European Journal of Medicinal Plants

23(1): 1-16, 2018; Article no.EJMP.40565
ISSN: 2231-0894, NLM ID: 101583475

Effect of Brewing Methods and Time on Secondary Metabolites, Total Flavonoid and Phenolic Content of Green and Roasted coffee *Coffea arabica*, *Coffea canephora* and Monsooned Malabar

Mansimran Kaur[1], Shivani Tyagi[2] and Namrata Kundu[1*]

[1]Helix BioGenesis Pvt Ltd, Noida, U.P., India.
[2]Mewar University, Mewar, Rajasthan, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author NK designed the study, performed the statistical analysis, wrote the protocol, and first draft of the manuscript. Authors MK and ST managed the analyses of the study. Author MK managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2018/40565
Editor(s):
(1) Ana Maria Queijeiro Lopez, Professor, Institute of Chemistry and Biotechnology, Federal University of Alagoas, Brazil.
(2) Marcello Iriti, Professor, Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Rafael Carlos Eloy Dias, Instituto Federal Catarinense, Brazil.
(2) Supreena Srisaikham, Burapha University Sakaeo Campus, Thailand.
(3) Socaci Sonia, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania.
(4) Mary Ann Foglio, Brasil.
(5) Esraa Ashraf Ahmed ElHawary, Ain Shams University, Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history/24181

Original Research Article

Received 31st January 2018
Accepted 12th April 2018
Published 16th April 2018

ABSTRACT

Coffea arabica, Coffea canephora (robusta) and monsooned malabar are the three types of coffee which are well known and used by most of population in India and all over the globe. For consuming coffee various brewing methods are used worldwide, French press, Espresso, Turkish coffee, being the common. Eleven brewing methods are introduced in the paper for brewing of green coffee, some of which are common for brewing tea.

Among all the mentioned brewing methods, Decoction method showed the best results, as maximum amount of Flavonoids and Phenols were found to be present in green coffee arabica, values being 69.24 mg QE/g of coffee and 108.67 mg QE/g of coffee respectively, whereas, for

**Corresponding author: E-mail: knamrata511@gmail.com;*

robusta it was found to be 47.47 mg QE/g of coffee and 93 mg QE/g of coffee respectively. Brewing time is also considered as one of the major factors for coffee brewing, as if the time is too short, all the flavours will not dissolve and if too long, it may dissolve undesirable components as well. An increasing trend was seen in flavonoid and phenolic content in both arabica (TPC up to 84.11 mg QE/g of coffee) and robusta (TPC up to 78 mg QE/g of coffee) along with monsooned malabar (TPC up to 84.88 mg QE/g of coffee) with increase in brewing time. Another factor considered is the solvent used for brewing. A comparison was made between water and alcohol. Both the coffees, green and roasted showed a significant difference in the values when brewed in ethanol and in water.

Results indicate that the brewing methods given may be used along with a variant brewing time depending on its phenolic content. Also, green coffee can be proved a boon with more health benefits as compared to roasted one.

Keywords: Green coffee; roasted coffee; phytochemicals; phenols; antioxidants.

ABBREVIATIONS

TPC : Total Phenolic Content
TFC : Total Flavonoid Content
QE : Quercetin Equivalent
GAE : Gallic Acid Equivalent
a : Absent
p : Present

1. INTRODUCTION

An evergreen arbour, Coffee, is a member of the Rubiaceae family, is derived from the name of the province 'Keffa", where shepherds from Abyssinia/Ethiopia discovered it in 6th century. Since then this beverage is one of the most consumed worldwide due to the pleasant taste, aroma, stimulant effect and health benefits [1-3]. Coffee plants consist of several species such as *Coffea canephora, Coffea liberica, Coffea excels* and *Coffea stenophylla* [4,5]. The most cultivated varieties are *Coffea arabica* (arabica) and *Coffea canephora* (robusta), which are used for commercial production, accounting for about 60% and 40% of the world coffee market, respectively, while *Coffea liberica* contributes less than 1% of marketed coffee [6-8]. Arabica and robusta may appear similar but there are a number of significant differences between them. Arabica requires different environmental conditions and produces less coffee per hectare than robusta, increasing the growth cost. They even differ in caffeine and chlorogenic acid content. Then there is a process that is applied to coffee beans, called monsooned malabar, these harvested coffee seeds are exposed to the monsoon rain and winds for a period of about three to four months, causing the beans to swell and lose the original acidity, resulting in a flavour profile with a practically neutral pH balance.

For many people, especially in western countries, coffee drinking is a part of their lifestyle and morning habit. Morning cup of coffee is a daily routine for millions of people worldwide, about 40% of the world's population starts the day this way [9,10]. Popularity of drinking of roasted or instant coffee infusions is a result of its stimulating effects, exceptional smell and taste [11].

The coffee that we know is produced by processing of the green coffee beans in several stages. Green coffee refers to raw or unroasted seeds (beans) of *Coffea* fruits [12-14]. The infusions are believed to accelerate metabolism, thus they can be helpful in reducing weight and preventing or overcoming obesity [15].

The main constituents of green coffee beans are carbohydrates (up to 50% of the dry weight), both soluble (galactomannan, arabinogallactan) and insoluble in water (cellulose), phenolic species, i.e. caffeine, cholorogenic acids, in addition to polysaccharides, proteins, polyphenols, melanoids, lipids and minerals [11, 16-,20]. However, the chemical composition and the biological activity of green coffee are highly affected by roasting [14]. In the process of roasting chlorogenic acids are particularly degraded as well the content of the phenolic compounds decrease along with the antioxidant activity [21-23].

Secondary metabolites from plants have important biological and pharmacological activities, such as anti-oxidative, anti-allergic, antibiotic, hypoglycemic and anti-carcinogenic [24-27]. Largest group of phytochemicals comprises the phenolic chemicals. Plant polyphenols are secondary metabolites that are widely distributed in higher plants [28]. The most important dietary phenolics are phenols acids,

polyphenols and flavonoids, the latter being the most studied group [29]. The flavonoid subclass of phenols includes minor flavonoids (flavonones and dihydroflavonols), flavones and flavonols [28]. Flavonoids have a benzo-γ-pyrone structure and are synthesized by phenylpropanoid pathway. Available reports tend to show that flavonoids are responsible for the variety of pharmacological activities [30,31], but the best described property of almost every group of flavonoids is their capacity to act as antioxidants. The antioxidant activity of flavonoids depends upon the arrangement of functional groups about the nuclear structure [32,33].

Brewing techniques and time have a great impact on the content of polyphenols. The difference in the presence of phytochemical in different methods depends upon steeping time of the infusion, type of species, type of solvent and temperature [34].

Although, the brewing time is given in the standard coffee brewing techniques used all over the world, the knowledge of extraction behaviour of the main coffee oxidants during time and various methods might induce to the technological factors with major impact on antioxidants extraction. There is no narrative data referring to the content and the effect of the method of preparing coffee infusions and time, on amount of phenolic and antioxidant content. Therefore these parameters can impact not only in coffee brews with higher antioxidant capacity but also coffee extracts with health properties that can be used as ingredients in functional foods. Thus, an attempt was made to establish a correlation among different brewing methods, brewing time and phenolic content in infusions.

1.1 Objective

Present study aimed to give various brewing methods for brewing of green coffee *Coffea arabica* and *Coffea canephora.* To give the best method among the prescribed brewing methods on the basis of its total flavonoid and phenolic content. Study was also focussed on the importance of brewing time and solvent used for brewing giving their phytochemical analysis and phenolic content.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Green and roasted coffee beans of *Coffea arabica, Coffea canephora* and roasted monsooned malabar were bought online from Amazon. The roasted arabica beans and roasted robusta beans were from Coorg (Karnataka) and Travancore (Kerala) respectively. Both were freshly roasted on the day before delivery. The monsooned malabar were the homestead spices from Western Ghats region of Kerala.

Samples collected were identified and authenticated Dr. Ravindra Kumar, Associate Professor of Plant Biotechnology, Botany Department, Hindu College, Delhi University, India.

2.1.1 Brewing Methods implemented for green coffee

Green coffee beans of coffee *Coffea arabica* and *Coffea canephora* were grounded to make powder in a regular grinder (Philips HL 7720) and used for all methods except for 10th i.e. overnight brewing for which coffee beans were directly used. Coffee infusions were prepared by following various recipies:

1. **Soft Infusion:** 1 g of coffee powder was weighed in weighing balance (High Precision Balance) added to 15 ml of distilled water and kept at 75-80℃ in Water bath (Thermo Scientific) for 3-5 minutes [35].
2. **Hard Infusion:** 1g of coffee powder was infused in 15ml of distilled water and kept 75-80℃ for 25-30 minutes [11].
3. Ambient Infusion: This method involves infusion of 1 g of coffee powder in 15 ml of distilled water at room temperature for 30-40 minutes [34].
4. **Cold Infusion:** 1 g of coffee powder was infused in distilled water at room temperature for 15 minutes and then refrigerated at 8-10℃ in Refrigerator (Thermo Scientific) for an hour [34].
5. **Decoction Method:** 1 g of coffee powder was added to 15ml of distilled water and boiled for 3-4 minutes and then cooled down for 30 seconds [34].
6. **Chilled Green Coffee:** 1 g of coffee powder was brewed in distilled boiling water for 2-5 minutes and was then kept in refrigerator at 8-10℃ for an hour [34].
7. **Cold Cocktail:** In this, 1 g of coffee powder was infused in 40% ethanol for 15 minutes and then filtered. Filtered infusion was refrigerated for an hour at 8-10℃ [34].
8. **Hot Cocktail:** In this alcoholic infusion, 1 g of coffee powder was added to 40%

ethanol and kept at room temperature for 15 minutes followed by heating up to 50-55℃ [34].

9. **Pour-over Method:** 1 g of coffee powder was directly kept on filter paper; hot water was poured over it and collected.
10. **Overnight Brewing:** 10 g of coffee beans were soaked in 150 ml of distilled water overnight. Mixture was boiled on high flame. After on boil, it was simmered on low flame for 15 minutes giving occasional stir. Mixture was cooled completely and filtered to remove beans.
11. **French Press:** For this, a press pot or a coffee plunger device (KMX Coffee Plunger) was used. The pot was placed on a flat surface, plunger was pulled out and 1 g of coffee powder was added and boiling hot water was gently poured inside. Then the plunger was reinserted in to the pot on the surface of the coffee beverage and plunged down after 5 minutes. Once the press plunger was put down, sample of coffee was taken for analysis [11].

2.1.2 Preliminary phytochemical screening for the presence of secondary metabolites

Both water and alcohol (ethanol) were used as solvents for the preparation of sample infusions for screening of phytochemicals. Whereas, time given for brewing was 1, 2, 4, 8 and 16 minutes. Only infusion with brewing time of 1 min and 16 min were screened phytochemically for the presence and absence of secondary metabolites (both green and roasted coffee) and thereafter Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were measured (green coffee).

1. **Test for Saponins: Foam Test:** To 1 ml (Pipette: Gilson) of brewed sample, 3ml of distilled water was added and shaken vigorously for 2 minutes. Frothing persist on warming indicates the presence of saponins [36].
2. **Test for Tannins: Ferric chloride Test:** To 1 ml of sample few drops of 1% $FeCl_3$ solution were added. Occurrence of blue black, green or blue green precipitate indicates the presence of tannins [37].
3. **Test for Steroids: Liebermann – Burchard Test:** To 1 ml of sample, 2-3 ml of chloroform was added and then equal volume of H_2SO_4 was added carefully along the sides to form two different layers. Turning of upper layer red and yellow green fluorescence at sulphuric acid layer indicates the presence of steroids [38].
4. **Test for Flavonoids: NaOH Test:** 2 ml 10% NaOH was added to 1 ml of sample. Intense Yellow colour obtained which changes to colourless on adding 1% HCl indicates the presence of flavonoids [39].
5. **Test for Terpenoids: Liebermann – Burchard Test:** To 1 ml of sample, 2-3 ml of chloroform was added and then equal volume of H_2SO_4 was added carefully along the sides to form two different layers. Formation of reddish-violet colour indicates the presence of Terpenoids [38].
6. **Test for Naphthoquione:** To 1 ml of sample, a few drops of 10% KOH were added. Formation of blue-black colour indicates presence of Naphthoquione.
7. **Test for Inulin:** To 1 ml of sample α-napthol and H_2SO_4 solution was added. Formation of brownish colour indicates the presence of Inulin.
8. **Test for Alkaloids: Wagner's Test:** 2 g of Iodine and 6 g of Potassium Iodide were dissolved in 100 ml of distilled water and stored in dark. To 1ml of solvent, 2ml of the prepared solution was added. Formation of brown and reddish brown colour indicates the presence of Alkaloids [40].
9. **Test for Phenols: Ferric chloride Test:** To 1 ml of sample 5 ml of distilled water and 1-2 drops of 1% $FeCl_3$ were added. A red blue-green or purple colour indicates the presence of Phenols [41].

2.1.3 Preparation of standard solution

1 g of Quercetin was dissolved in 100 ml of methanol separately to get 1% solution of Quercetin (10 mg/ml) termed as standard solution.

2.1.4 Determination of total flavonoid content (TFC)

Total flavonoids of the infusions were determined by using aluminium chloride spectrophotometric analysis [42] with slight modifications. 100 µl of the samples were raised up to 1 ml and then diluted by 2 ml of methanol. Mixture was mixed with 0.1 ml of 10% (w/v) aluminium chloride solution and 0.1 ml of 1 M potassium acetate solution. The mixture was kept at room temperature for 30 minutes. Then the maximum absorbance was measured at 415 nm using UV-

Visible spectrophotometer (Hitachi High Technologies). TFC was calculated from the calibration curve, and the results were expressed as mg of Quercetin equivalent per g weight [43].

2.1.5 Determination of total phenolic content (TPC)

Total phenolics were determined by using Folin-Ciocalteau reagent [44] with slight modifications. 100 µl of the coffee infusions were made up to 1ml with distilled water, mixed thoroughly with 2.5ml of 20% (w/v) sodium carbonate for 10 min, followed by the addition of 200 µl of Folin-Ciocalteau reagent. The mixture was allowed to stand further for 30 min in dark at room temperature and absorbance was measured at 750nm using UV-Visible spectrophotometer. TPC was calculated from the calibration curve, and the results were expressed as mg of Quercetin equivalent per g weight [45].

2.2 Statistical Evaluation

To estimate the accuracy of the experimental data, each experiment was performed in triplicates, and the result was expressed as the mean ± standard deviation of three replications. $P < 0.05$ was considered as statistically significant.

3. RESULTS AND DISCUSSION

Arabica coffee (*Coffea arabica*) and robusta coffee (*Coffea canephora*) have different growing conditions and chemical composition, and thereafter organoleptic properties, particularly taste and smell, of their beans and infusions are also different [46].

Green and roasted coffee *Coffea arabica* and *Coffea canephora* and roasted monsooned malabar coffee were brewed for different times i.e. 1, 2, 4, 8 and 16 minutes in distilled water and ethanol. The coffees brewed for 1 minute and 16 minute were initially screened phytochemically to detect the presence and absence of different secondary metabolites.

Phytochemicals are various active compounds found in plants which have been used in a variety of industrial as well as commercial applications, since synthetic compounds do have side effects. Phytochemicals of plants show different biological activities, which play an important role in the protection against chronic diseases [47].

Phytochemical screening was done to observe the presence of secondary metabolites like saponins, tannins, terpenoids, steroids, napthoquione, inulin, flavonoids, alkaloids and phenols. Initially saponins were found to be absent in both green coffee arabica and robusta regardless of the solvent in which it was brewed but it was present in roasted robusta and roasted monsooned malabar brewed in water for 1 min and 16 min. However roasted robusta and roasted malabar in ethanol gave no positive results for saponins and roasted arabica brewed for 16 min in water was positive for the same. Tannins and Inulin were present in all the samples for both the solvents. Steroids were seen positive in all the brews except green coffee robusta brewed in ethanol for both the times and also in roasted monsooned malabar brewed in ethanol for 1 minute. Terpenoids were found to be absent in all the samples with no exceptions. However napthoquione showed an irregular trend as it was completely absent in both green coffee arabica and robusta brewed in water and ethanol. It was present in roasted coffee arabica brewed for 1 min in water and ethanol but was absent in 16 - minute brew of both. The roasted coffee robusta and roasted malabar brewed for 1 and 16 min in water were seen positive for napthoquione but the same brewed in ethanol showed no results. Previous studies suggest that tannins, terpenoids, flavonoids and alkaloids were present in green coffee, while saponins were absent in the sample under investigation [48].

Alkaloids, flavonoids and phenols were present in all the samples. Infact, green coffee robusta brewed in distilled water showed a double positive for all the three metabolites. Also the yellow colour of the precipitate in the test for flavonoids was even more intense in coffees brewed for 16 mins in distilled water than the ones brewed for 1 minute. This gave us the idea of increase in flavonoid content with increase in brewing time. Also same trend was seen in phenols. Therefore, we further subjected all the coffees i.e. green and roasted coffee arabica and robusta along with roasted monsooned malabar for quantitative analysis of TFC and TPC (Table 2- Table 11).

In plants, there are about 8000 known phenolic compounds with aromatic ring produced as secondary metabolites. They protect the plant against pathogens and abiotic stress such as changes in temperature, water content, exposure to UV light and deficiency of mineral nutrients.

The level of individual phenolic compounds normally depends on the maturity of the beans and a smaller degree, on the composition of the soil, climatic conditions and agricultural practices related to the coffee bush [17,49].

Total flavonoids content and total phenolic content was determined by spectrophotometric analysis using aluminium chloride and sodium carbonate respectively. Quercetin was used as the standard for both (Figs. 5 and 6).

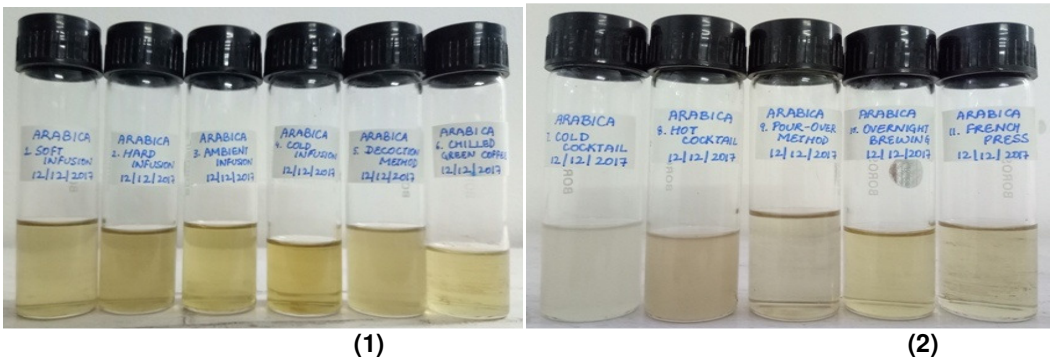
Different brewing methods were performed for green coffee arabica and robusta. These infusions were then subjected to determine the total flavonoids and phenols (Figs. 1 and 2).

For green coffee arabica, the decoction method was reported to have the maximum flavonoid content of 69.24 mg QE/g of coffee, second being the chilled green coffee of value 61.65mg QE/g of coffee. Lowest being the pour over method i.e. 7.53 mg QE/g of coffee. Rest of the methods lying in between these two were having flavonoid content in the range of 14-36 mg QE/g of coffee. Similar trend was seen in the phenolic content with decoction method having the maximum of 108.67 mg QE/g of coffee, chilled green coffee being the next having 101 mg QE/g of coffee and pour over method having the lowest of value 72.78 mg QE/g of coffee (Table 1 and Fig. 2).

Same brewing methods were performed for green coffee robusta, but the values seen were quite different from green coffee arabica, the best being overnight brewing method with value of 57 mg QE/g of coffee. Next value being 53.23 mg QE/g of coffee for hard infusion method. However lowest flavonoid content was seen in cold cocktail method i.e. 10.35 mg QE/g of coffee and the pour over method being very close to it with value 10.59 mg QE/g of coffee. The phenolic content was obviously more than the flavonoid content but had a different trend. The overnight brewing method and chilled green coffee had similar value of 93.33 mg QE/g of coffee. There was a slight difference in the values of cold cocktail method and pour over method i.e. 71.89 mg QE/g and 71 mg QE/g of coffee respectively (Table 1 and Fig. 2).

Table 1. TFC and TPC of various brewing methods implemented in green coffee *Coffea arabica* and *Coffea canephora*

Brewing methods	Total flavonoid content (mg QE / g of coffee)		Total phenolic content (mg QE / g of coffee)	
	Arabica	Robusta	Arabica	Robusta
Soft Infusion	27.18	27.65	94	78.33
Hard Infusion	35.76	53.23	96	89.11
Ambient Infusion	27.47	21.3	93	77
Cold Infusion	27.53	24.76	89.78	77.44
Decoction method	69.24	47.47	108.67	93
Chilled Green Coffee	61.65	45.24	101	93.33
Cold Cocktail	13.59	10.35	83.56	71.89
Hot Cocktail	25.77	11.65	92.44	73.78
Pour over method	7.53	10.59	72.78	71
Overnight brewing	22.06	57	88.67	93.33
French Press	29.94	18.18	100.78	74.11



(1) **(2)**

Fig. 1. (1) and (2) Representing the green coffee extracts of arabica prepared after all the eleven brewing methods implemented

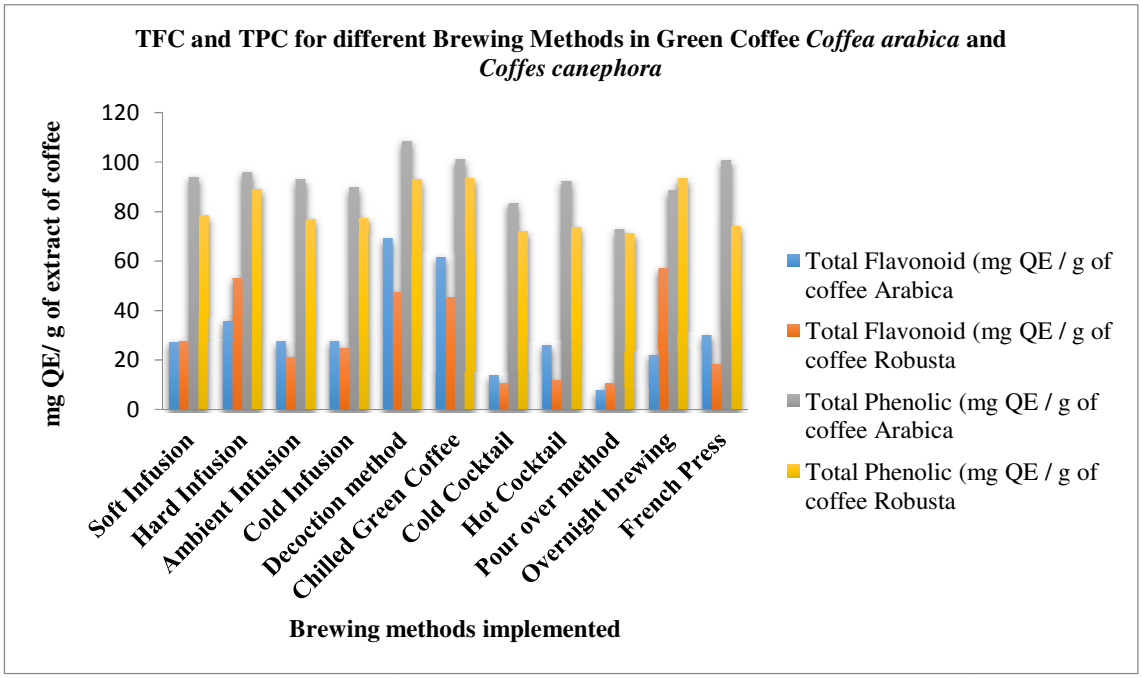


Fig. 2. TFC and TPC in various brewing methods followed for *Coffea arabica* and *Coffea canephora*

It is important that the brewing time must be exactly controlled. Improper brewing time can be one of the reasons that people get different results while preparing coffee. If the extraction time is shortened, we'll fail to dissolve the essential flavour compounds. Again, over-extraction of the same will dissolve too many of the undesirable compounds [50].

Table 2. Results showing Phytochemical screening for the presence of mentioned secondary metabolites of green coffee arabica brewed in distilled water for 1 and 16 minutes

Secondary metabolite	1 min	16 min
Saponins	a	a
Tannins	p	p
Steroids	p	p
Flavonoids	p	p
Terpenoids	a	a
Napthoquione	a	a
Inulin	p	p
Alkaloids	p	p
Phenols	p	p

There is important exception to "exact brewing time". About 90% of the solubles are extracted during the early phase of the brewing process [50].

The amount of time that the water is in contact with the coffee grounds is another important flavour factor. In a drip system, the contact time should be approximately 5 minutes. If one is making your coffee using a French Press, the contact time should be 2-4 minutes. Espresso has an especially brief brew time - the coffee is in contact with the water for only 20-30 seconds. Cold brew, on the other hand, should steep overnight (about 12 hours) [51].

Table 3. Results showing phytochemical screening for the presence of mentioned secondary metabolites of green coffee arabica brewed in ethanol for 1 and 16 minutes

Secondary metabolite	1 min	16 min
Saponins	a	a
Tannins	p	p
Steroids	p	p
Flavonoids	p	p
Terpenoids	a	a
Napthoquione	a	a
Inulin	a	p
Alkaloids	p	p
Phenols	p	p

After different brewing methods for green coffee arabica and robusta, all the coffees including

green and roasted coffee arabica, robusta and roasted monsooned Malabar were brewed for 1, 2, 4, 8 and 16 minutes in distilled water and ethanol and then subjected for quantification of flavonoids and phenols.

Table 4. Results showing phytochemical screening for the presence of mentioned secondary metabolites of green coffee robusta brewed in distilled water for 1 and 16 minutes

Secondary metabolite	1 min	16 min
Saponins	a	a
Tannins	p	p
Steroids	p	p
Flavonoids	p	p
Terpenoids	a	a
Napthoquione	a	a
Inulin	p	p
Alkaloids	p	p
Phenols	p	p

Table 5. Results showing phytochemical screening for the presence of mentioned secondary metabolites of green coffee robusta brewed in ethanol for 1 and 16 minutes

Secondary metabolite	1 min	16 min
Saponins	a	a
Tannins	p	p
Steroids	a	a
Flavonoids	p	p
Terpenoids	a	a
Napthoquione	a	a
Inulin	p	p
Alkaloids	p	p
Phenols	p	p

Table 6. Results showing phytochemical screening for the presence of mentioned secondary metabolites of roasted coffee arabica brewed in distilled water for 1 and 16 minutes

Secondary metabolite	1 min	16 min
Saponins	p	a
Tannins	p	p
Steroids	p	p
Flavonoids	p	p
Terpenoids	a	a
Napthoquione	p	a
Inulin	p	p
Alkaloids	p	p
Phenols	p	p

An increasing trend was seen in the flavonoid and phenolic content and with increase in time of brewing with distilled water. However, brewing in ethanol showed different results. It seemed to increase first and then started decreasing, with 4 min brewing showing the maximum value in case of roasted coffee, but for green coffee the trend was same as observed with distilled water.

Table 7. Results showing phytochemical screening for the presence of mentioned secondary metabolites of roasted coffee arabica brewed in ethanol for 1 and 16 minutes

Secondary metabolite	1 min	16 min
Saponins	p	a
Tannins	p	p
Steroids	p	p
Flavonoids	p	p
Terpenoids	a	a
Napthoquione	p	a
Inulin	p	p
Alkaloids	p	p
Phenols	p	p

Table 8. Results showing phytochemical screening for the presence of mentioned secondary metabolites of roasted coffee robusta brewed in distilled water for 1 and 16 minutes

Secondary metabolite	1 min	16 min
Saponins	p	p
Tannins	p	p
Steroids	p	p
Flavonoids	p	p
Terpenoids	a	a
Napthoquione	p	p
Inulin	p	p
Alkaloids	p	p
Phenols	p	p

TPC and TFC might be related with antioxidant activity [52]. Green coffee arabica exhibited more amounts of flavonoids and phenols than green coffee robusta. However roasted coffee robusta showed more amounts of polyphenols than roasted coffee arabica for brewing in distilled water, while reverse in case of brewing with ethanol as a solvent. As far as green and roasted coffee of the same species is compared, roasted coffee gave the presence of more phenols as compared to green, when brewed in distilled water. But, using ethanol as a brewing solvent showed more presence of phenols in case of green than in roasted.

Table 9. Results showing phytochemical screening for the presence of mentioned secondary metabolites of roasted coffee robusta brewed in ethanol for 1 and 16 minutes.

Secondary metabolite	1 min	16 min
Saponins	a	a
Tannins	p	p
Steroids	p	p
Flavonoids	p	p
Terpenoids	a	a
Napthoquione	a	a
Inulin	p	p
Alkaloids	p	p
Phenols	p	p

Flavonoids content varied from 11.9 to 34.5 mg QE/g of coffee and 21.1 to 35.3 mg QE/g of coffee for green and roasted coffee *Coffea canephora* respectively (Table 10 and 15) (Fig. 8 and 10), when brewed in distilled water. Same trend was observed for the phenolic content i.e. 62.8 to 78 mg QE/g of coffee and 83 to 92.5 mg QE/g of coffee for green and roasted robusta coffee respectively. When solvent was changed from distilled water to alcohol, the trend in flavonoid and phenolic content was reversed. TFC varied from 6.5 to 10.1 mg QE/g of coffee for green coffee robusta and 3.6 to 7.6 mg QE/g of coffee for roasted coffee robusta. In the present study, TPC was found to be present in more amounts and increased with time in green coffee robusta than roasted coffee robusta when brewed in alcohol as a solvent i.e. 63.3 to 68.9 mg QE/g of coffee. Whereas, for roasted coffee robusta, a decreasing trend was found for brewing in ethanol i.e. 64.6 to 55.5 mg QE/g of coffee which was quite different from TPC in ethanolic extract of arabica green coffee from Toraja, Lintong, and Mandailing were 28.31, 13.74, and 70.55 g GAE/100 g [52]. In another study, it was reported that TPC in arabica coffee which were extracted using isopropanol-water (80:20), (70:30), (60:40) were 23.29, 28.71, 32.19% GAE, while TPC in robusta coffee were 22.89, 26.19, 31.71% GAE. TFC in the present study demonstrated that ethanolic arabica coffee extract of Toraja, Lintong and Mandailing were 3.57, 3.60, 5.86 g QE/100 g and their TCC 0.17, 0.28, 0.24 g BE/100 g respectively [53].

As far as *Coffea arabica* was kept into consideration, initially the brewing trend with distilled water was same as seen in *Coffea canephora*. Both TFC and TPC were found to be present more for roasted coffee arabica, being 19.5 to 35.1 mg QE/g of coffee and 82 to 88 mg QE/g of coffee respectively as compared to green coffee, where both TFC and TPC increased with brewing time i.e. 18.9 to 40.1 mg QE/g of coffee and 71.6 to 83.4 mg QE/g of coffee respectively. When brewed in ethanol for the given time intervals, both flavonoid and phenolics were found to be more in green coffee then in roasted coffee. Flavonoids in green and roasted coffee when brewed in ethanol were increased from 10.9 to 14.7 mg QE/g of coffee and 3.8 to 8.2 mg QE/g of coffee respectively. Phenolic content for green coffee was found to be maximum when brewed for 2 mins, and afterwards it decreased to 79.8 mg QE/g of coffee and then it was found to be 84.1 mg QE/g of coffee. Therefore, no consistent trend was observed. In case as roasted arabica coffee, the phenolic content was found to be increase from 1 min to 4 min i.e. 66 to 71.7 mg QE/g of coffee, and then it decreased to 63.3 mg QE/g coffee with increase in time to 16 mins (Table 14 and 16) (Figs. 9 and 11).

Table 10. Results showing phytochemical screening for the presence of mentioned secondary metabolites of roasted coffee monsooned malabar brewed in distilled water for 1 and 16 minutes

Secondary Metabolite	1 min	16 min
Saponins	p	p
Tannins	p	p
Steroids	p	p
Flavonoids	p	p
Terpenoids	a	a
Napthoquione	p	p
Inulin	p	p
Alkaloids	p	p
Phenols	p	P

Table 11. Results showing phytochemical screening for the presence of mentioned secondary metabolites of roasted coffee monsooned malabar brewed in ethanol for 1 and 16 minutes

Secondary metabolite	1 min	16 min
Saponins	a	a
Tannins	p	p
Steroids	a	p
Flavonoids	p	p
Terpenoids	a	a
Napthoquione	a	a
Inulin	p	p
Alkaloids	p	p
Phenols	p	p

Table 12. TFC and TPC variation in roasted coffee monsooned malabar with time and solvents.

Time	Water		Ethanol	
	TFC	TPC	TFC	TPC
1 min	17.11	71.55	3.58	60
2 min	20.76	71.88	4.82	64.66
4 min	21.52	73.33	5	65.66
8 min	25.58	84.55	7	64.55
16 min	35.35	84.88	9.23	61.77

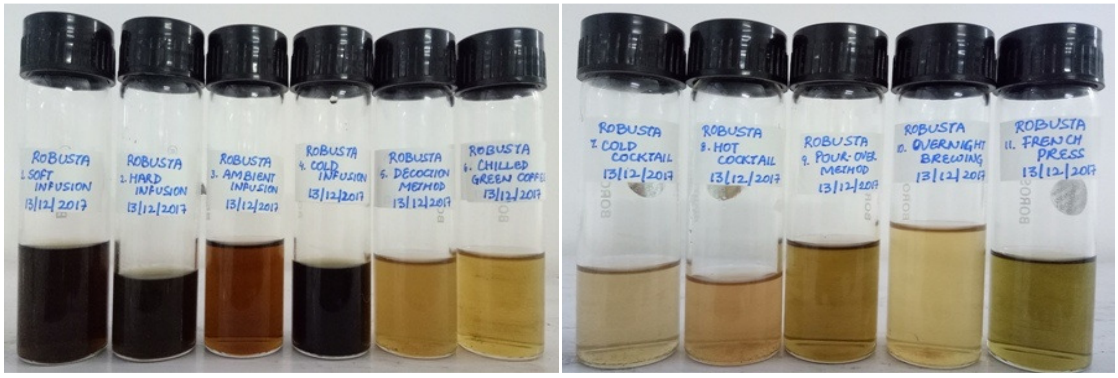
Units: mg QE/g of coffee

For monsooned malabar, total flavonoids and phenolics increased with time when brewed in distilled water i.e. 17.1 to 35.3 mg QE/g of coffee and 71.5 to 84.8 mg QE/g of coffee respectively. Same trend was observed for flavonoids when brewed in ethanol- 3.5 to 9.2 mg QE/g of coffee. But as phenols are considered when brewed in alcohol, the content increased till 4 min brewing – from 60 to 65.6 mg QE/g coffee and then it decreased till 16 min - 61.7 mg QE/g of coffee (Table 12 and Fig. 7).

Table 13. TFC and TPC variation in green coffee robusta with time and solvents

Time	Water		Ethanol	
	TFC	TPC	TFC	TPC
1 min	11.89	62.78	6.41	63.33
2 min	13.35	63.89	7.3	70.78
4 min	24.41	74.11	20.41	77.33
8 min	33.18	77.22	13.23	68.22
16 min	34.47	78	10.12	68.89

Units: mg QE/g of coffee



(3) **(4)**

Fig. 3. (3) and (4) representing the green coffee extracts of robusta prepared after all the eleven brewing methods implemented.

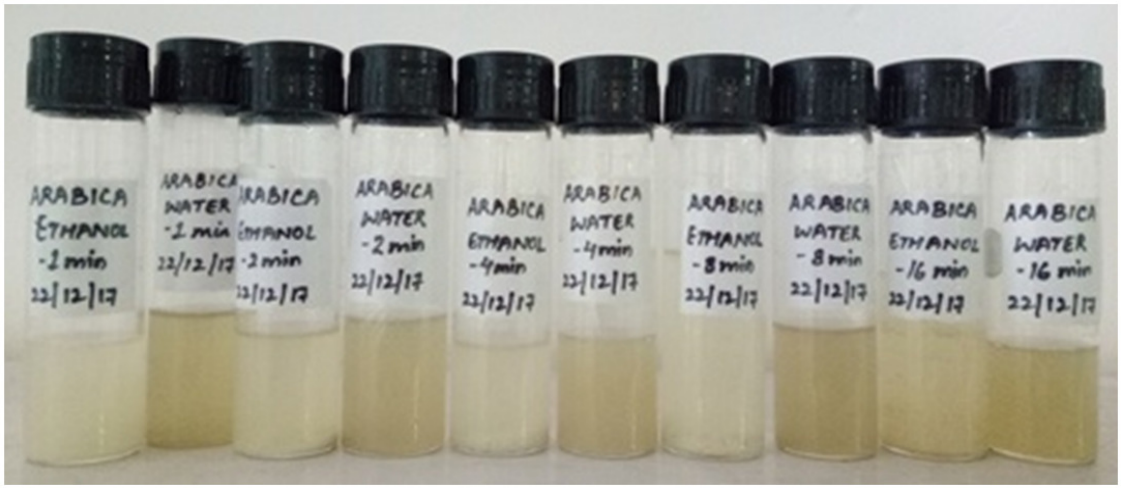


Fig. 4. Showing coffee extracts prepared after brewing for 1, 2, 4, 8 and 16 minutes of green coffee arabica in ethanol and distilled water

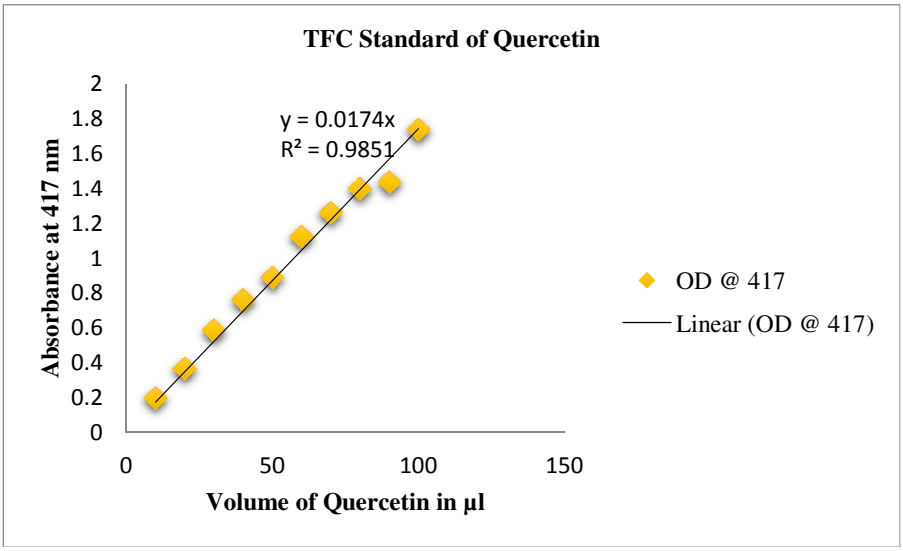


Fig. 5. Standard for TFC

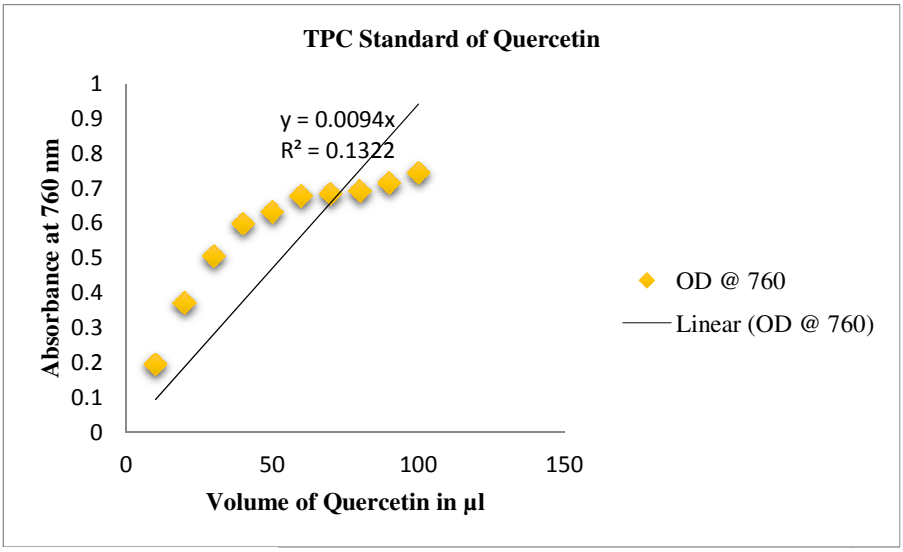


Fig. 6. Standard for TPC

Table 14. TFC and TPC variation in green coffee arabica with time and solvents

Time	Water		Ethanol	
	TFC	TPC	TFC	TPC
1 min	18.94	71.67	10.94	76.56
2 min	22.47	79	14.3	87.56
4 min	32.76	82	19.35	83.89
8 min	37.53	83	17.23	79.89
16 min	40.18	83.44	14.65	84.11

Units: mg QE/g of coffee

Table 15. TFC and TPC variation in roasted coffee robusta with time and solvents

Time	Water		Ethanol	
	TFC	TPC	TFC	TPC
1 min	21.11	83	3.64	64.66
2 min	26.35	88.22	5.23	64.44
4 min	31.52	89.88	6.29	63.55
8 min	33.47	90.22	7.47	60.88
16 min	35.35	92.55	7.64	55.55

Units: mg QE/g of coffee

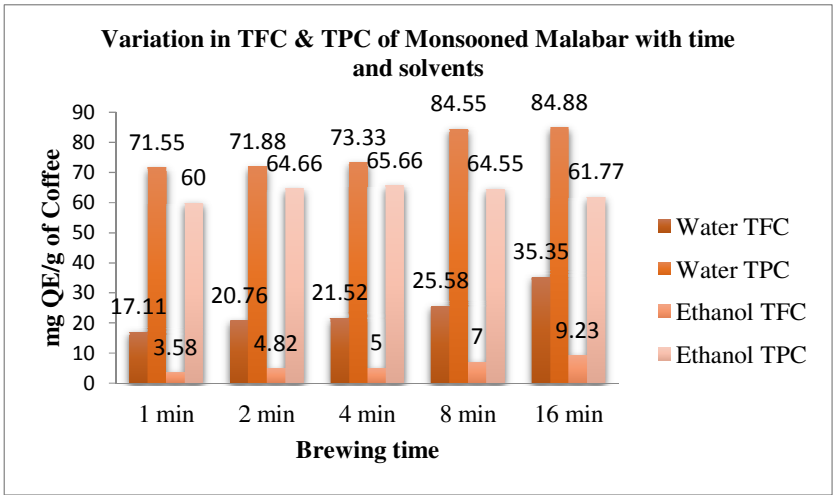


Fig. 7. TFC and TPC variation in roasted coffee monsooned malabar with time and solvents

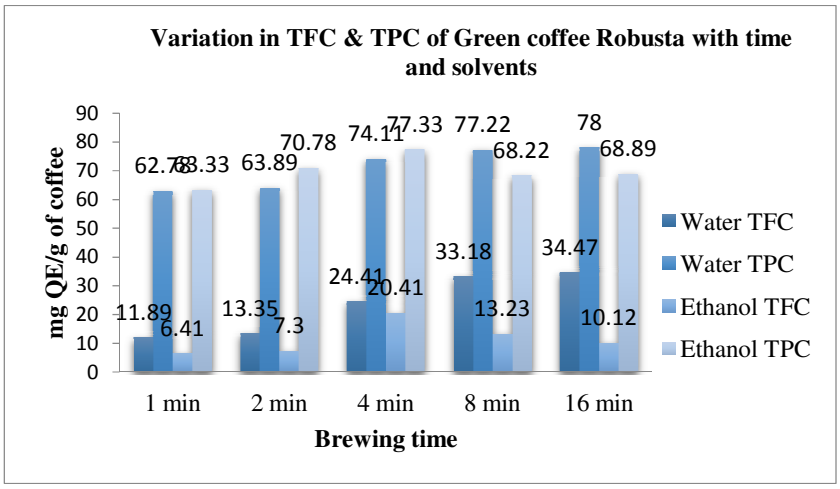


Fig. 8. TFC and TPC variation in green coffee robusta with time and solvents

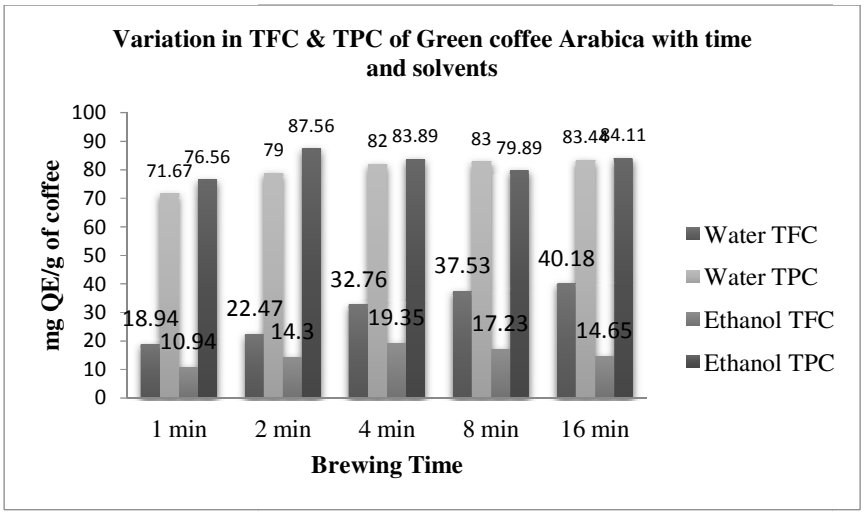


Fig. 9. TFC and TPC variation in green coffee arabica with time and solvents

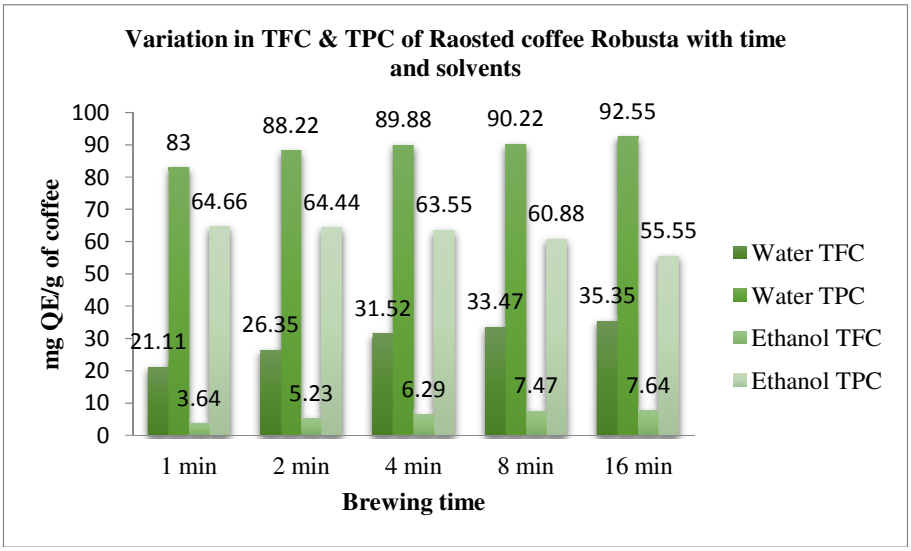


Fig. 10. TFC and TPC variation in roasted coffee robusta with time and solvents

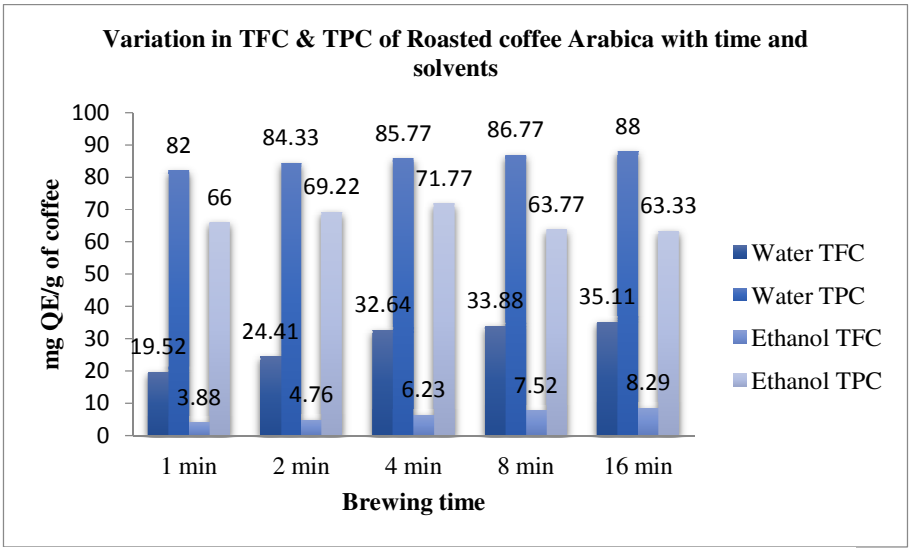


Fig. 11. TFC and TPC variation in roasted coffee arabica with time and solvents

Table 16. TFC and TPC variation in roasted coffee arabica with time and solvents

Time	Water		Ethanol	
	TFC	TPC	TFC	TPC
1 min	19.52	82	3.88	66
2 min	24.41	84.33	4.76	69.22
4 min	32.64	85.77	6.23	71.77
8 min	33.88	86.77	7.52	63.77
16 min	35.11	88	8.29	63.33

Units: mg QE/g of coffee

4. CONCLUSION

We, the authors reach a conclusion that instead of using the general brewing methods that are used till date, the mentioned methods in the paper can also be used for brewing coffee according to taste. Moving towards brewing time, it may be increased according to purpose for which one is consuming coffee for. Apart from that, we would like to conclude that alcohol may also be used as a solvent for brewing for both green and roasted coffee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Saura-Calixto F, Goni I. Antioxidant capacity of the Spanish Mediterranean diet. Food Chem. 2006;94:442-447.
2. Monente C, Ludwig IA, Irigoyen A, de Pena MP, Cid C. Assessment of total (free and bound) phenolic compounds in spent coffee extracts. J Agri Food Chem. 2015;63:4327-4334.
3. Gebeyehu T, Bikila SL. Determination of caffeine content and antioxidant activity of coffee. Am J App Chem. 2015;3:69-76.
4. AC Charrier, J Berthaund. Botanical classification of coffee. Coffee: Botany, Biochemistry and Production of Beans and Beverage. M.N. Clifford and K.C. Willson, Eds., Croom Helm, London, UK, 1985;13-47.
5. A Farah and TF Dos Santo. The coffee plant and beans. Coffee in Health and Disease Prevention. Academic Press, New York, NY, USA. 2015;5-10.
6. Rodrigues NP, de Jesus Garcia Salva T, Bragagnolo N. Influence of influence of coffee genotype on bioactive compounds and the in vitro capacity to scavenge reactive oxygen and nitrogen species. J Agri Food Chem. 2015;63:4815-4826.
7. International coffee organization. Coffee Market Report; 2014.
8. Schievano E, Finotello C, de Angelis E, Mammi S, Navarini L. Rapid authentication of coffee blends and quantification of 16-O-methylcafestol in roasted coffee beans by nuclear magnetic resonance. J Agri Food Chem. 2014;62:12309-12314.
9. Mussatto SI, Machado EMS, Martins S, Teixeira JA. Production, consumption and application of coffee and its industrial residues. Food Bioprocess Tech. 2011;4: 661-672.
10. Oliveira M, Casal S, Morais S, Alves C, Dias F, Ramos S, Mendes E, Delerue-Matos C, Oliveira BPP. Intra- and interspecific mineral composition variability of commercial coffees and coffee substitutes. Contribution to mineral intake. Food Chem. 2012;130:702-709.
11. Ewelina S, Pwael P, Anna SM. The content of Ca, Cu, Fe, Mg and Mn and antioxidant activity of green coffee brews. Food Chem. 2015;182:302-308.
12. Farah A, dos Santos TF. The coffee plant and beans: An introduction. In V.R. Preedy (Ed.). Coffee in health and disease prevention. Elsevier Inc: Academic Press. 2015;5-10.
13. Valentin J, Watling RJ. Provenance establishment of coffee using solution ICP-MS and ICP-AES. Food Chem. 2013;141: 98-104.
14. Sevcan S, Selda M, Murat Y, Munevver A. Elemental composition of green coffee and its contribution to dietary intake. Food Chem. 2017;215:92-100.
15. Shimoda H, Seki E, Aitani M. Inhibitory effect of green coffee bean extract on fat accumulation and body weight gain in mice. BMC Complementary and Alt Med. 2006;6:9.
16. Bicchi CP, Binello AE, Pellegrho GM, Vanni AC. Characterization of green and roasted coffees through the chlorogenic acid fraction by HPLC-UV and principal component analysis. J of Agri and Food Chem. 1995;43:1549-1555.
17. Chu YF. Coffee: Emerging health effects and disease prevention (1^{st} ed.) Oxford: Wiley – Blackwell; 2012.
18. Fischer M, Reimann S, Trovato V, Rodgwell RJ. Polysaccharides of green arabica and robusta coffee beans. Carbohydrate Res. 2001;330:93-101.
19. Naidu MM, Sulochanamma G, Sampathu SR and Srinivas P. Studies on extraction and antioxidant potential of green coffee. Food Chem. 2008;107:377-384.
20. Wei F, Furihata K, Koda M, Hu F, Kato R, Miyakawa T. ^{13}C – NMR based Metabolomics for the classification of green coffee beans according to variety and origin. J of Agri and Food Chem. 2012; 60:10118-10125.
21. Brezova V, Slebodova A; Stasko A. Coffee as a source of antioxidants: An EPR study. Food Chem. 2009;114:859-868.
22. Koseoglu YP, Hacibekiroglu I, Kolak U. Effect of roasting on antioxidant and anticholinesterase capacities of coffee. J of Food and Nut Res. 2014;53:232-239.
23. Wei F, Tanokura M. Chemical changes in the components of coffee beans during roasting. In V.R. Preedy (Ed.). Coffee in health and disease prevention. Elsevier Inc: Academic Press. 2015;83-91.
24. Milan S. Stanković. Total phenolic content, Flavonoid concentration and antioxidant

activity of *Marrubium peregrinum* L. extracts. Kragujevac J of Science. 2011; 33:63-72.

25. Borneo R, Leon EA, Aguirre A, Ribotta P, Cantero JJ. Antioxidant capacity of medicinal plants from the Province of Cordoba (Argentina) and their in vitro testing in model food system. Food Chem. 2008;112:664-670.
26. Katalinic V, Milos M, Kulisic T, Jukic M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem. 2004;94:550-557.
27. Mulabagal V, Tsay H. Plant cell cultures - an alternative and efficient source for the production of biologically important secondary metabolites. Int J App Sci and Engg and Tech. 2004;2:29-48.
28. Cora JD, J Bruce G. Phytochemicals: Neutraceuticals and human health. J of the Sci of Food and Agri. 2000;80:1744-1756.
29. King A and Young G. Characteristics and occurrence of phenolic phytochemicals. J Am Diet Assoc. 1999;99:213-218.
30. Mahomoodally MF, Gurib-Fakim A, Subratty AH. Antimicrobial activities and phytochemical profiles of endemic medicinal plants of Mauritius. Pharma Bio. 2005;43(3):237-242.
31. Pandey AK. Anti-staphylococcal activity of a pan-tropical aggressive and obnoxious weed *Parihenium histerophorus*: An in vitro study. National Academy Science Letters. 2007;30:11-12,383–386.
32. Kelly EH, Anthony RT, and Dennis JB. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. J of Nut Biochem. 2002; 13(10):572–584.
33. Pandey AK, Mishra AK, Mishra A. Antifungal and antioxidative potential of oil and extracts derived from leaves of Indian spice plant *Cinnamomum tamala.* Cellular and Mol Bio. 2012;58:142–147.
34. Safdar N, Sarfaraz A, Kazmi Z and Yasmin A. Ten different brewing methods of green tea: comparative antioxidant study. J of App Bio Biotech. 2016;4(03):033-040.
35. Wolska J, Janda K, Jakubezyk K, Szymkowiak M, Chlubek D, Gutowska I. Levels of antioxidant activity and fluoride content in coffee infusions of Arabica, Robusta and Green coffee beans in accordance to their brewing methods. Bio of Trace Elements Res. 2017;179:327-333.
36. Kumar A, Ilavarasn R, Jayachandran T, Decaraman M, Aravindhan P, Padmanaban N, Krishnan MRV. Phytochemical investigation on a tropical plant. Pak J of Nut. 2009;8:83-85.
37. Treare GE, Evans WC. Pharmacognosy 17th edition, Bahive Tinal, London. 1985; 149.
38. Gibbs RD. Chemotaxonomy of flowering plants. McGill Queen's University Press, Montreal and London. 1974;1.
39. Ajayi IA, Ajibade O, Oderinde RA. Preliminary phytochemical analysis of some plant seeds. Res J of Plant Sci. 2011; 1,3.
40. Geetha TS, Geetha N. Phytochemical screening, quantitative analysis of primary and secondary metabolites of *Cymbopogan citrates* (DC) stapf. Leaves from Kodaikanal hills, Tamilnadu. Int J of Pharma Tech Res. 2014;6(2):521-529.
41. Devi PS, Satyanarayana B, Naidu MT. Phytochemical screening for secondary metabolites in *Boswellia serrata* Roxb. and *Wrightia tinctoria* (Roxb.) R.Br. Notulae Scientia Biologicae. 2014;6(4):474-477.
42. Chang CC, Yang MH, Wen HM. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J of Food Drug Analysis. 2002;10:178-182.
43. Quy DD, Artik EA, Phuong LN, Lein HH, Felycia ES, Suryadi I, Yi-Hsu J. Effect of extraction solvent on total phenol content, total flavonoids content and antioxidant activity of *Limnophila aromatic.* J of Food and Drug Analysis. 2013;1-7.
44. Singleton VL, Joseph AR. Colorimetry of total phenolics with phosphomolybdic - phosphotungstic acid reagents. Am J of Enology and Viticulture. 1965;16:144-158.
45. Kaur C and Kapoor HC. Anti-oxidant activity and total phenolic content of some Asian vegetables. Int J of Food Sci and Tech. 2002;37:153-161.
46. Vignoli JA, Viegas MC, Bassoli DG and de Benassi T. Roasting process affects differently the bioactive compounds and the antioxidant activity of arabica and robusta coffees. Food Res Inter J. 2014; 61:279-285.
47. Oloyed OI. Chemical profile of unripe pulp of *Carica pagaya*. Pak J of Nut. 2005;4: 379-381.
48. Raoof GFA, Mohamed KY, Mohammed HM. Phytochemical evaluation, anti-obesity and antihyperlipidemic effects of combined administration of green coffee. Cinnamon and Ginger. Plant. 2017; 5(5):80-84.

49. Farah A, Donangelo CM. Phenolic compounds in coffee. Braz J of Plant Physio. 2006;18:23-36.
50. Available:https://blackbearcoffee.com/resources/87
51. Available:http://www.ncausa.org/About-Coffee/How-to-Brew-Coffee
52. Irda F, Annisa, Komar R. Antioxidants activities of Arabica green coffee from three regions using ABTS and DPPH asaays. Asian J of Pharma and Clinical Res. 2016;9(2):189-193.
53. Naidu MM, Sulochanamma G, Sampathu SR, Srinivas P. Studies on extraction and antioxidant potential of green coffee. Food Chem. 2008;107: 377-384.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/24181