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Optimization of Instant Controlled Pressure Drop Dic-Assisted-Solvent Extraction of Total Phenols of Green Coffee Beans

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Abstract

The present work deals with optimization of Instant Controlled Pressure Drop (DIC) assisted solvent extraction of total phenols (TP) from green coffee beans (GCBs). DIC has been used as a specific unit operation for decontamination and texturing with specific impacts on water extraction of bioactive molecules from plant materials. Reflux solvent system up to 97°C,

was used to extract TP from raw and DIC treated GCBs. TP were determined using spectrophotometric Folin–Ciocalteu method. Response surface methodology (RSM) was used to optimize DIC processing conditions P (saturated steam pressure), t (heating time t) and W (initial water content of GCBs). A comparative study confirmed that between various solvent types (water, methanol, ethanol, isopropanol and acetone), methanol was the best solvent for both DIC-treated and untreated GCBs. RSM was used also to optimize the extraction variables (methanol proportion 43-77%, extraction temperature 26.5-68.5°C, and extraction time 20-120 min). The overall results revealed that DIC treatment showed obvious advantages in term of high yield and efficiency to recover polyphenols from GCBs. The optimum of DIC assisted solvent extraction of TP was estimated from RS analysis as ranged up to 20.3% dry basis from beans DIC-treated at (P=0.6 MPa, W = 33% db, and t = 84 s) compared to 7.8% from untreated raw material. Methanol concentration at 43%, extraction temperature at 69°C, and extraction time 120 min were found to be the optimum parameters for TP extraction from the DIC treated GCBs. The remarkable enhanced extraction of TP may be related partly to a greater extent of cell rupture and expansion of the plant material by DIC treatment.

Keywords: Green coffee beans, Total Polyphenols Content, Instant Controlled Pressure Drop, Optimization, Response Surface Methodology (RSM)

1. Introduction

Polyphenols are an important category of compounds; they constitute one of the most widespread groups of substances in plants. Fruit represent the main sources of polyphenols, but vegetables, leguminous plants, and cereals, beverages, such as wine, tea and coffee are also important sources. Polyphenols include wide variety of molecules that contain at least one aromatic ring with one or more hydroxyl groups in addition to other substituents. The importance of polyphenols is due to their positive contribution to cellular processes within the body; they have strong antioxidant nature, which is based on their ability to absorb free radicals. They protect against the oxidation of high-density lipids, while helping to remove problematic low-density lipids (Nawaz et al., 2006), they also have anti-ulcer, anti-carcinogenic, and anti-mutagenic activities (Singh et al., 2009). Polyphenols can be divided into several classes according to the number of phenol rings that they contain and to the structural elements that bind these rings to one another. The main groups of polyphenols are: flavonoids, phenolic acids, phenolic alcohols, stilbenes and lignans (Tapiero et al., 2002; Antolovich et al., 2000). A number of phenolics are linked to polysaccharides in the cell wall of plant materials, especially pectins. Hence, their availability is based on the degradation and release of phenolics (Rani and Appaiah, 2012).

Polyphenols correct determination is pivotal nowadays and involves their extraction from the sample, analytical separation, identification, quantification and interpretation of the data. Conventional heat-reflux extraction methods using water or organic solvents are generally used to extract polyphenols from plant materials. Assisted extraction processes that use innovative technologies such microwaves (Upadhyay et al., 2012; Biswas et al., 2012), ultrasound (Adje et al., 2010) and DIC (Kamal et al., 2012) along with solvent are recently also used. The Assisted-extraction method provided short duration and high efficiency to recover the total polyphenols in comparison with conventional heat-reflux extraction. High voltage electrical discharges could also accelerate polyphenol extraction (Boussetta et al., 2009).

Wide range of analytical methods can be used to determine polyphenols from plant materials. The most used techniques have been chromatographic techniques such as Reverse Phase HPLC (Hecimovic et al., 2011; Alves et al., 2010; Mnatsakanyan et al., 2010), capillary electroseparation methodologies (Hurtado-Fernández et al., 2010), cyclic voltammetry (Paul et al., 2003), spectroscopic methods (Stecher et al., 2003) and immobilized enzyme-based biosensors (Narang et al., 2011).

Coffee is a functional beverage (Esquivel and Jimenez, 2011), it the third most widely consumed beverage in the world, after water and tea (Villanueva et al., 2006). Coffee is generally processed in many ways, which include fermentation of berries and roasting of seeds. These processes influence the final concentration of polyphenols in coffee beverage. However, highest antioxidant activity was observed in coffee brews obtained from medium and light roasted coffee (Hecimovic et al., 2011; Vignoli et al., 2011). Dark roasting, showed to lead to oxidation and thermal degradation of phenolic compounds. Phenolics degradation results in the formation of compounds which show lower antioxidant activity than the

originals (Sacchetti et al., 2009). Nevertheless, independently of manufacture process, phenolic compounds are mainly found in green coffee beans (GCBs) as chlorogenic acids (up to 12% of solids) (Clifford, 2000; Ky et al., 2001; Duarte et al., 2010; Alonso-Salces et al., 2009; belitz et al., 2009), and the final beverage contains consistently high concentration of chlorogenic acid, 5-*O*-caffeoylquinic acid (5-CQA), and its isomers 3- and 4-CQA, accompanied by various related compounds (Ferrazzano et al., 2009; Ferruzzi, 2010).

On the other hand, DIC is a controlled high temperature and short time pretreatment process has been applied successfully to food and non-food materials, it showed promising results in improving kinetics of some processes including drying, extraction and decontamination and to get better functional and organoleptic quality (Allaf et al., 2010; Besombes et al., 2009).

In previous studies, we have employed the DIC process on green coffee beans as a pre-treatment-texturing step prior to roasting. Remarkable expansion characteristics were detected in the treated beans, which make them lighter and more porous (Kamal et al., 2008). The technology was used also for caffeine extraction, and new solvent-free environmentally decaffeination process has been defined through direct extraction, and completed decaffeination from aqueous extraction was estimated at 99.5% compared to 58% extracted from the untreated raw beans (Kamal et al., 2012).

The objective of this study was to detect the effect of the DIC treatment on total phenol extraction from green coffee beans. The quality of the extracts was monitored through determination of the contents of polyphenols. It is in this context that Response Surface Methodology was used to model the effect of DIC parameters on total phenolic content, and to optimize the extraction conditions.

2. Materials and Methods

2.1 Reagents and chemicals

All the chemicals used in this work were of analytical grade obtained from Fluka Chemie GmbH, Buchs, Switzerland.

2.2 Plant material

The plant material used was Ethiopian green coffee beans (GCBs) purchased from local market. The beans were grinded to 7-mm particles, and then soaked in water bath at ambient temperature to reach predefined values of water content. Moisture content was measured using the oven method. The measurements were triplicated and conducted on a 3 g of GCBs, placed in a thin layer glass capsule and dried in the oven at 105.5°C for 24 h. The moisture content was determined between the initial raw material value of 11.00%±0.2% db (dry basis: g H_2O/100 g dry matter) and 35±0.2% db for the most soaked beans.

2.3 Treatment protocol

The treatment used in this study is illustrated in Figure 1.

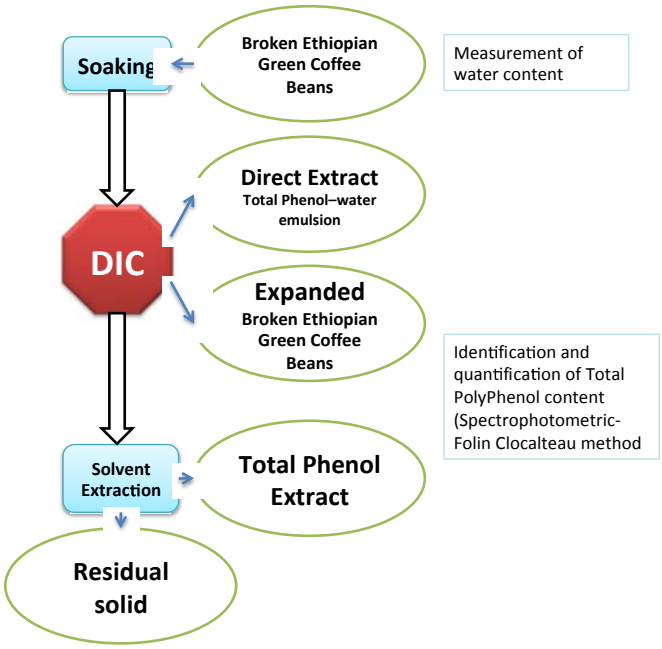

Figure 1. Treatment protocol and assessments of green coffee beans through instant controlled pressure drop DIC-assisted solvent extraction process

2.3.1 Instant Controlled Pressure Drop DIC treatment and equipment

DIC reactors have been presented in numerous articles (Besombes et al., 2010; Kamal and Allaf, 2011; Ben Amor et al., 2009). In the present work, the reactor used was a new developed version of DIC apparatus (Micro-DIC provided from ABCAR-DIC Process, La Rochelle, France). It is a 30 cc processing vessel, a 7-liter vacuum tank connected with a water ring vacuum pump allowing the pressure to reach 5 kPa. A pneumatic valve ensures an "instant" connection between the vacuum tank and the processing vessel; it can open in less than 50 ms. Other valves control the flow of steam and compressed air within the processing vessel. DIC process is usually carried out by establishing high-pressure / high temperature saturated steam (0.1 to 0.7 MPa relating with 100 to 165°C during 5 to 60 s). This is followed by an instant pressure drop towards a vacuum at about 5 kPa at a rate $\Delta P/\Delta t$ higher than 0.5 MPa s^{-1}.

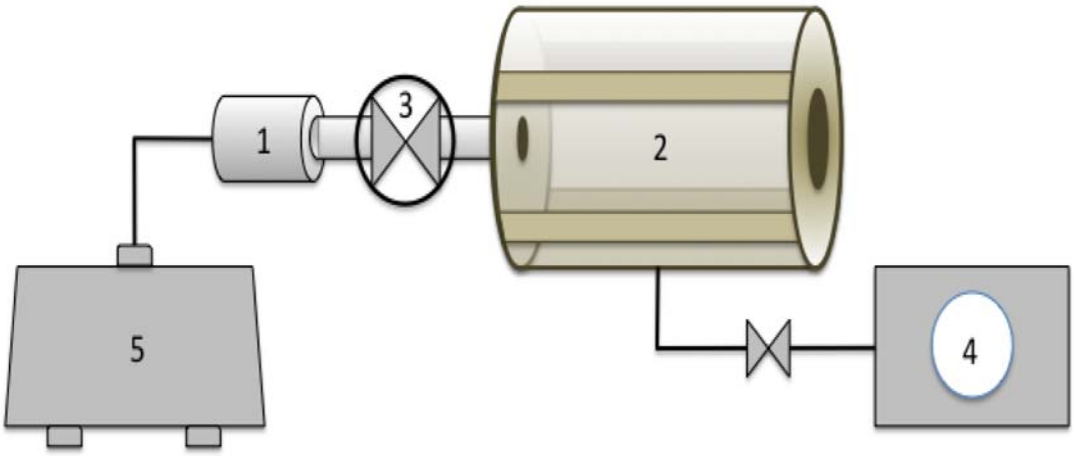

Figure 2. Schematic presentation of DIC reactor: 1- treatment vessel; 2- vacuum tank with transparent Jacket; 3- controlled instant pressure drop valve; 4- water ring vacuum pump; 5- steam generator

Preliminary experiments were performed to define the range of various operating parameters. In a second stage, DIC extraction was carried out using a specific experimental design to identify and compare the effects of saturated steam pressure, process (heating) time and initial moisture content, chosen as operating parameters (or independent variables). The extracted product (direct extract) was collected as an emulsion of water and other compounds from the vacuum tank. The amount of total phenols present in this emulsion was quantified.

2.3.2 Solid–Liquid extraction procedure

Reflux water distillation–extraction was used as a solid-liquid extraction technique to extract TPC from raw and GCBs treated previously by DIC. A mixture of 1 g of concerned beans, distilled water (20 ml) was heated inside a round button flask (50 ml) using a thermostatic bath set at 97°C. The reflux distillation set-up was provided by magnetic agitation to ensure that the concentration of the extracted beans and the temperature of the solution were uniform within the flask. The crude extracts were filtered immediately after 20 min extraction using R-C membrane filter 0.45 µm (Sartorius Stedim Biotech GmbH/Germany). The amount of total phenols present in the aqueous extracts was quantified using UV-Visible spectroscopy.

The collective TPC value (from direct extract and the corresponding aqueous extract) from each sample was considered as the main response for quantifying the performances of the DIC-assisted extraction process.

2.4 Assessment methods

2.4.1 Determination of water content

The water content was determined by drying three samples (3 g of raw material) in drying oven at 105.5°C until a constant weight was obtained.

2.4.2 Determination of Total Phenols Content (TPC)

The level of total phenols in the crude extracts was determined using spectrophotometric Folin–Ciocalteu method according to (Lapornik et al. 2005) with some modifications using gallic acid as standard. In the Folin-Ciocalteu method, the phenols are oxidized by a mixture of two strong inorganic oxidants, phosphotungstic and phosphomolybendic acids, found in the Folin-Ciocalteu reagent. This results in the production of a complex molybdenum-tungsten blue colored solution. The phenols oxidize rapidly in the alkaline solution, which first converts them to phenolate ions.

0.5 ml of diluted samples were added into test tubes followed by 2.5 ml of Folin-Ciocalteu reagent (10%, v/v). After 30 s to 5 min, 2 ml of 20% of sodium carbonate solution was added. All test tubes with the mixture were caped and shaken for 10 s and put on to incubation in a water bath at 45^{o}C for 5 minutes. Absorbance was measured after 30 min at 765 nm (Helios Omega UV/VIS Thermo Scientific Merk and Co. Spectrophotometer) against blank sample.

Blank sample was prepared with water instead of the extract. Determination of total phenolic compounds was carried out in a triplicate and calculated from the calibration curve obtained with Gallic acid, which was used as standard. The calibration equation for Gallic acid (R^2=0.9955) is shown in equation 1:

$$y = 0.0092x+0.0144 \quad \text{Eq.1}$$

The results were expressed as milligrams of Gallic acid equivalents (GAE) per g of dry base GCBs (mg GA eq./g db).

2.5 Experimental design

Response Surface Methodology RSM was used to optimize both DIC treatment, and TPC solvent extraction. In each case, a three-factor five-level (−α, −1, 0, 1, α) rotatable central composite experiment design was defined with:

- Factorials points (2^n=2^3): 8 points (-1/-1/-1; -1/-1/+1; -1/+1/-1; -1/+1/-1; +1/-1/-1; +1/-1/+1; +1/+1/-1; +1/+1/-1)
- Star points (2*n): 6 points (-α/0/0; + α/0/0; 0/-α/0; 0/+α/0; 0/0/-α; and 0/0/+α)
- Eight repetitions of the central points (0,0,0)

It results in 22 experimental trials. The value of α (axial distance) depending on the number of parameters considered (n) is calculated as $\alpha = \sqrt[4]{2^n} = 2^{3^{0.25}} = 1.681792831$.

The three DIC-independent variables were the saturated steam pressure (P in MPa), the heating time (t in s), and the water content of GCBs (W in %db). According to preliminary experiments, they ranged 0.1/0.6 MPa, 14/86 s, and 11%/35% db, respectively. The other operating parameters such as the final vacuum pressure, the double jacket temperature, etc. were kept constant.

The three independent variables of aqueous/methanol solvent extraction were defined according to the bibliography (Naidu et al., 2008), and through the results obtained from preliminary experiments. They were: the solvent ratio, which was methanol/water proportion:

S, the extraction temperature: T (°C), and the extraction time: t (min). They were ranged between (43-77 %), (26.5-68.5°C), and (20–120 min), respectively. The liquid-to-solid ratio (14 ml/g) and the particle size (0.40–0.60 mm) were kept constant.

The two experimental designs are shown in Table 1 and 2

3. Results and discussion

3.1 TPC assay

Results of TPC assay were expressed as Gallic acid equivalent (i.e. x mg of Gallic acid per 1 g db of Coffee beans). TPC of each sample was calculated from calibration curve of Gallic acid (Eq.1), where the calibration equation was determined to be, whereby y = absorbance at 765 nm and x = concentration of total phenolic compounds in mg per 1 g db green coffee beans. Total phenols content values of water extracts and solvent extracts of raw and DIC treated coffee beans are listed in table 1 and table 2 respectively.

Table 1. Independent variables and their coded and natural levels employed in a 5-level central composite design and the response values of total phenols content (TPC) of green coffee beans water extracts

Trial N°	DIC parameters						Response
	Saturated steam pressure P		Water content dry basis W		processing time t		TPC (mg GA eq./g db)
	Coded level	Natural level (MPa)	Coded level	Natural level (% db)	Coded level	Natural level (s)	
1	+α	0.6	0	23.00%	0	50	158.19±3.4
2	0	0.35	0	23.00%	+α	84	119.52±5.8
3	0	0.35	0	23.00%	0	50	104.93±3.9
4	1	0.5	1	30.00%	1	70	156.93±3.3
5	0	0.35	+α	35.00%	0	50	71.72± 2.4
6	0	0.35	0	23.00%	0	50	106.10±2.1
7	1	0.5	1	30.00%	-1	30	110.41±3.5
8	1	0.5	-1	16.00%	1	70	115.87±2.7
9	0	0.35	0	23.00%	0	50	102.74±3.4
10	1	0.5	-1	16.00%	-1	30	111.92±2.6
11	-1	0.2	1	30.00%	1	70	69.08±3.1
12	0	0.35	0	23.00%	0	50	104.71±2.9
13	-1	0.2	1	30.00%	-1	30	64.75±2.1
14	-1	0.2	-1	16.00%	1	70	72.25±2.4
15	0	0.35	0	23.00%	0	50	105.56±4.3
16	0	0.35	-α	11.00%	0	50	67.78±2.6
17	-α	0.1	0	23.00%	0	50	29.23±2.2
18	0	0.35	0	23.00%	0	50	100,34±2.6
19	-1	0.2	-1	16.00%	-1	30	78.56±3.1
20	0	0.35	0	23.00%	-α	16	56.10±2.5
21	0	0.35	0	23.00%	0	50	102.71±3.8
22	0	0.35	0	23.00%	0	50	102.14±3.4
Raw mat.				11.00%			77.99±2.8

The statistical analysis of the results was carried out using the analysis design procedure of Statgraphics Plus software for Windows (1995, version 5.1, Levallois-Perret, France). Variance (ANOVA) was performed to determine significant differences between independent variables ($P \leq 0.05$). Experimental data were analyzed to fit a second order polynomial model. Model and regression coefficient were considered significant when p-values were lower than 0.05. The experimental design with response values of TPC is shown in table 1 and the response analysis of TPC is shown in Figure 3.

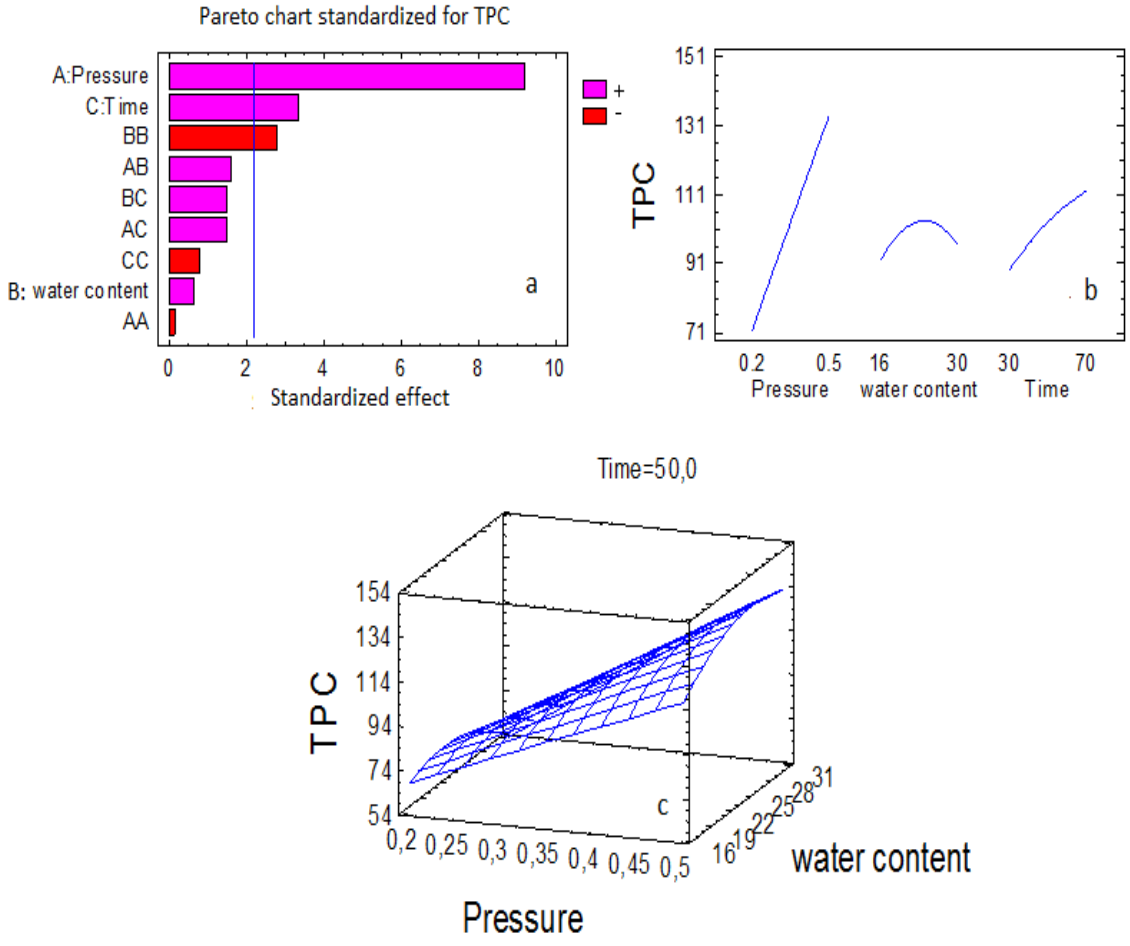

Figure 3. Pareto chart (a), general trends (b), and response surface (c) of DIC operating parameters in the treatment of GCBs to intensify the aqueous extraction of TPC

Multiple linear regressions using the second-order polynomial model (Eq. 2) were performed on the results of Table 1. Good fits were achieved and most of the responses' variability was explained by the model; the coefficient of multiple determination (R^2) being 90.27 for total phenols content. The polynomial empirical model estimated is as the following:

$$TPC=28.23-42.74P+3.91W-0.666t-18.1285P^2+2.185Pt-0.1812W^2+0.0475Wt-0.0062t^2 \quad \text{Eq.2}$$

Variables in their coded form (table 1) permitted a direct interpretability of the effects (linear, quadratic, and interaction) of the independent variables, and the surface plots (figure. 3) facilitated the visualization of the statistically significant factors. The analysis was statistically significant and suggested that the parameters of the model can explain the

experimental variation for TPC in relation to the average response.

Regarding processing pressure and heating time, linear effects were verified to be statistically significant with positive response for TPC, while water content linear effect seemed to be not significant. Nevertheless, quadratic effect of water content was significant. The negative quadratic effect of water content for TPC, indicating that there is a maximum in the TPC extraction at a certain water content of GCBs and the yield in TP starts to decrease above that water content value.

TPC experimental results for the DIC treated beans showed that total phenolic contents from the crude extracts of the DIC treated GCBs were ranged up to 15.82% at (P=0.6 MPa, W=23% db, t=50 s) compared to 7.80 from the aqueous extract of the raw untreated beans. Meanwhile, an optimum TPC value of 20.33% could be achieved when optimizing the DIC processing conditions at DIC processing parameters (P=0.6 MPa, W=33% db, t=84 s) as reflected from the Response surface analysis results. The RSM analysis results of TPC of water extracts confirmed that there could be an increase of TPC yield of about 261% for the DIC treated green coffee beans compared to the untreated raw material.

3.2 Optimization of solvent extraction parameters of total phenols from DIC treated GCBs

In the present work, optimization of solvent extraction of total phenols was carried out using GCBs treated previously by DIC at relatively moderate conditions (P=0.2 MPa, W=16%, t=30 s). The conditions were selected and employed in order to prove that even moderate DIC processing variables could enhance remarkable extractability of TP from GCBs. TPC values estimated represent TP in crude solvent extracts as listed in Table 2.

Table 2. Independent variables and their coded and natural levels employed in a 3-variable 5-level central composite design to optimize the effect of extraction variables on total phenols content (TPC) from solvent extracts of DIC treated GCBs (P=0.2 MPa, W= 16%, t= 30 s)

Trial N°	Extraction parameters						Response
	Solvent proportion (MeOH)		Extraction time		Extraction temperature		TPC (mg GA eq./g db)
	Coded level	Natural level %	Coded level	Natural level min.	Coded level	Natural level °C	
1	+α	77	0	70	0	47.5	79.89±2.6
2	0	60	0	70	+α	68.5	95.73±3.0
3	0	60	0	70	0	47.5	90.43±3.2
4	1	70	1	100	1	60	88.23±2.2
5	0	60	+α	120	0	47.5	75.37±2.8
6	0	60	0	70	0	47.5	90.36±2.7
7	1	70	1	100	-1	35	77.18±1.3
8	1	70	-1	40	1	60	79.12±2.1
9	0	60	0	70	0	47.5	90.62±2.2
10	1	70	-1	40	-1	35	68.91±2.8
11	-1	50	1	100	1	60	139.03±3.5
12	0	60	0	70	0	47.5	90.30±3.1
13	-1	50	1	100	-1	35	100.77±2.4
14	-1	50	-1	40	1	60	65.29±2.9
15	0	60	0	70	0	47.5	90.49±2.7
16	0	60	-α	20	0	47.5	47.19±2.2
17	-α	43	0	70	0	47.5	111.95±3.1
18	0	60	0	70	0	47.5	90.43±2.3
19	-1	50	-1	40	-1	35	61.45±2.1
20	0	60	0	70	- α	26.5	84.61±2.9
21	0	60	0	70	0	47.5	90.43±3.1
22	0	60	0	70	0	47.5	90.43±3.3

3.2.1 Effect of Solvent Type

The extraction of polyphenols is dependent upon two actions, the dissolution of each polyphenolic compound at the cellular level in the plant material matrix, and their diffusion in the external solvent medium. Extractability of the solvent mainly depends on the solubility of the compounds in the solvent system, the mass transfer kinetics of the product and the strength of the solute/matrix interactions (Upadhyay et al., 2010). Many of the phenolic compounds of coffee are reported to be soluble in polar solvents and the choice of solvents depends on the number of hydroxyl groups of the phenolics. Accordingly, methanol, ethanol, propanol, acetone, ethyl acetate, dimethylformamide and their combinations has been generally used for extraction of polyphenols (Naczk and Shahidi, 2006). However, alcohols were found to be more efficient than water in extraction of polyphenols from different plant materials, but methanol was the best solvent for polyphenols extraction. (Adil et al., 2007; Mussatto et al., 2011).

DIC treated GCBs were treated in different solvent mixtures (water, 60% methanol, 60% ethanol, 60% isopropanol and 60% acetone) at 14 ml/g db for 2 hours at 22.5± 0.3°C. The

crude solvent extracts were filtered and then evaluated spectrochemically for TPC assay. The results estimated are illustrated in Figure 4; they confirmed that aqueous methanol offered the highest TPC yield (10.48%); followed by aqueous acetone (9.72%), aqueous ethanol (7.91%), aqueous isopropanol (7.44%), and by water (6.32%). Methanol seemed to be the best for polyphenols extraction from GCBs. These results perfectly corroborate those reported by Adil et al. (2007), and Mussatto et al. (2011).

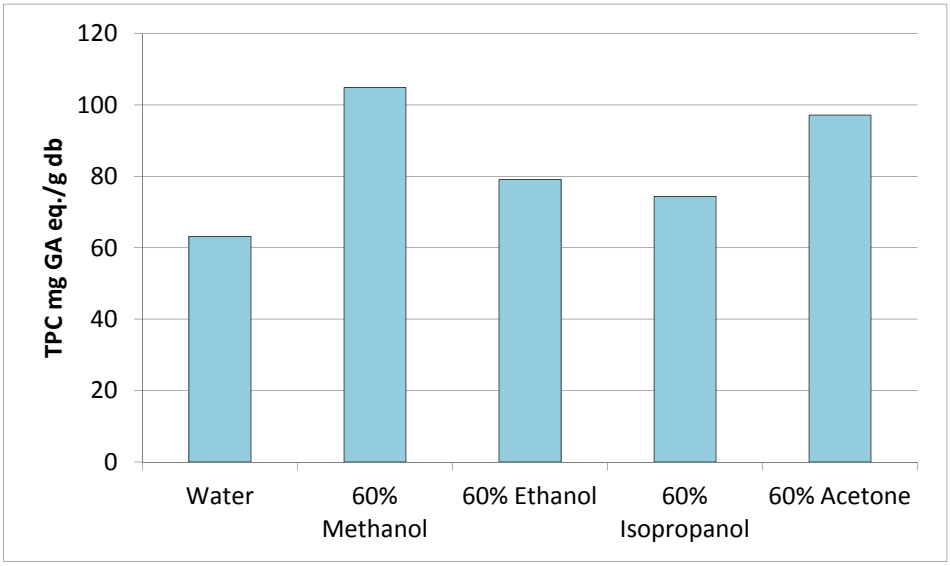

Figure 4. TPC DIC-assisted extraction of Total Phenol from expanded coffee beans with different solvents

This behavior can be explained by the fact that phenolic compounds are often more soluble in organic solvents less polar than water. However, it was reported that low TPC (0.16%) was recovered from spent coffee grounds using 60% methanol in a solvent/solid ratio of 40 ml/g dry weight material, during 90 min (Mussatto et al., 2011), while higher TPC was estimated from defatted Arabica and Robusta green coffee beans (31.7–32.2%) as was explored by (Naidu et al., 2008) when isopropanol and water in ratio of 60:40 was employed at room temp for 5 hours.

According to our results, aqueous methanol has been chosen as solvent for the experiments carried out to optimize the extraction process of total phenols.

3.2.2 Optimization of TPC extraction parameters using RSM

Multiple linear regressions using the second-order polynomial model (Eq. 3) were performed on the results of Table 2. The model was established with high coefficient of determinations, (R^2) being 0.9181 for total phenols content, which means a close agreement between the experimental results and those predicted by the model. The model could efficiently be used for a rapid prediction of the extraction results to be achieved when using methanol concentration, extraction time and temperature in the range of values here studied.

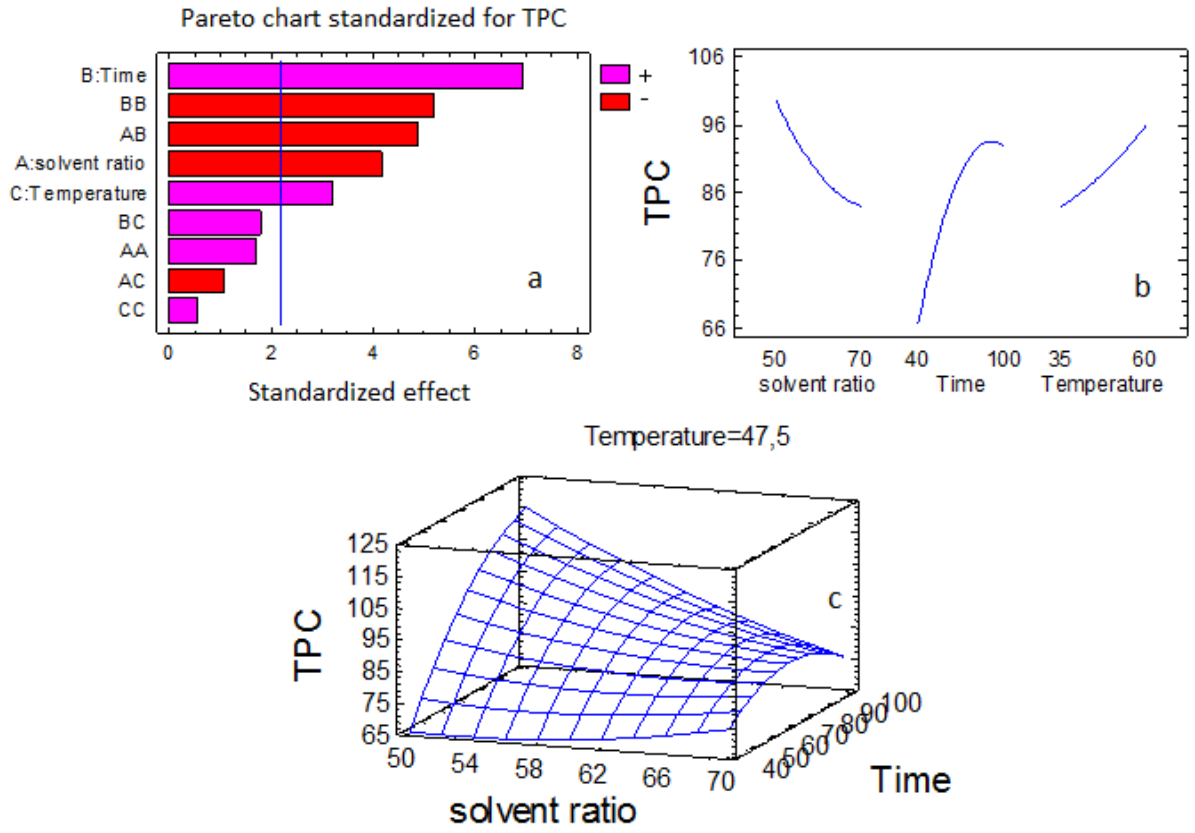

Figure 5. Pareto chart of DIC extraction parameters for TPC (a), general trends (b), and response surface (c) for TPC from crude extracts of DIC treated GCBs

The software generated the following regression equation which demonstrates the empirical relationship between solvent (MeOH) concentration (S), time (t), and temperature (T):

$$TPC=-30.506-0.655S+3.689t+0.291T+0.0304S^2-0.0398St-0.021ST-0.012T^2+0.0118tT+0.006t^2 \quad \text{Eq.3}$$

The analysis was statistically significant and suggested that the three parameters of the model can explain the experimental variation for TPC in relation to the average response.

Regarding the methanol proportion, linear effects were verified to be statistically significant for TPC. A negative effect of methanol proportion was obtained for TPC, indicating that the TPC extracted decreases with increasing % methanol. Using 43% methanol as solvent for polyphenols recovery seemed to produce the optimum TPC yield.

A linear positive effect was interestingly found for a positive effect of extraction temperature, and a negative effect of solvent ratio, both on TPC as response. The negative effect of methanol proportion tended generally to increase TPC at lower levels of methanol proportion.

Regarding the effect of extraction temperature, a linear positive effect was detected for TPC, which confirms that the increase in temperature improves the phenolic yields. Similar trend was observed in extraction of total phenols from other plant materials (Bucić-Kojic et al., 2007; Silva et al., 2007; Pompeu et al., 2009). The increase in temperature may favor the

extraction by enhancing the solubility of the phenolic compounds, increasing the diffusion coefficient, and decreasing the viscosity coefficient (Liyana-Pathirana and Shahidi, 2005). Raising the temperature of the extractant might soften the tissues and weaken the phenol–protein and phenol–polysaccharide linkages, leading to migration of the polyphenols into the solvent.

Indeed, a higher temperature increases the solubility and diffusion coefficient of polyphenols allowing higher extraction rate. However, an upper limit must however be respected to avoid degradation of thermo-sensitive polyphenols. The oxidation, hydrolysis and isomerization, which are the most widely suggested degradation pathways for phenolics, are usually accelerated when the phenols are subjected to higher temperatures (Pinelo et al., 2008). A positive quadratic effect of extraction temperature may indicate that the polyphenols increases with the increase in extraction temperature theoretically up to 100%.

On the other hand, the influence of temperature on extraction rate of TPC was evaluated throughout extraction of predetermined amounts of the DIC treated GCBs with 60% Methanol at different temperatures (26.5, 35, 47.5, and 68.5°C) for 70 min.

By assuming the solvent extraction kinetics as a first order reaction, the extraction of TPC would evolve as:

$$\frac{TPC_{\infty}-TPC}{TPC_{\infty}} = exp(-kt)$$

Eq. 4

$$LN\left(\frac{TPC_{\infty}-TPC}{TPC_{\infty}}\right) = -kt$$

Eq.5

Thus, the temperature influence would give a relationship between the initial TPC extraction rate k and extraction temperatures as was assessed by linearized Arrhenius equation:

$$k = -k_o exp\left(-\frac{E_A}{RT}\right)$$

Eq.6

Where E_a (kJ mol^{-1}) is the activation energy of the reaction, R the universal gas constant (8.3145 J mol^{-1} K^{-1}), T the temperature (K) and k_o the frequency factor (min^{-1}) the pre-exponential constant.

Table 2. Temperature effect on TPC solvent extraction: 0,7 g of DIC treated GCBs extracted by 10 ml (methanol/water) 60:100 for 70 min at different temperatures

Temperature°C	TPC (mg Ga/g db)	Ln TPC	TPC ratio	LN(TPC ratio)	k (min^{-1})	LN(k)
26,5	84,6	4,44	0,39	-0,94	0,0134	-4,31
35	86,87	4,46	0,376	-0,98	0,0140	-4,27
47,5	90,02	4,50	0,352	-1,04	0,0149	-4,21
68,5	95,7	4,56	0,312	-1,17	0,0167	-4,09

The corresponding Arrhenius plot is illustrated in

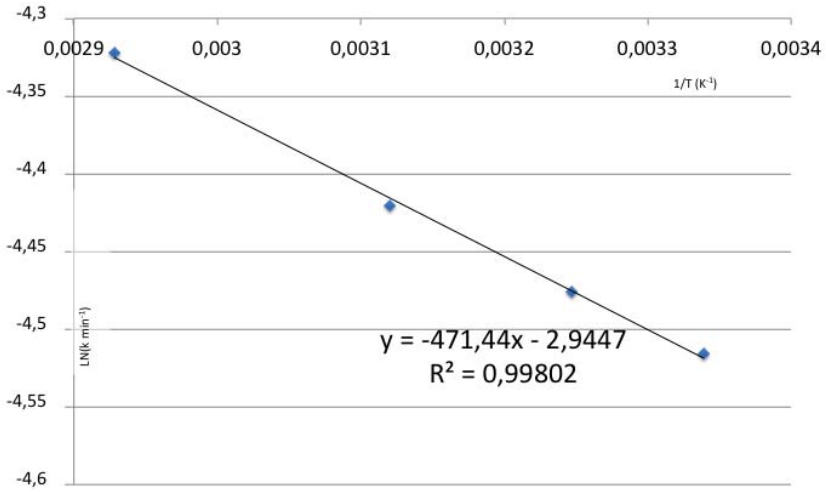

Figure 6 with TPC_{∞}=158.19 mg Ga/g db. The results implied that extraction rate tends to increase with temperature. A correlation coefficient of 0.998 was obtained. The activation energy of the extraction process within the temperature range (26.5-68.5°C) was 3.92 kJ/mole.

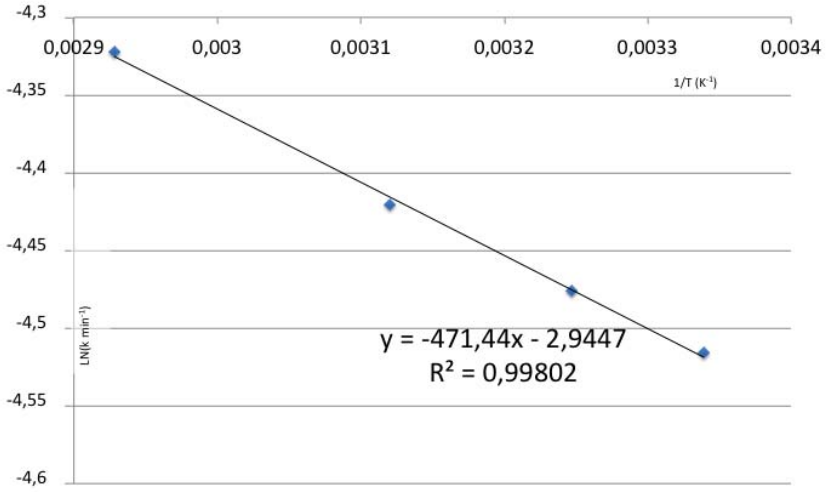

Figure 6. Arrhenius plot illustrates the effect of temperature on TPC extracted from DIC treated GCBs

The RSM results of DIC treated GCBs showed that optimum total phenols content of 17.4% could be extracted using as solvent a water:methanol (57%:43%) solution for 120 min at

68.5°C, compared to 13.9% estimated experimentally using 50% methanol for 100 min at 60°C.

4. Conclusions

The data presented in this manuscript unambiguously demonstrates that DIC pretreatment of green coffee beans leads to increase twice more the yield of total phenols extracted than that from untreated raw material. The results also confirmed that optimization of the extraction parameters (solvent proportion, time and temperature) is critical for accurate extraction of TPC. Precise optimizations of the DIC operating variables and extraction parameters allowed us to recover the maximum of phenols content from green coffee beans. The overall results obtained confirmed that DIC treatment had obvious advantages in terms of high efficiency to recover polyphenols from green coffee beans; TPC optimum could be achieved when extract the GCBs with 43% methanol for 120 min at 68.5°C. The remarkable enhanced extraction of TPC may be related partly to a greater extent of cell rupture and expansion of the plant material by DIC treatment.

References

Adil, I.H., Çetin, H.I., Yener, M.E., & Bayindirl,i A. (2007). Subcritical (carbon dioxide+ethanol) extraction of polyphenols from apple and peach pomaces, and determination of the antioxidant activities of the extracts. *J. Supercrit. Fluids, 43(*1), 55–63. http://dx.doi.org/10.1016/j.supflu.2007.04.012

Adjé, F., Lozano, Y.F., Lozano, P., Adima, A., Chemat, F., & Gaydou, E.M. (2010). Optimization of anthocyanin, flavonol and phenolic acid extractions from Delonix regia tree flowers using ultrasound-assisted water extraction. *Industrial Crops and Products, 32*(3), 439-444. http://dx.doi.org/10.1016/j.indcrop.2010.06.011

Allaf, K., Besombes, C., Berka- Zougali, B., Kristiawan, M., Sobolik, V., & Allaf, T.S.V. (2011). Instant controlled pressure drop (DIC) technology in plant extraction processes. In: "Enhancing Extraction Processes in the Food Industry". (PP. 255-302) Series on "Contemporary Food Engineering", Taylor & Francis–CRC Press, London (UK), ISBN: 9781439845936.

Alonso-Salces, R.M., Guillou, C., & Berrueta, L.A. (2009). Liquid chromatography coupled with ultraviolet absorbance detection, electrospray ionization, collision-induced dissociation and tandem mass spectrometry on a triple quadrupole for the on-line characterization of polyphenols and methylxanthines in green coffee beans. *Rapid Communications in Mass Spectrometry, 23*(3), 363–383. http://dx.doi.org/10.1002/rcm.3884

Alves, R.C., Almeida, I.M.C., Casa,l S., & Oliveira, M.B.P.P. (2010). Method development and validation for isoflavones quantification in coffee. *Food Chemistry, 122*(3), 914-919. http://dx.doi.org/10.1016/j.foodchem.2010.03.061

Antolovich, M., Prenzler, P., Robards, K., & Ryan, D. (2000). Sample preparation in the determination of phenolic compounds in fruits. *Analyst, 125*(5), 989–1009. http://dx.doi.org/10.1039/B000080I

Belitz, H.-D., Grosch, W., & Schieberle, P. (2009). *Food chemistry,* (4th ed.), Springer, Heidelberg-Germany (Chapter 21).

BenAmor, B., & Allaf, K. (2009). Impact of texturing using instant pressure drop treatment prior to solvent extraction of anthocyanins from Malaysian Roselle (Hibiscus sabdariffa). *Food Chemistry, 115*(3), 820-825. http://dx.doi.org/10.1016/j.foodchem.2008.12.094

Biswas, A., Sutivisedsak, N., Cheng, H.N., Willett, J.L., Lesch, W.C., & Tangsrud, R.R. (2012). Extraction and analysis of antioxidant capacity in eight edible beans. *International Journal of Food Agriculture and the Environment, 10*(1), 89-96.

Boussetta, N., Lanoisellé, J., Bedel-Cloutour, C., & Vorobiev, E. (2009). Extraction of soluble matter from grape pomace by high voltage electrical discharges for polyphenol recovery: Effect of sulphur dioxide and thermal treatments. *Journal of Food Engineering, 95*(1), 192-198. http://dx.doi.org/10.1016/j.jfoodeng.2009.04.030

Bucić-Kojić, A., Planinić, M., Tomas, S., Bilić, M., & Velić, D. (2007). Study of solid–liquid extraction kinetics of total polyphenols from grape seeds. *Journal of Food Engineering, 81*(1), 236-242. http://dx.doi.org/10.1016/j.jfoodeng.2006.10.027

Clifford, M.N. (2000). Chlorogenic acids and other cinnamates: nature, occurrence, dietary burden, absorption and metabolism. *J Sci Food Agric, 80*(7), 1033–1043. http://dx.doi.org/10.1002/(SICI)1097-0010(20000515

Duarte, G.S., Pereira, A.A., & Farah, A. (2010). Chlorogenic acids and other relevant compounds in Brazilian coffees processed by semi-dry and wet post-harvesting methods. *Food Chemistry, 118* (3), 851-855. http://dx.doi.org/10.1016/j.foodchem.2009.05.042

Esquivel, P., & Jimenez, V.M. (2011). Functional properties of coffee and coffee by-products. Food Research International. (online) available. http://www5.ocn.ne.jp/~ajca/cosic23.6-20.pdf (June 12, 2011).

Ferrazzano, G.F., Amato, I., Ingenito, A., De Natale, A., & Pollio A. (2009). Anti-cariogenic effects of polyphenols from plant stimulant beverages (cocoa, coffee, tea). *Fitoterapia, 80*(5). 255-262. http://dx.doi.org/10.1016/j.fitote.2009.04.006

Ferruzzi, M.G. (2010). The influence of beverage composition on delivery of phenolic compounds from coffee and tea. *Physiol Behav., 100*(1), 33-41. http://dx.doi.org/10.1016/j.physbeh.2010.01.035

Hečimović, I., Belščak-Cvitanović, A., Horžić, D., & 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. http://dx.doi.org/10.1016/j.foodchem.2011.05.059

Kamal, I., Gelicus, A., & Allaf, K. (2012). Impact of instant controlled pressure drop (DIC) treatment on drying kinetics and caffeine extraction from green coffee beans. *Journal of food research, 1*(1), 24- 47. http://dx.doi.org/10.5539/jfr.v1n1p24

Kamal, I.M., Sobolik, V., Kristiawan, M., Mounir, S.M., & Allaf, K. (2008). Structure expansion of green coffee beans using instantaneous controlled pressure drop process, *Innovative Food Science & Emerging Technologies, 9*(4), 534-541. http://dx.doi.org/10.1016/j.ifset.2008.01.004

Ky, C.L., Louarn, J., Dussert, S., Guyot, B., Hamon, S., & Noirot, M. (2001). Caffeine, rigonelline, chlorogenic acids and sucrose diversity in wild Coffea arabica L. and C. canephora P. ccessions. *Food Chem, 75*(2), 223–230. http://dx.doi.org/10.1016/S0308-8146(01)00204-7

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

Liyana-Pathirana, C., & Shahidi, F. (2005). Optimization of extraction of phenolic compounds from wheat using response surface methodology. *Food Chem., 93*(1), 47–56. http://dx.doi.org/10.1016/j.foodchem.2004.08.050

Mazzafera P., & M.B. Silvarolla. (2010). Caffeine content variation in single green Arabica coffee seeds. *Seed Science Research, 20* (3), 163–167. http://dx.doi.org/10.1017/S0960258510000140

Mnatsakanyan, M., Goodie, T.A., Conlan, X.A., Francis, P.S. McDermott, G.P., Barnett, N.W., Shock, D., Gritti, F., Guiochon, G., & Shalliker, R.A. (2010). High performance liquid chromatography with two simultaneous on-line antioxidant assays: Evaluation and comparison of espresso coffees. *Talanta, 81*(3). 837-842. http://dx.doi.org/10.1016/j.talanta.2010.01.024

Mussatto, S.I., Ballesteros, L.F., Martins, S., & Teixeira, J. A. (2011). Extraction of antioxidant phenolic compounds from spent coffee grounds. *Separation and Purification Technology, 83*(11), 173-179. http://dx.doi.org/10.1016/j.seppur.2011.09.036

Naczk, M., & Shahidi, F. (2006). Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *Journal of Pharmaceutical and Biomedical Analysis, 41*(5), 1523-1542. http://dx.doi.org/10.1016/j.jpba.2006.04.002

Naidu, M.M., Sulochanamma, G., Sampathu, S.R., & Srinivas, P. (2008). Studies on extraction and antioxidant potential of green coffee. *Food Chemistry, 107*(1), 377-384. http://dx.doi.org/10.1016/j.foodchem.2007.08.056

Napolitano, A., Fogliano, V., Tafuri, A., & Ritieni, A. (2007). Natural occurrence of Ochratoxin A and antioxidant activities of green and roasted coffees and corresponding byproducts. *Journal of Agricultural and Food Chemistry, 55*(25), 10499–10504. http://pubs.acs.org/doi/pdf/10.1021/jf071959%2B.

Narang, J., Chauhan, N., Singh, A., & Pundir, C.S. (2011). A nylon membrane based amperometric biosensor for polyphenol determination. *Journal of Molecular Catalysis B: Enzymatic, 72*(3-4). 276-281. http://dx.doi.org/10.1016/j.molcatb.2011.06.016

Nawaz, H., Shi, J., Mittal, G. S., & Kakuda, Y. (2006). Extraction of polyphenols from grape seeds and concentration by ultrafiltration. *Sep. Purif. Technol, 48*(2). 176-181. http://dx.doi.org/10.1016/j.seppur.2005.07.006

Paul, A. Kilmartin, & Chyong, F. Hsu. (2003). Characterisation of polyphenols in green, oolong, and black teas, and in coffee, using cyclic voltammetry. *Food Chemistry, 82*(4). 501-512. http://dx.doi.org/10.1016/S0308-8146(03)00066-9

Pinelo, M., Zornoza, B., & Meyer, A.S.(2008). Selective release of phenols from apple skin: Mass transfer kinetics during solvent and enzyme-assisted extraction . *Separation and Purification Technology, 63*(3), 620-627. http://dx.doi.org/10.1016/j.seppur.2008.07.007

Pompeu, D.R., Silva, E.M., & Rogez, H. (2009). Optimisation of the solvent extraction of phenolic antioxidants from fruits of Euterpe oleracea using Response Surface Methodology. *Bioresource Technology, 100*(23). 6076-6082. http://dx.doi.org/10.1016/j.biortech.2009.03.083

Rani, M.U., Appaiah, K.A.A., Hurtado-Fernández, E., Gómez-Romero, M., Carrasco-Pancorbo, A., & Fernández-Gutiérrez, A. (2012). Gluconacetobacter hansenii UAC09-mediated transformation of polyphenols and pectin of coffee cherry husk extract. *Food Chemistry, 130*(2), 243-247. http://dx.doi.org/10.1016/j.foodchem.2011.07.021

Sacchetti, G., Mattia, C. D., Pittia, P., & , D. (2009). Effect of roasting degree, equivalent thermal effect and coffee type on the radical scavenging activity of coffee brews and their phenolic fraction. *Journal of Food Engineering, 90*(1), 74-80. http://dx.doi.org/10.1016/j.jfoodeng.2008.06.005

Santini A., Ferracane R., Mikušová P., Eged S., Šrobárová A., Meca G., Mañes J., & Ritieni A. (2011). Influence of different coffee drink preparations on ochratoxin A content and evaluation of the antioxidant activity and caffeine variations. *Food Control, 22*(8), 1240-1245. http://dx.doi.org/10.1016/j.foodcont.2011.01.024

Silva, E.M., Rogez, H., & Larondelle, Y . (2007). Optimization of extraction of phenolics from Inga edulis leaves using response surface methodology . *Separation and Purification Technology,. 55*(3), 381-387. http://dx.doi.org/10.1016/j.seppur.2007.01.008

Singh, B.N., Singh, B.R., Singh, R.L., Prakash , D.,. Singh, D.P, Sarma, B.K., Upadhyay ,G., & Singh, H.B. (2009). Polyphenolics from various extracts/fractions of red onion (Allium cepa) peel with potent antioxidant and antimutagenic activities. *Food and Chemical Toxicology, 47*(6), 1161–1167. http://dx.doi.org/10.1016/j.fct.2009.02.004

Stecher, G., Huck, C.W., Stöggl, W.M., & Bonn, G.K. (2003). Phytoanalysis: a challenge in phytomics. *Trands in Analytical Chemistry, 22*(1), 1–14. http://dx.doi.org/10.1016/S0165-9936(03)00108-0

Tapiero, H., Tew, K.D., Ba, G.N., & Mathé, G. (2010). Application and potential of capillary electroseparation methods to determine antioxidant phenolic compounds from plant food

material. *Journal of Pharmaceutical and Biomedical Analysis, 53*(5), 1130-1160. http://dx.doi.org/10.1016/j.jpba.2010.07.028

Upadhyay, R., Ramalakshmi, K., & Rao, L.J.M. (2010). Microwave-assisted extraction of chlorogenic acids from green coffee beans. *Food Chemistry, 130*(1), 184-188. http://dx.doi.org/10.1016/j.foodchem.2011.06.057

Vignoli, J.A., Bassoli, D.G., & Benassi, M.T. (2011). Antioxidant activity, polyphenols, caffeine and melanoidins in soluble coffee: The influence of processing conditions and raw material. *Food Chemistry, 124*(3), 863-868. http://dx.doi.org/10.1016/j.foodchem.2010.07.008