kinetics of extraction was compared in order to study stability of polyphenol population in green and roasted coffee beans.

2. MATERIALS AND METHODS

Green and roasted coffee beans (*Coffea arabica*) were purchased from local market. Distilled water was used in the entire analysis. All chemicals used were of analytical grade.

2.1. Sample preparation and solvent extraction

Green and roasted coffee beans were sterilized at 120°C for 20 min in hot air oven. Sterilized beans were ground to 50 mesh size and stored at -20°C till use. 1g coffee powder was taken in 500 ml stoppered Erlenmeyer flasks and extracted with methanol in an orbital shaker for predetermined period of time. The solution was centrifuged (9000 rpm, 15°C, 10 min) and supernatant was filtered through whatman filter paper no: 41, separated and stored at -20 °C till analysis was performed.

2.2. Total Phenolic Content

The total phenolic content was determined using Folin- Ciocalteu method as performed by Singleton and Rossi, (1965). Calibration curve was obtained using gallic acid as standard (50 – 500 mg/l). Total phenolic content was expressed in mg Gallic acid equivalence (GAE) per gram dry coffee beans.

$$\textbf{mg GAE/g Dry smaple} = [\text{GA (mg/ml)}] * \frac{\text{Extract volume (ml)}}{\text{Dry mass (g)}}$$

2.3. Antioxidant radical scavenging activity (DPPH)

The 1,1- diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity method is extensively used to determine antioxidant capacity of polyphenols in purified as well as natural plant extracts (Ozgen et al., 2006). The antioxidant activity of coffee extracts were determined using the by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay (Arnous et al., 2001). Radical scavenging activity was calculated using the following relation:

$$\%\Delta\, \boldsymbol{A_{515}} = \left[\frac{A_{515}\ (t=0) - A_{515}\ (t=30)}{A_{515}\ (t=0)}\right]$$

Calibration curve was produced using Butylated Hydroxy Toluene (BHT) as standard. Antioxidant activity was expressed as M BHT/ g dry coffee beans.

2.4. Statistical optimization

Influence of three main extraction variables (solvent concentration, solvent: solid and time of extraction) on extraction efficiency was studied and the extraction conditions were optimized using a 2^3 central composite design.

A 95% significance level ($p<0.05$) was assumed to find out statistical significance of the variables studied. For green coffee beans the experimental design was: 70- 90% methanol, 30-50 ml/g solvent and 6- 10 h extraction time. Roasted coffee beans were analyzed between 70-90% methanol, 40-60 ml/g solvent: solid ratio and 2-6 h time of extraction. The coded and actual values for the experimental designs for green and roasted coffee beans are given in Table 1 and 2 respectively. Statistical analysis of the data as well as the determination of the conditions able to maximize the extraction results were performed using Design expert (version 6.0.10). Graphical optimization of the extraction conditions to maximize polyphenol extraction and antioxidant activity was performed. The data obtained was fitted into quadratic equations by eliminating statistically insignificant terms and statistical significance was checked by referring their R^2 (Coefficient of determination) values. Assays done to validate optimized extraction conditions for total polyphenols and DPPH radical scavenging activity were done in triplicates.

2.5. Extraction kinetics

The optimized conditions got from the statistical analysis were used for the determination of kinetics of extraction. A comparison between the extraction kinetics of green and roasted coffee beans was done to assess impact of roasting on stability of polyphenols and to differentiate extraction patterns between the two. Total Polyphenol Index (TPI) was calculated as a measure of amount of polyphenol extracted into solvent at regular intervals of 1 h for a total time of 12 h. The obtained results were fitted to polynomial equations using CoStat.

Total Polyphenol Index (TPI) = A_{765} * Dilution factor

3. RESULTS AND DISCUSSION

Sample preparation with respect to particle size has been taken care of in the present study, setting the value at 50 mesh (300 micron) to ensure maximum surface area and thus high extraction efficiency. Polyphenols are readily soluble in organic solvents that are less polar (Kim and Lee, 2002; Liu and Ang, 2000). Extraction of phenolics from plant sources such as black tea, barley, tubers, citrus peel and some medicinal plants have shown ethanol and methanol to be effective (Chirinos et al., 2007; Jamal and Barkat, 2010; Rehman, 2006; Turkmen et al., 2006). Methanol was chosen in the present study due to its reported efficiency in extraction from plant material and it being economical (Kim and Lee, 2002).

Response surface methodology, 2^3 central composite design was used for optimization of three critical variables in polyphenol extraction- solvent percentage, solvent- solid ratio and time of extraction. Conditions that yield phenol rich extract with high antioxidant activity were established. Table 1 and 2 lists the experimental designs for green and roasted coffee beans and their responses total polyphenol content and radical scavenging activity (DPPH assay). Statistical analysis of the responses showed significant influence of all three variables on polyphenol content as well as antioxidant capacity (green / roasted) with a 'p value' less than 0.05. It is seen from the Table 1 that the total polyphenol content and antioxidant capacity is high in experiments with high solvent- solid ratio and time of extraction. A similar trend is also observed in case of roasted coffee beans (Table 2). The highest polyphenol content on an average was 58 mg GAE/ g dry bean (Table 1-experiment 10, 18, 20) in green beans and 78 mg GAE/g dry beans (Table 2- experiment 5, 8, 10) in roasted coffee beans. Results show that the polyphenol content and the radical scavenging activity are high in roasted coffee beans compared to green beans. This can be explained by the formation of polyphenolic substances and other maillard reaction products during roasting which adds to and help in maintaining the phenolic content of coffee beans (Borrelli et al., 2002; Daglia et al., 2000; Del Castillo et al., 2002; Nicoli et al., 1997a, b, 1999). According to Nicoli et al., (1997a), intermediate maillard reaction products show higher activity than the final products. In this case the roasted beans have higher polyphenolic content as well as radical scavenging activity compared to the green beans, indicating higher content of polyphenols formed during roasting. Similar results were observed by Pérez-Hernández et al., (2012) who reported a value of 55.84± 2.40 mg GAE/ g for ultra sonnicated green coffee beans (*Coffea arabica*). Naidu et al., (2008) reported 32.19 ± 0.63 mg GAE/g for the same variety when extracted with isopropanol. Kreicbergs et al., (2011) reported a maximum of 37 mg GAE/g when studying polyphenols extracted with water from various coffee brands. A positive nonlinear relationship was observed in our experiments between TPC and DPPH radical scavenging activity in green and roasted coffee beans.

The results were fitted to quadratic equations as a function of the three variables; omitting statistically insignificant ($p> 0.05$) terms (Table 3 and 4). All equations were of quadratic fit and had high R^2 values showing that the observed outcomes are replicated by the model and that there is high degree of correlation between experimental and predicted results. Table 3 shows that linear (A, B, and C) and quadratic terms (A^2, B^2, C^2) are significant with p value < 0.05 for both polyphenol content and radical scavenging activity. The same trend was observed in roasted coffee beans. The variable with the largest effect on total polyphenols in green beans was the quadratic term A^2 (methanol concentration) and that of scavenging activity was found to be linear term B (solvent: solid ratio). In case

of roasted coffee beans, the quadratic term C^2 (time) has the largest effect for both total polyphenols and radical scavenging activity (Table 4).

3D contour plots showing the variation of responses as a function of three variables is shown in Fig 1 and 2. Both green and roasted beans show an increase in TPC on initial rise of methanol concentration and solid- solvent ratio. After reaching a plateau, TPC takes up a falling trend, a steeper one in case of roasted coffee beans. Radical scavenging activity follows a direct linear relationship with solid solvent ratio in green beans, and a rise- fall trend in roasted beans. Radical scavenging activity decreases with increasing methanol % in green and roasted coffee beans. The observed variation between the beans is due to the change in polyphenolic composition during roasting.

A graphical optimization of the extraction conditions was done for both green and roasted coffee using an overlay plot to find out region of maximum polyphenol yield and radical scavenging activity. The time of extraction was fixed to be 8 and 2 h for green and roasted coffee beans after which no change/ decrease in polyphenol content was observed. Figure 3 and 4 shows the overlapping region that satisfies this criterion. A point within the overlapping region was chosen to validate the optimized conditions (Table 5). The validation experiments were performed in triplicates and showed excellent compliance with the predicted values. Optimized conditions showed that % methanol and solvent: solid ratio was almost same for green and roasted coffee beans, the major difference being time of extraction. Naidu et al., (2008) reported that 60% isopropanol yielded high polyphenol extract from green coffee beans. Similarly, 60% methanol was sufficient to extract out polyphenols from spent coffee grounds (Mussatto et al., 2011). Also 44- 50 ml/g solvent solid ratio was able to extract out maximum polyphenols. The combined use of water with methanol creates a moderately polar medium and gives greater yield compared to water or methanol alone (Chirinos et al., 2007; Kim and Lee, 2002; Lapomik et al., 2005; Lui and Ang, 2000; Musa et al., 2011).

A major difference in optimized extraction conditions with respect to time of extraction indicates huge stability variation and was studied by analyzing kinetics of extraction at the optimized conditions. Fig 5 shows the trend of polyphenol leaching into the solvent. Green coffee beans showed an increasing trend till 8^{th} hour after which it reaches a plateau and the polyphenol content remains almost same till the end. Whereas, roasted coffee beans achieves maximum extraction by 4^{th} hour and then tends to fall continuously with a polyphenol content less than initial value at the end of 12 h. From this it can be concluded that the polyphenols present in green coffee beans are relatively more stable than the ones in roasted coffee beans. Roasting of coffee beans (180–200 ^{0}C) results in profound changes in polyphenol content and its biological activity (Hecimovic et al., 2011). Roasting leads to degradation of existing polyphenols and formation of less stable and volatile maillard reaction products and other compounds having antioxidant activity (Hecimovic et al., 2011, Moreira et al., 2000). Also extended extraction times lead to degradation/ oxidation of extracted polyphenols (Biesaga and Pyrzynska, 2013; Davidov- Pardo and Marin Arroyo, 2011). Cämmerer and Kroh (2006) observed reduction in radical scavenging activity of roasted coffee beans in contact with atmospheric oxygen. The same authors also demonstrated the stability of Chlorogenic acids (the major polyphenol in green coffee beans) in the presence of atmospheric oxygen. The above reasons could be the possible explanation for the observed decrease in antioxidant content during the extraction after attaining maximum. This emphasizes the need to estimate polyphenols immediately after 4 hours of extraction and that care should be taken to minimize atmospheric exposure. The results were fitted to polynomial equations as shown in table 6 having high R^2 values.

5. CONCLUSION

The extraction of polyphenols for estimation from green and roasted coffee beans (*Coffea arabica)* was optimized using response surface methodology. The fitted models had high coefficient of determinations R^2 and validation studies showed high predictability, indicating that it can be used for navigating the range studied for reliable analytical estimation. Thus, the optimized extraction conditions can be used as a reliable method for estimating polyphenol content from the studied samples. Also, kinetics of polyphenol extraction was compared between green and roasted coffee beans which helped in establishing stability differences of antioxidant compounds found in the two groups. These results will be useful in the preparation of phenolic conserves applicable for food processing and preservation.

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Table 1

2^3 central composite design showing experimental design for extraction of polyphenols from green coffee bean

Experiment	Variables- real (coded)			Responses [a]	
	Methanol concentration (%)	Solvent :Solid ratio (ml/g)	Time (h)	TPC (mg GAE/ g dry bean)	DPPH (M BHT/ g dry beans)
1	80 (0)	40 (0)	8 (0)	54.23	4.11
2	80 (0)	40 (0)	8 (0)	55.29	4.50
3	80 (0)	40 (0)	8 (0)	55.86	4.11
4	70 (-1)	50 (1)	10 (1)	43.75	3.84
5	70 (-1)	30 (-1)	6 (-1)	40.62	3.51
6	80 (0)	23.18 (-1.682)	8 (0)	48.43	2.04
7	90 (1)	30 (-1)	10 (1)	42.32	2.28
8	80 (0)	40 (0)	4.64 (-1.682)	42.58	3.68
9	80 (0)	56.82 (1.682)	8 90)	44.04	4.11
10	80 (0)	40 0)	8 (0)	58.29	4.00
11	70 (-1)	30 (-1)	10 (1)	46.61	2.18
12	90 (1)	50 (1)	10 (1)	33.75	3.74
13	80 (0)	40 (0)	11.36 (1.682)	50.24	2.94
14	90 (1)	50 (1)	6 (-1)	35.54	5.12
15	96.82 (1.682)	40 (0)	8 (0)	40.71	3.28
16	63.18 (-1.682)	40 (0)	8 (0)	38.56	3.28
17	70 (-1)	50 (1)	6 (-1)	40.89	4.41
18	80 (0)	40 (0)	8 (0)	60.71	4.40
19	90 (1)	30 (-1)	6 (-1)	41.25	2.93
20	80 (0)	40 (0)	8 (0)	57.00	4.37

TPC- Total polyphenol content; DPPH- Radical scavenging activity by DPPH assay
[a] Each response value represents the mean of three replicates

Table 2
2^3 central composite design showing experimental design for extraction of polyphenols from roasted coffee bean

Experiment	Variables real (coded)			Responses [a]	
	Methanol concentration (%)	Solvent :Solid ratio (ml/g)	Time (h)	TPC (mg GAE/ g dry bean)	DPPH (M BHT/ g dry beans)
1	63.18 (-1.682)	50 (0)	4 (0)	53.39	6.05
2	80 (0)	66.82 (1.682)	4 (0)	43.20	6.24
3	70 (-1)	60 (1)	2 (-1)	31.50	5.14
4	96.82 (1.682)	50 (0)	4 (0)	24.82	4.68
5	80 (0)	50(0)	4 (0)	73.39	6.94
6	90 (1)	60 (1)	2 (-1)	12.65	3.76
7	90 (1)	60 (1)	6 (1)	26.79	5.06
8	80 (0)	50 (0)	4 (0)	78.04	7.23
9	80 (0)	50 (0)	4 (0)	70.44	7.06
10	80 (0)	50 (0)	4 (0)	75.89	7.56

11	80 (0)	50 (0)	7.36 (1.682)	48.75	5.99
12	90 (1)	40 (-1)	2 (-1)	26.71	3.72
13	70 (-1)	40 (-1)	6 (1)	57.00	5.89
14	70 (-1)	40 (-1)	2 (-1)	39.86	4.93
15	80 (0)	33.18 (-1.682)	4 (0)	38.75	5.00
16	80 (0)	50 (0)	0.64 (-1.682)	16.25	3.95
17	70 (-1)	60 (1)	6 (1)	44.36	5.93
18	90 (1)	40 (-1)	6 (1)	54.71	4.64
19	80 (0)	50 (0)	4 (0)	68.75	7.35
20	80 (0)	50 (0)	4 (0)	69.24	6.49

TPC- Total polyphenol content; DPPH- Radical scavenging activity by DPPH assay
[a] Each response value represents the mean of three replicates

Table 3 Mathematical models fitted to experimental data for green coffee beans

Response	**Model equation**	**R^2**
Total Polyphenolic Compounds (mg GAE/ g dry beans)	TPC = +56.99 – (1.13* A) – (1.78 * B) + (1.54 * C) – (6.68 * A^2) – (4.35* B^2)- (4.28* C^2)	0.92
Antioxidant activity (M BHT/g dry beans)	DPPH = +4.24 + (0.011* A)+ (0.71* B) – (0.38* C) – (0.27* A^2) – (0.34 * B^2) – (0.26 * C^2)	0.91

TPC- Total Polyphenol Content; DPPH- ,1- diphenyl-2-picrylhydrazyl radical scavenging activity; A- % Methanol; B- Solvent to solid ration (ml/g); C- Time of extraction (hr)

Table 4 Mathematical models fitted to experimental data for roasted coffee beans

Response	**Model equation**	**R^2**
Total Polyphenolic Compounds (mg GAE/ g dry beans)	TPC = +72.59 – (7.32 * A) – (4.06* B)+ (9.28 * C) –(11.58 * A^2) – (10.92* B^2)- (13.91* C^2)	0.95
Antioxidant activity (M BHT/g dry beans)	DPPH = +7.12 – (0.51 * A)+ (0.21* B)+ (0.54* C) –(0.68* A^2) – (0.59* B^2) – (0.82* C^2)	0.94

TPC- Total Polyphenol Content; DPPH- ,1- diphenyl-2-picrylhydrazyl radical scavenging activity; A- % Methanol ; B- Solvent to solid ration (ml/g); C- Time of extraction (h)

Table 5 Validation of optimized conditions for green and roasted coffee beans

Sample	**Experiments**	**Variables**			**Response**	
		Methanol concentration (%)	**Solvent :Solid ratio (ml/g)**	**Time (h)**	**TPC (mg GAE/ g dry bean)**	**DPPH (M BHT/ g dry beans)**
Green coffee beans	Predicted	79.59	44.32	8	55.46	4.48
	Actual	79.59	44.32	8	54.96[a]	4.42 [a]
Roasted	Predicted	78.84	51.66	4	72.35	7.18

coffee beans	Actual	78.84	51.66	4	71.94 [a]	7.05 [a]

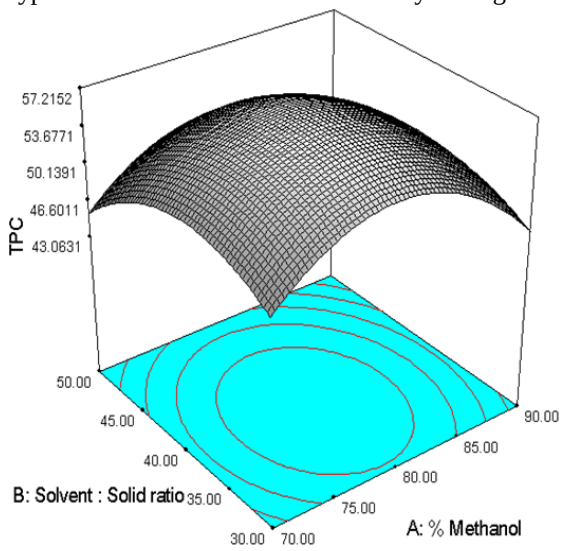
TPC- Total Polyphenol Content, BHT- Butylated Hydroxy Toluene, GAE- Gallic acid equivalence, DPPH- 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity.
[a] mean value of 3 replicates

Table 6 Model kinetic equations for solvent extraction in green and roasted coffee beans.

Sample	**Model equation**	**R^2**
Green coffee beans	y= .681+0.191 x-.0082 x^2	0.97
Roasted coffee beans	y=1.687+0.674 x-.0089 x^2	0.86

y= Total Polyphenol Index (mg/ml extract); x= Time (h)

Figure 1
Response surfaces depicting effect of methanol concentration and solvent: solid ratio on the total extractable polyphenols and their antioxidant activity from green coffee beans.



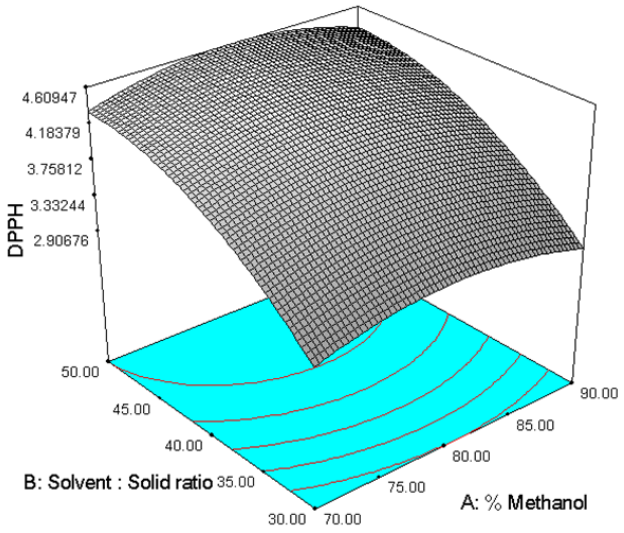


Figure 2
Response surfaces depicting effect of methanol concentration and solvent: solid ratio on the total extractable polyphenols and their antioxidant activity from roasted coffee beans.

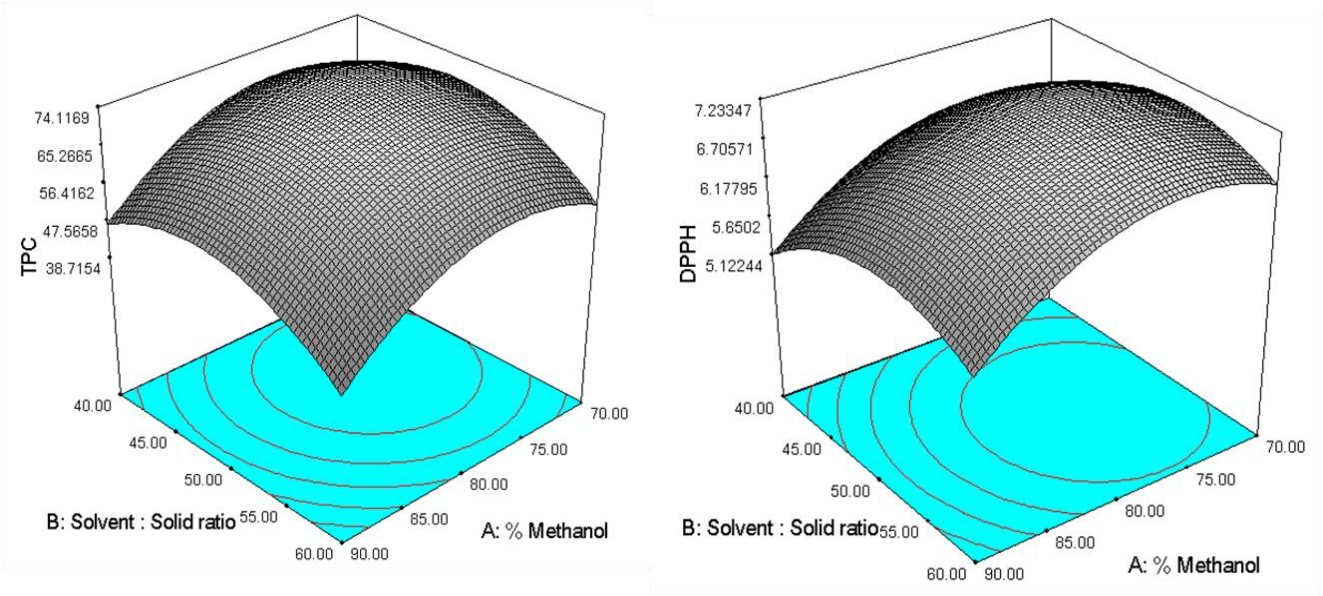


Figure 3
Superimposed plot depicting region of maximum extractable polyphenols (TPC) with antioxidant activity (DPPH) from green coffee beans as a function of the methanol concentration and solvent/solid ratio.

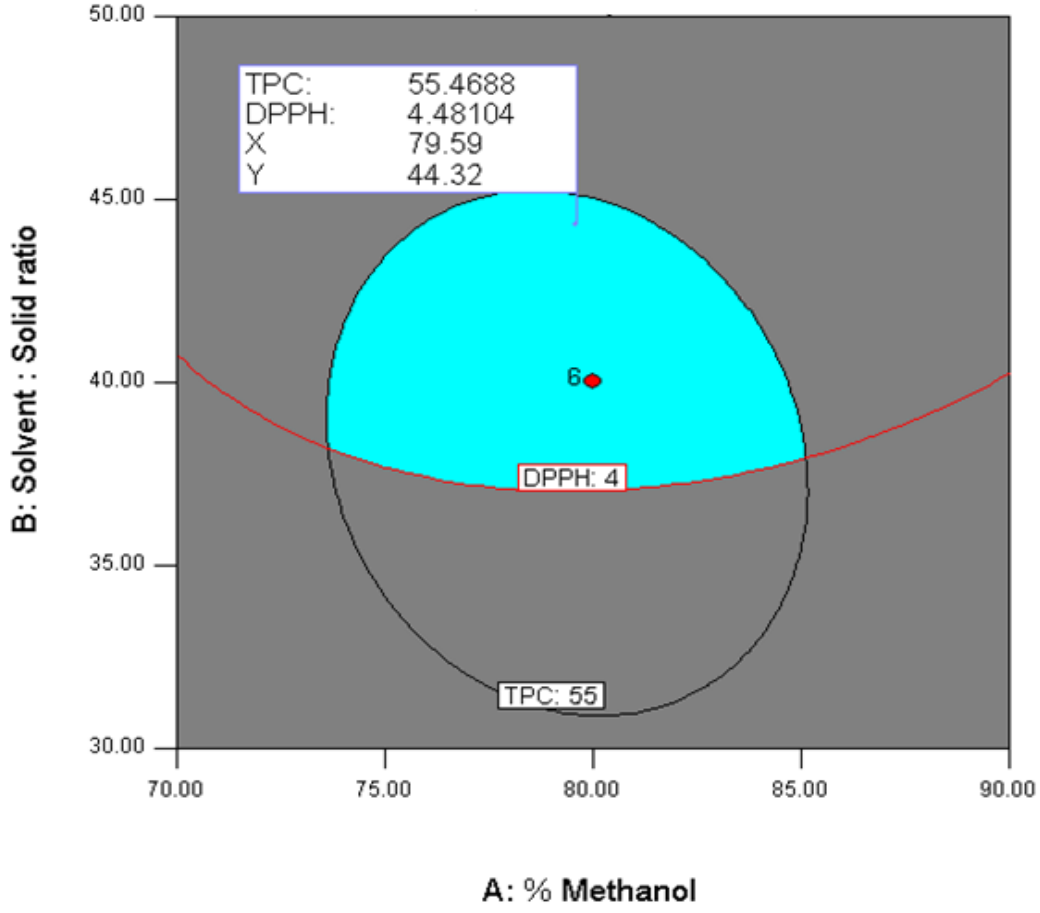


Figure 4
Superimposed plot depicting region of maximum extractable polyphenols (TPC) with antioxidant activity (DPPH) from roasted coffee beans as a function of the methanol concentration and solvent/solid ratio.

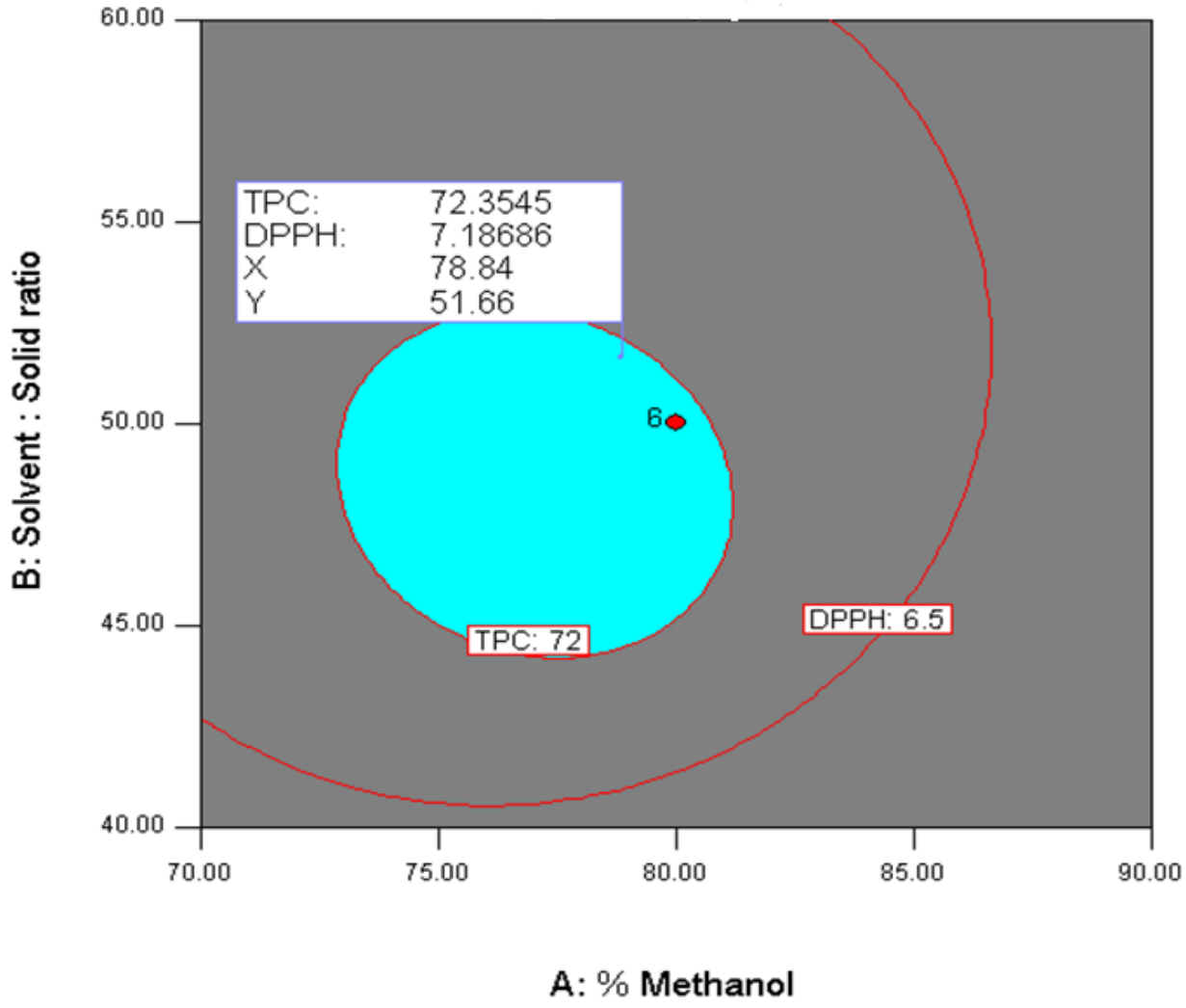
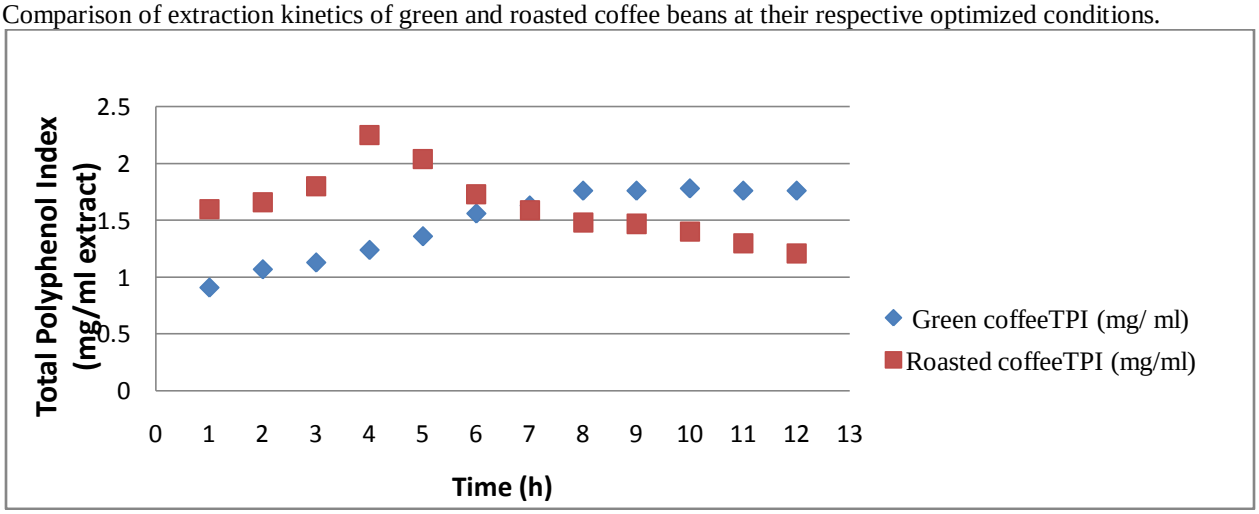


Figure 5
Comparison of extraction kinetics of green and roasted coffee beans at their respective optimized conditions.



REFERENCES

Arellano-González, M. A., Ramírez-Coronel, M. A., Torres-Mancera, M., Pérez-Morales, G. G., and Saucedo-Castañeda, G. (2011): Antioxidant activity of fermented and nonfermented coffee (Coffea arabica) pulp extracts. Food Technology and Biotechnology., 49(3): 374-378.

Arnous, A., Makris, D. P., and Kefalas, P. (2001): Effect of principal polyphenolic components in relation to antioxidant characteristics of aged red wines. Journal of Agricultural and Food Chemistry., 49(12): 5736-5742.

Belay, A., and Gholap, A. V. (2009): Characterization and determination of chlorogenic acids (CGA) in coffee beans by UV-Vis spectroscopy. African Journal of Pure and Applied Chemistry., 3(11): 34-240.

Biesaga, M., and Pyrzyńska, K. (2013): Stability of bioactive polyphenols from honey during different extraction methods. Food chemistry., 136(1): 46-54.

Borrelli, R. C., Visconti, A., Mennella, C., Anese, M., and Fogliano, V. (2002): Chemical characterization and antioxidant properties of coffee melanoidins. Journal of Agricultural and Food Chemistry., 50(22): 6527-6533.

Cämmerer, B., and Kroh, L. W. (2006): Antioxidant activity of coffee brews. European Food Research and Technology., 223(4): 469-474.

Chirinos, R., Rogez, H., Campos, D., Pedreschi, R., and Larondelle, Y. (2007). Optimization of extraction conditions of antioxidant phenolic compounds from mashua (< i> Tropaeolum tuberosum</i> Ruíz & Pavón) tubers. Separation and Purification Technology., 55(2): 217-225.

Daglia, M., Papetti, A., Gregotti, C., Bertè, F., and Gazzani, G. (2000). In vitro antioxidant and ex vivo protective activities of green and roasted coffee. Journal of Agricultural and Food Chemistry., 48(5): 1449-1454.

Davidov-Pardo, G., Arozarena, I., and Marín-Arroyo, M. R. (2011): Stability of polyphenolic extracts from grape seeds after thermal treatments. European Food Research and Technology., 232(2): 211-220.

del Castillo, M. D., Ames, J. M.,and Gordon, M. H. (2002): Effect of roasting on the antioxidant activity of coffee brews. Journal of Agricultural and Food Chemistry., 50(13): 3698-3703.

Escribano-Bailon, M. T., Santos-Buelga, and C. E. L. E. S. T. I. N. O. (2003): Polyphenol extraction from foods. Methods in polyphenol analysis., 1-16.

Farah, A., de Paulis, T., Moreira, D. P., Trugo, L. C., and Martin, P. R. (2006): Chlorogenic acids and lactones in regular and water-decaffeinated arabica coffees. Journal of agricultural and food chemistry., 54(2): 374-381.

Farah, A., de Paulis, T., Trugo, L. C., and Martin, P. R. (2005): Effect of roasting on the formation of chlorogenic acid lactones in coffee. Journal of agricultural and food chemistry., 53(5): 1505-1513.

Glei, M., Kirmse, A., Habermann, N., Persin, C., and Pool-Zobel, B. L. (2006): Bread enriched with green coffee extract has chemoprotective and antigenotoxic activities in human cells. Nutrition and cancer., 56(2): 182-192.

Hečimović, I., Belščak-Cvitanović, A., Horžić, D., and Komes, D. (2011): Comparative study of polyphenols and caffeine in different coffee varieties affected by the degree of roasting. Food chemistry., 129(3): 991-1000.

Jamal, P., Ahmad Barkat, A., and Amid, A. (2010): Distribution of phenolics in various Malaysian medicinal plants. Journal of Applied Sciences., 10(21): 2658-2662.

Dai, J., and Mumper, R. J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules., 15(10): 7313-7352.

Kim, D. O., and Lee, C. Y. (2002): Extraction and isolation of polyphenolics. Current protocols in food analytical chemistry.

Kreicbergs, V., Dimins, F., Mikelsone, V., and Cinkmanis, I. (2011): Biologically active compounds in roasted coffee: In Proceedings of the 6th Baltic Conference on Food Science and Technology FOODBALT-2011: Innovations for food science and production: Jelgava, Latvia: pp. 110-115.

Lapornik, B., Prošek, M., and Golc Wondra, A. (2005): Comparison of extracts prepared from plant by-products using different solvents and extraction time. Journal of Food Engineering., 71(2): 214-222.

Liu, F. F., Ang, C. Y., and Springer, D. (2000): Optimization of extraction conditions for active components in Hypericum perforatum using response surface methodology. Journal of agricultural and food chemistry., 48(8): 3364-3371.

Madhava Naidu, M., Sulochanamma, G., Sampathu, S. R., and Srinivas, P. (2008): Studies on extraction and antioxidant potential of green coffee. Food Chemistry., 107(1): 377-384.

Moreira, R. F. A., Trugo, L. C., and De Maria, C. A. B. (2000): Componentes voláteis do café torrado. Parte II: compostos alifáticos, alicíclicos e aromáticos. Química nova., 23(2): 195-203.

Musa, K. H., Abdullah, A., Jusoh, K., and Subramaniam, V. (2011): Antioxidant activity of pink-flesh guava (Psidium guajava L.): effect of extraction techniques and solvents. Food Analytical Methods., 4(1): 100-107.

Mussatto, S. I., Ballesteros, L. F., Martins, S., and Teixeira, J. A. (2011): Extraction of antioxidant phenolic compounds from spent coffee grounds. Separation and Purification Technology., 83: 173-179.

Naczk, M., and Shahidi, F. (2004): Extraction and analysis of phenolics in food. Journal of Chromatography A., 1054(1): 95-111.
Nicoli, M. C., Anese, M., Manzocco, L., and Lerici, C. R. (1997a): Antioxidant properties of coffee brews in relation to the roasting degree. LWT-Food Science and Technology., 30(3): 292-297.
Nicoli, M. C., Anese, M., and Parpinel, M. (1999): Influence of processing on the antioxidant properties of fruit and vegetables. Trends in Food Science & Technology., 10(3): 94-100.
Nicoli, M. C., Anese, M., Parpinel, M. T., Franceschi, S., and Lerici, C. R. (1997b): Loss and/or formation of antioxidants during food processing and storage. Cancer Letters., 114(1): 71-74.
Nissen, L. R., Byrne, D. V., Bertelsen, G., and Skibsted, L. H. (2004): The antioxidative activity of plant extracts in cooked pork patties as evaluated by descriptive sensory profiling and chemical analysis. Meat Science., 68(3): 485-495.
Ozgen, M., Durgaç, C., Serçe, S., and Kaya, C. (2008): Chemical and antioxidant properties of pomegranate cultivars grown in the Mediterranean region of Turkey. Food Chemistry., 111(3): 703-706.
Pérez-Hernández, L. M., Chávez-Quiroz, K., Medina-Juárez, L. Á., and Gámez Meza, N. (2012): Phenolic Characterization, Melanoidins, and Antioxidant Activity of Some Commercial Coffees from Coffea arabica and Coffea canephora. Journal of the Mexican Chemical Society., 56(4): 430-435.
Pinelo, M., Tress, A. G., Pedersen, M., Arnous, A., and Meyer, A. S. (2007): Effect of cellulases, solvent type and particle size distribution on the extraction of chlorogenic acid and other phenols from spent coffee grounds. Am. J. Food Technol., 2: 641-651.
Zia-ur-Rehman. (2006): Citrus peel extract-A natural source of antioxidant. Food Chemistry., 99(3): 450-454.
Tsao, R. (2010): Chemistry and biochemistry of dietary polyphenols. Nutrients., 2(12): 1231-1246.
Routray, W., and Orsat, V. (2012): Microwave-assisted extraction of flavonoids: a review. Food and Bioprocess Technology., 5(2): 409-424.
Singleton, V. L., and Rossi, J. A. (1965): Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture., 16(3): 144-158.
Stalikas, C. D. (2007): Extraction, separation, and detection methods for phenolic acids and flavonoids. Journal of separation science., 30(18): 3268-3295.
Turkmen, N., Sari, F., and Velioglu, Y. S. (2006): Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin–Ciocalteu methods. Food Chemistry., 99(4): 835-841.