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Analytical evaluation of espresso spent coffee grounds as a source of bioactive compounds

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Abstract
Coffee is the most popular beverages worldwide. But the waste produced from coffee industry creates a problem for environment due to presence of high organic compounds. Utilization of these compounds is the best way to minimize the environmental pollution and also to develop quality products. So present study was aimed to evaluate the chemical composition of spent coffee grounds generated from espresso machine. Therefore, Arabica and Robusta coffee were selected for analysis. Moisture content was found in the rage of 4.6% to 3.5% for spent coffee grounds generated from espresso machine. Total soluble solid was determined and was found 14% and 13% for Arabica and Robusta espresso spent coffee grounds. Robusta espresso spent coffee grounds contained higher amount of ash content i.e. 1.9% than Arabica (1.2%). Soluble protein content was found 0.35% and 0.32% for Arabica and Robusta espresso spent coffee grounds respectively. Lipid content was found high in both Arabica and Robusta spent coffee that were 12 and 10%. Caffeine content was found in Arabica and Robusta espresso spent coffee i.e. 0.27 and 0.28%. Arabica and Robusta spent coffee showed good source of chlorogenic acid i.e 1.4% and 1.5% respectively. Espresso spent coffee grounds showed higher antioxidant activity as determined by DPPH free radical scavenging activity.

Keywords: Espresso spent coffee grounds, caffeine, chlorogenic acid, polyphenol content, antioxidant activity

Introduction

Coffee is the second largest traded commodity in the world next to crude oil. It belongs to Rubiaceae family that contains more than 70 species but two of them are of significant economic importance namely, Arabica (*Coffea Arabica*) and Robusta (*Coffea canephora*). Coffee is the widely consumed beverages in the world. Coffee consumption in the world is increased from 158771 thousand 60kg bags in the year 2016/2017 to 166346 thousand 60kg bags in the year 2020/2021 (ICO, 2022) [14]. Due to high demand for coffee consumption, large amount of waste is produced from processing of green coffee bean, roasting of green bean, processing from instant coffee production and the direct preparation of beverages in cafeterias, restaurants, or homes (Mussatto *et al*., 2011, Jimenez-Zamora *et al*., 2015) [28, 19]. Spent coffee grounds (SCG) is the solid residue which remains after roasted coffee beans are grinded and brewed, both in coffee shops chains and in industry, for obtaining instant coffee and represents the most abundant coffee by-product (45%) (Murthy and Naidu, 2012) [27]. Spent coffee grounds is a source of important bioactive compounds i.e. phenolics, lipids, proteins, lignin, cellulose, hemicellulose and other polysaccharides (Kourmentza *et al*., 2018) [21]. Spent coffee grounds contains high Dietary fibre (43%) which includes, resistant starch, oligosaccharides and manno-oligosaccharides (Campos-Vega *et al*., 2015b; Vázquez-Sánchez *et al*., 2018; Tian *et al*., 2017) [8, 38, 37]. There are also present some bioactive secondary metabolites, such as diterpenes, sterols, chlorogenic acids, flavonoids and caffeine (Clifford, 1985) [9].

Antioxidant, antimicrobial, antiproliferative and prebiotic properties of spent coffee were reported by several authors, using different solvents and methods (Bravo *et al*., 2012; Ramalakshmi *et al*., 2009, Jim´enez-Zamora *et al*., 2015, Sousa *et al*., 2015, Balzano *et al*. 2020) [7, 19, 34, 3]. Chlorogenic and caffeic acids (Maydata, 2002) [26] contributes towards antioxidant properties of spent coffee grounds. Besides chlorogenic acid and its derivatives, caffeine is a major biological active compound of spent coffee grounds (Cruz *et al*., 2012b) [10] ranging from 3.59 to 8.09 mg/g of spent coffee (Bravo *et al.* 2012) [7].

Spent coffee grounds creates an impact on environment if it is disposed in landfills (Mata *et al*., 2018) [25] as SCG contains polyphenols and tannins, which produces toxicity to the environment. Considering the negative impact of SCG disposed of in landfills, recently utilization of spent coffee waste is in high demand for production of functional food, biodiesel, to reduce heavy metal contamination (Kourmentza *et al*., 2018, Kovalcik *et al*., 2018, Janissen and Huynh, 2018, Iriondo-DeHond *et al*., 2019) [21, 22, 18, 17]. Despite its Italian roots, the popularity of espresso coffee has spread all around the world (Petracco, 2001) [30]. Therefore, present study was undertaken to evaluate the nutritional composition and antioxidant properties of spent coffee grounds generated from espresso machine.

Material and Methods

Selection of the coffee bean

Arabica parchment A grade and Robusta parchment AB grade green coffee beans from Tamil Nadu were selected for the present study.

Roasting of green coffee bean

Roasting was done in a 100 gm capacity drum roaster at coffee quality lab, coffee board, Bengaluru at temperature of 210 to 220 ˚C (for dark roast). The green coffee beans were fed into a rotating drum roaster and exposed to above mentioned temperature for 10-15 min. The color range was selected for roasted coffee powder in between 90 to 100 CTn on the instrument of Neuhaus Neotec.

Grinding of roasted coffee bean

Grinding is the process of making the roasted coffee into powder form. It was done by using Mahnkonig grinder at ditting 5 grind size for espresso.

Preparation for espresso

Espresso is a strong shot of coffee which is brewed under the high temperature of water (temperature 93 ^oC) with time 30 sec. For one espresso shot, 8 gm coffee powder was taken in porta filter which is tamped by under 30 pounds pressure (manually by Barista). The quantity of one espresso shot was 30ml with the brew ratio 1:2 (coffee: water)

After taking a espresso shot whatever powder is left under the porta filter which is called spent coffee that were taken for next process of drying. Spent coffee powder was collected in plate and kept it at temperature 60^{o}C for 48 hrs in Hot air Oven. After drying, the dried powder was packed in polythene bags and kept at room temperature for determining further analytical process.

Methods

Nutritional composition analysis

Moisture estimation

Moisture content was determined by methods of IS 2791: 1992. 2g of spent coffee powder were taken and dried at 95±2 ^{o}C for 2 hrs. The samples were cooled in desiccators and dry weights were taken. The process of heating and weighing was repeated until difference between two successive weights is less than 1 mg. Percentage of Moisture was calculated by following formulae.

$$\text{Moisture content (\%)} = 100(M_1-M_2)/(M_1-M)$$

Where

M= Mass in g of empty dish.

M_1= Mass in g of dish with the material before drying.

M_2= Mass in g of dish with the material after drying.

Water soluble matter

Water Soluble matter was determined by methods of IS 30-77: 1992. Spent coffee powder (2gm) was refluxed with hot water (200ml) over low flame for 1 hr. It was cooled and made up to 250 ml. The solution was filtered through what man No 1 filter paper. The filtrate (50 ml) was transferred to weigh petri dish and evaporated to dryness on water bath. The dried sample in the petri dish was heated at 100± 20 C for 1 hour and cooled in a dessicator and weight was taken. The process of heating and weighing was repeated until difference between two successive weights is less than 1 mg.

$$\text{Water Soluble Matter} = 50000(M_2-M_1)/M(100-X)$$

Where

M= Calculated mass in g, of sample taken for test.

M_1= Mass in g, of the empty dish.

M_2= Mass in g, of dish with dried water soluble matter.

X= Moisture in percentage.

Total ash

Ash Content was determined by methods of IS 30-77:1992. 5g of sample was taken in porcelain dish and kept at 100±2 ^{o}C until water is expelled and heated slowly under flame until swelling ceases. After that samples were kept in a muffle furnace at 550 ±100 C until grey ash results. Samples were cooled in desiccators and weights of samples were taken. The process of heating and weighing was repeated until difference between two successive weights is less than 1 mg.

$$\text{Total Ash content} = 1000(M_2-M)/(M_1-M)\ (100-X)$$

Where

M= mass in g of empty dish.

M_1= mass in g, of dish with the material.

M_2= mass in g, of dish with ash.

X = Moisture in percentage.

Lipid

Total lipid content was analysed by soxhlet extraction method (Folstar, 1985) [12]. Spent coffee powder (10g) was extracted with hexane (1:10) in a soxhlet apparatus for 16 hr on a boiling water bath. The solvent extract was desolventised in a rotovapour and solvent was removed by keeping the extract in vacuum dessicator. The solvent extract free from traces of solvent was finally weighed and the amount of total lipid content was calculated.

Total soluble protein

Protein content was determined by bradford method (1976). Spent coffee powder (0.1g) was mixed with 5 ml of 0.1N NaOH solution and ground in a mortar using pestle. The solution was filtered using Whatman No1 filter paper and filtrate was collected. Bradford's reagent (Commassie blue G-250) was prepared by dissolving 20 mg of Bradford Reagent in 10 mL of 95% ethanol. To this 50 ml distilled water and 20 ml of o-phosphoric acid were added. The solution was made up to 200 ml with distilled water. Sample solution (80µL) was mixed with Bradford's reagent (3.6ml) and diluted to 4 ml using distilled water. The absorbance of this solution was measured at a wavelength of 595nm and protein content was obtained from the standard graph. Standard protein graph was prepared using Bovine's serum albumin (BSA) standard.

Caffeine content

Caffeine content was determined by the methods of AOAC, (2000) [1]. Spent coffee powder (5g) was extracted along with magnesium oxide (3g) and distilled water (100ml) for 45 min with distilled water and filtered. The filtrate was extracted with chloroform, the extract was desolventized and the absorbance was measured at 275 nm in a spectrophotometer. The quantity of caffeine was calculated using a standard graph prepared from caffeine reference sample

$$\text{Caffeine (\%)} = \frac{\text{Average} \times \text{Sample absorbance} \times \text{Total volume} \times 100}{10{,}000 \times \text{sample volume} \times \text{sample weight}}$$

Chlorogenic acid

Chlorogenic acid was estimated by UV spectrophotometry before and after lead acetate treatment of the coffee extract, followed by measurement of the absorbance at 325nm (AOAC, 2000) [1].

Total polyphenols

Total polyphenol content of the spent coffee samples was determined using Folin-Ciocalteu's reagent. Spent coffee powder (0.5g) was taken in methanol: water (70:30, 10 mL) solution in a graduated test tube and heated on a water bath (70 ^{o}C) for 10min. The sample was subjected to centrifugation for 3500 rpm for 10min. and the supernatant was separated. Saturated sodium carbonate solution (5mL) and Folin-Ciocalteu's reagent (0.2mL) were added to the sample solution and made up to 10mL with distilled water. The solution was incubated at room temperature for 60min. and the absorbance of this solution measured at 765nm. The total polyphenol content of coffee samples is expressed as gallic acid equivalents (Swain and Hillis, 1959) [36].

Antioxidant activity

Crude extract preparation

Extraction for antioxidant activity is followed by procedure of Jeszka-Skowronn *et al*. 2016. 0.5 gm of spent coffee powder was taken in 20ml of distilled water and kept at 94 °C for 10 min and extract was cooled and centrifuged for 5 min at 4500rpm Supernatant is taken used for antioxidant activity measurement.

Antioxidant Activity: Antioxidant activity was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method of Shimada, Fujikawa, Yahara and Nakamura (1992). 100µl of coffee extracts was added to 5 ml of 0.1 mm methanolic solution of DPPH. The mixed solution was shaken vigorously and kept in dark at room temperature for 20 min. Absorbance was taken at 517 nm. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula:

$$\text{Radical Scavenging activity (\%)} = \frac{\text{Control optical density} - \text{Sample optical density} * 100}{\text{Control optical density}}$$

Statistical analysis

Results are expressed as mean ± standard deviation (at least three replicates).

Result and Discussion

Nutritional composition analysis

Moisture content

Moisture content was determined by oven dry method and it is presented in table 1. The determination of moisture content is very important as moisture content limits the storage of the material. Higher amount of moisture decreases the shelf life of material and also deteriorates the quality of food materials. In our study, moisture content of spent coffee powder obtained from espresso machine was found in the range of 3.5 to 4.6%. Severini *et al*. (2020) [33] and Martinez Saez *et al*., (2017) [24] reported 3.34% and 3.60% of moisture content in spent espresso coffee ground (SECG) which is closely related to our results.

Total soluble solids (TSS)

Total soluble solids of spent coffee was found for Arabica 14% and Robusta 13% (Table 1). As per study of Cruz *et al.* (2012) [10], TSS values ranged from 11.6 to 27.5% in espresso spent coffee powder of different blends of Arabica and Robusta coffee. TSS depends on the coffee/water ratio, grinding size and percolation temperature. As per our study and other reported study, espresso coffee spent grounds are rich in TSS content. Therefore, extraction efficiency of soluble coffee components from espresso spent grounds is higher than other industrial spent coffee grounds which is around 6.6% (Yen, 2005) [39].

Total Ash

Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agent which provides a measure of the total amount of mineral within a food.

Ash content of spent coffee powder was found 1.2% for Arabica and 1.9% for Robusta (Table 1). Ballesteros *et al*., (2014) [2] reported the total ash content of 1.3% in spent coffee waste which was also showing similar results with the present study value for Arabica spent coffee. Our studied values also were in similar the range of ash content values reported by Cruz *et al*. (2012) [10]. Roasted coffee contains total ash around 4.6% (Belitz *et al*., 2004) [5]. Espresso spent coffee grounds is also rich source of minerals which can be of good significance in the improvement of soil quality (Kondamudi *et al*., 2008) [20]

Lipid content

Lipid content of spent coffee powder was found in the range of 10 to 12% (Table 1). Similar findings also were reported by Cruz *et al*. (2012) [10] i.e. 9.3 to 16.2% lipid content. Spent coffee powder of both Arabica and Robusta showed higher content of lipid as compared to spent coffee generated from instant coffee Industry i.e. 2.29% reported by Ballesteros *et al*. (2014) [2].

The lipid is a substance of biological origin that is soluble in nonpolar solvent, it comprise group of naturally occurring molecules that include fats, wax, sterols, fat soluble vitamins (such as vitamins A, D, E, K) monoglycerides, diglycerides, triglycerides and phospholipids. The biological function of lipids includes storing energy. As lipids are not efficiently extracted in coffee brew, the majority of lipids are retained in spent coffee (Ratnayake *et al*., 1993) [32].

Our study showed higher content of lipid in spent coffee grounds. So, espresso spent coffee grounds can be used as a source of lipids which can be utilized for extraction of oil to be used in cosmetic industry as well as other industries also.

Soluble protein content

Protein content of spent coffee powder was determined by Bradford method and sample readings were compared with standard graph made from different concentrations of BSA standards. The spent coffee powder of Arabica and Robusta possessed protein content of 0.35% and 0.32% respectively as it is presented in Table 1. The green coffee bean approximately contains 11-16% proteins and free amino acids. The content of protein in roasted beans was reported to be 9%, of which 1.5% is soluble in water and the remaining 7.5% part insoluble (Spiller, 1998) [35]. The suitability of the proteins in SCG as industrial protein and functional ingredient in food and feed production (emulsifier, gelling properties, nutritional value) is considered poor due to thermal degradation of protein and incorporation of proteins or amino acids in the formation of melanoidins and phenolics during roasting (Bekedam *et al*. 2008a) [4].

Caffeine content

Caffeine (1,3,7-trimethyl-xanthine) is a major alkaloid that is present in coffee. Coffee contains higher content of caffeine as compared to other beverages. Although the caffeine content in coffee waste is lower than that in coffee beans, a large amount of caffeine still remains. So, caffeine present in spent coffee waste generated from espresso machine can be utilized for the development of caffeinated drinks.

Caffeine content of Arabica spent coffee and Robusta spent coffee was found 0.28% for Arabica spent coffee and 0.27% for Robusta spent coffee (Table 2). Our findings is within the similar range of caffeine content reported by Cruz *et al*., 2012 [10] i.e. ranging from 194.0 to 787.7 mg/100 g (DW), with a mean amount of 452.6 mg/100 g (DW). In raw Arabica coffee, caffeine can be found in values varying between 0.8 and 1.4% (w/w), whereas for Robusta these amounts vary between 1.7 and 4.0% (w/w) (Mussatto *et al*., 2011) [28].

Chlorogenic acid

The chlorogenic acid is essential phytochemical compound found in coffee beans. It has so many health benefits as it can reduce blood sugar levels and potentially exert an anti-diabetic effect. It has also been implicated in weight loss and exerting an anti-obesity effect. Coffee waste in terms of spent coffee also provides a good source of chlorogenic acid. So, it can be utilized as food supplement and also in food industry as preservatives.

The present study showed chlorogenic acids content of Arabica and Robusta espresso spent coffee grounds 1.41% and 1.54% respectively (Table 2). The results of spent coffee powder were compared with the study made by Mussatto *et al*. (2011b) [28]. The chlorogenic acid content spent coffee powder was found higher than the results compared to Mussatto *et al*. (2011b) [28]. They have found chlorogenic acids in spent coffee powder 479 mg/100gm for Arabica and 686 gm/100gm for Robusta.

Total polyphenols content

The spent coffee powder possessed polyphenols content 0.44% and 0.52% for Arabica and Robusta respectively (Table 2). So, the results of spent coffee powder were compared with the study made by Bravo *et al*. (2012) [7]. The polyphenol content of spent coffee powder was found higher than the results of present study which is 15.79 and 19.12 mg GA/g of spent coffee for Arabica and Robusta coffees, respectively.

The phenolic compounds are the major determinant of antioxidant potentials found in high concentrations in plant (Balasundram *et al*., 2006). Recently interest in plant derived natural products has grown mainly because synthetic antioxidant suffers from several side effects. Spent coffee grounds contain several human health related compound such as phenolics with demonstrated antioxidant, antibacterial, antiviral, anti-inflammatory and anti-carcinogenic activities ((Dorsey and Jones, 2017, Fumagalli *et al*., 2016, Magoni *et al*., 2018, Sousa *et al*., 2015) [11, 13, 23, 34].

Anti-oxidant activity determination

The antioxidant activity of spent coffee grounds was evaluated using the1,1-diphenyl-2-picrylhydrazil radical scavenging method. Antioxidant reacts with DPPH, which is a stable free radical and converts it to α-α-diphenyl-β-picrylhydrazine. The degree of discoloration indicates the scavenging potential of the antioxidant increases. Robusta spent coffee powder showed higher antioxidant activity i.e. 82.51% than Arabica which was 58.08% (Figure 1). Similar finding also were reported by bravo *et al*. (2012) [7] i.e. 59.2-85.6%.

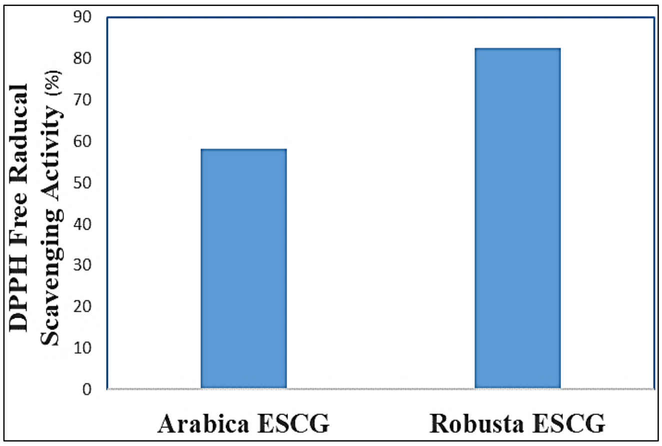

Fig 1: DPPH free radical scavenging activity of Espresso spent coffee grounds (ESCG)

Table 1: Nutritional composition of espresso spent coffee grounds

Sample	Moisture content (%)	Water soluble matter (%)	Ash content (%)	Lipid content (%)	Protein content (%)
Arabica spent coffee powder	4.6 ±0.87	14±0.89	1.2±0.12	12±0.67	0.35±0.02
Robusta spent coffee powder	3.5± 0.76	13± 1.2	1.9±0.032	10±0.78	0.32±0.01

Table 2: Caffeine, Chlorogenic acid, total polyphenol content and DPPH free radical scavenging activity of espresso spent coffee grounds

Sample	Caffeine (%)	Chlorogenic acid (%)	Total polyphenols (%)	DPPH free radical scavenging activity (%)
Arabica spent coffee powder	0.28±0.001	1.41±0.02	0.44±0.06	58.08+ 1.23
Robusta spent coffee powder	0.27±0.005	1.54±0.06	0.52±0.07	82.51+ 2.01

Conclusion

Present study was conducted to evaluate the nutritional as well as functional properties of spent coffee waste produced from espresso machine. Lipid content was found high in both Arabica and Robusta spent coffee that were 12% and 10%. Arabica and Robusta espresso spent coffee grounds contained similar caffeine content (0.27 and 0.28%). It may be due to most of the caffeine was extracted in decoction prepared from espresso machine. Arabica and Robusta spent coffee showed good source of chlorogenic acid i.e. 1.4 and 1.5% respectively. Robusta spent coffee grounds showed higher antioxidant activity (82.51%) by DPPH free radical scavenging activity as compared to Arabica (58.08%). Espresso spent coffee is a good source of nutritional as well as functional compounds. So, these could be used as preservatives in food formulations, as natural antioxidant sources for application in food and pharmaceutical products, or as raw material to obtain new functional ingredients for food industry. Espresso spent coffee grounds have characteristics that make possible their reutilization in different industrial fields.

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