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FULL PAPER

Influence of pectinase–assisted extraction time on the antioxidant capacity of Spent Coffee Ground (SCG)

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Abstract

Spent Coffee Ground (SCG) comprises a range of functional components with high antioxidant potential and health benefits. Although SCG has many advantages, antioxidant values obtained using enzymes is still scarce. Several studies reported that the use of enzyme-assisted extraction techniques could improve the detection of antioxidant compounds in samples of by-products. In this study, SCG was extracted using enzyme-assisted techniques for different periods (0, 20, 40, 60, 80 and 100 mins). The total antioxidant capacity was measured using Peroxidase, Catalase, Ferric reducing antioxidant power (FRAP), Superoxide radical scavenging assay (SRSA) and 2,2-diphenyl-2-picrylhydrazyl (DPPH) analysis. 60 mins extraction was found to exhibit antioxidant activity in DPPH, SRSA, Catalase and Peroxidase of 84.18±7.01%, 32.83±6.75%, 76.65±5.52% and 75.01±7.67%, respectively. Besides that, the extraction also gave a high amount of content with 267.17±26.69 mg TAE/mL and 58.30±2.36 mg QE/g in total phenolic content and total flavonoid content respectively. Flavonoids such as quercetin, kaempferol, rutin, gallic acid, catechin, epigallocatechin, p-Coumaric acid and myricetin were found to be present in SCG extract. A high amount of catechin was found in all periods of extraction, whereby 60 mins had the highest concentration of 1741.65 mg/L, while 40 mins had the lowest concentration which was 389.85 mg/L. In conclusion, 60 mins extraction using a pectinase-assisted method was the best extraction period. Additionally, the individual flavonoid catechin in SCG showed that it has potential as an antioxidant. Finally, there is a strong correlation between antioxidative activity and both phenolic and flavonoid content.

1. Introduction

Coffee is one of the most popular beverages in the world, with an annual production of 156.6 million 60-kilogram bags in 2016 (Blinová *et al*., 2017). *Coffee arabica* (Arabica) and *Coffee canephora* var. *robusta* (Robusta) are the two main species of the genus Coffee that are cultivated for commercial production. Coffee manufacturing is the industrial process of converting the raw fruit of the coffee plant to finished coffee. Such systems generate a lot of solid waste or by-product and wastewater. The solid by-products from the production and preparation of coffee are used-up on coffee grounds which have been developed after the brewing process. It contains various functional components with high antioxidant potential and health benefits (Kim *et al*., 2016). This makes them an important source of raw materials for a variety of applications such as nutraceutical products.

The use of an enzyme to enhance the extraction of phenolic antioxidant compounds has been reported for food products and by-products (Syed, 2010). Some of the phenols contained in vegetables and fruits appear to be entangled with the plant cell wall polysaccharides via tight hydrophilic and hydrophobic bonds. The release of those phenolics can be enhanced via an enzyme-catalysed degradation of the cell-wall polysaccharides. Enzyme-assisted extraction is considered a mild, efficient, and environmentally friendly method, with

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several benefits, such as low energy consumption and easy operational standards (Zhao *et al*., 2016). The aid of enzymes, which have been employed frequently as biocatalysts to obtain target compounds from various plants, can improve the extraction efficiency, yield and biological activities of polysaccharides (Karaki *et al*., 2016).

Antioxidants are inhibitors of the process of oxidation, even at somewhat small concentrations and thus have various physiological roles in the body. Antioxidant components of the plant material act as radical scavengers and help in converting the radicals to less reactive species. A variety of free radical scavenging antioxidants are found in dietary sources such as fruit, vegetables and tea (Kumar *et al*., 2014). The role of antioxidants, as this definition suggests, is to prevent damage to cellular components that emerge as a consequence of chemical reactions involving which generates free radicals.

It is composed of many chemical compositions which makes them an interesting source of raw materials for different applications. Still, no attempts have been made to use it as a resource to produce value-added food ingredients (Kim *et al*., 2016). SCG have commercial value and are currently disposed of as solid waste and to a limited extent, used as fertilizers. This research will bring light to the potential of SCG for its antioxidant properties. Zuorro and Lavecchia (2013), suggested that SCG collected from the brewed method had a high content of total phenolics and could be easily recovered by an aqueous extraction procedure. Thus, this research aimed to extract antioxidant compounds from SCG at different time extraction, namely 20, 40, 60 80 and 100 mins and to evaluate the use of SCG as potential food ingredients.

2. Materials and methods

2.1 Spent coffee sample preparation

Coffee (*Coffea arabica* L.) was purchased from Kilang Kopi Fama, Banting (Selangor, Malaysia). The roasted coffee beans were ground using a coffee grinder for 90 s to a course-size ground. About 500 mL of hot water (98°C) was added to 40 g of coarse ground coffee in a French coffee press. The plunger was slowly pushed down after brewing for 3 mins (Zainol *et al*., 2020). The coffee was poured out, and the spent coffee ground (SCG) was collected and dried on a tray in the cabinet dryer for 24 hrs at 60°C. The dried SCGs were placed in ambient, dark containers in an airtight container prior to the extraction process (Zainol *et al*., 2020).

2.2 Pectinase-assisted extraction

Dried SCG (1 g) was added to 0.1 mL of 0.2 M pectinase and 15 mL of 200 mM sodium acetate buffer at pH 5.5 (Zohdi and Amid, 2013). The mixture was left to be incubated at 37 ° C for different periods, namely 0 min, 20 mins, 40 mins, 60 mins, 80 mins and 100 mins. After the designated time, the extracted SCG sample was placed in a 90°C water bath for 5 mins to stop the enzymatic hydrolysis. The extracted SCG sample was then centrifuged for 10 mins at 7500 rpm at 4°C. The supernatant was filtered using Whatman paper No. 1 and stored in amber bottles and kept in the refrigerator prior to further analysis.

2.3 Determination of antioxidative properties

2.3.1 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

DPPH solution at the concentration of 6.1×10^{-5} M was prepared in ethanol. About 75 µL of the diluted extract was mixed with 3 mL of the DPPH solution. After 1 hr, the absorbance was recorded at 515 nm (Malik *et al*., 2017). All operations were conducted in dark or dim light. The inhibition percentage (IP) of the DPPH by the extract was calculated according to this formula:

$$IP (\%) = [(A0\ min - A60\ min)/A0\ min] \times 100$$

Where A0 min is the absorbance of the blank at t = 0 min, and A60 min is the absorbance of samples at 60 min. The results were expressed as µmol Trolox equivalent (TE) per gram of sample on a dry basis.

2.3.2 Ferric reducing antioxidant power (FRAP) assay

The total antioxidant potential of a sample was determined using the ferric reducing ability of FRAP assay by Chong *et al*. (2018) as a measure of antioxidant activity. A potential antioxidant will reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}); the latter forms a blue complex (Fe^{2+}/TPTZ), which increases the absorption at 593 nm. Briefly, the FRAP reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ at 10:1:1 (v/v/v). FRAP reagent (3 mL) was added to each test tube containing 0.1 mL diluted sample and mixed thoroughly. The absorbance value of the reacted mixture was observed at 593 nm after 0 min. The results were expressed as µmol Trolox equivalent/g dried weight samples.

2.3.3 Superoxide radical scavenging assay (SRSA)

The activity of SRSA was assayed according to the method of Kanungo *et al*. (2015) with modification. The

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reaction mixture was prepared by mixing 2.6 mL of 50 mM phosphate buffer (pH 8.2) and 90 µL of 30 mM Pyrogallol, which was dissolved in 10 mM HCl. The reaction mixture was then left to react at 25°C for 4 mins, and finally, 100 µL of 0.2 M ascorbic acid was added to the mixture and the samples were measured against a blank at 325 nm.

2.3.4 Catalase test

Catalase activity was measured in a reaction mixture (3 mL) containing 100 mM sodium phosphate buffer (pH 6.8, 2 mL), 30 mM H_2O_2 (0.5 mL), and 0.5 mL sample extract (Kanungo *et al*., 2015). The decrease in absorbance due to hydrogen peroxide depletion was recorded at 240 nm by Uv/Vis spectrophotometer. Catalase activity was calculated by using the extinction coefficient of 40 M/cm^{-1} for H_2O_2 at 240 nm and was expressed as nKat moles of H_2O_2 decomposes per second/mL^{-1} of enzyme extraction.

2.3.5 Peroxidase test

Peroxidase activity was measured in a reaction mixture (3 mL) containing 100 mM potassium phosphate buffer (pH 7.0, 2.8 mL), 10 mM H_2O_2 (50 µl), 0.018M guaiacol (50 µL), and 100 µL sample extraction, according to the method of Kanungo *et al*. (2015) with modifications. The same method was repeated for α-tocopherol and β-carotene, which acts as a positive standard to compare with sample solutions. The increase in absorbance due to the formation of tetra-guaiacol was recorded at 436 nm.

2.4 Total phenolic content

The total phenolic content in SCG samples extracted using the pectinase-assisted technique at different times was determined using the Folin-Ciocalteu method (Ng *et al*., 2019). An aliquot (1 mL) of every sample was diluted into 50 mL of stock solution. From the stock solution, 1 mL was added to 17.9 mL of distilled water in and 0.5 mL of Folin-Ciocalteu reagent and left to stand for 1 min. Then, 1.5 mL of 20% sodium carbonate was added to the mixture. The prepared sample was then left at room temperature for 2 hrs in the dark. The resulting solution was measured at 765 nm, resulting in mg GAE per gram of sample extract (mg GAE/g) expressed as gallic acid equivalent.

2.5 Total flavonoid content

The content of flavonoids in the extracts was determined using the method of Hau *et al*. (2018) with slight modifications. 50 mg samples were mixed with 1.5 mL methanol, 0.1 mL 10% aluminium chloride, 0.1 mL 1M Potassium Acetate and 2.8 mL distilled water. The mixture was then incubated at room temperature for 30 mins. The absorbance of the reaction mixture was measured at 415 nm. The final result was expressed as mg of quercetin equivalent (QE) per gram of sample extract (mg QE/g).

2.6 Determination flavonoids using high-performance liquid chromatography (HPLC)

Sample (5 mg) was refluxed in 6 M HCl at 90°C for 2 hrs, with 60% (v/v) aqueous methanol (Mohd Zainol *et al*., 2009). HPLC was performed using an Analytical High -Performance Liquid Chromatography (HPLC) (Shimadzu, Japan) with a 4 solvent delivery system quaternary pump (LPG 3400 SD) equipped with a diode array detector (DAD 3000) with 5 cm flow cell. The separation was achieved by a reversed-phase Acclaim TM 120 C_{18} column (5 µm particle size, i.d. 4.6 x 250 mm). The stock solution of concentration 40 ppm was prepared by dissolving phenolic acid and flavonoids in 0.5 mL HPLC-grade methanol (60%) followed by sonication for 10 min. The resulting volume was made up to 1 mm with the solvent for the mobile phase (acetonitrile and 1% aqueous acetic acid 1:9). The standards and samples were filtered through a 0.45 µm PVDR-syringe filter. The mobile phase was degassed prior to the introduction to the HPLC system for analysis. A photodiode array UV detector detected the flavonoids at three different wavelengths (272, 280 and 310 nm) according to the absorption maxima of the analyzed compounds. Each compound was identified by its retention time and by spiking with standards under the same conditions (Seal, 2016; Shin *et al*., 2019).

2.7 Statistical analysis

The resulting data were expressed as a mean±standard deviation. Statistical comparisons with one-way variance analysis (ANOVA) were performed using Fisher's Least Significant Difference (LSD) test and values of $p < 0.05$ were considered significant using Minitab 16 Statistical Software (Mamat *et al*., 2018).

3. Results and discussion

3.1 Determination of antioxidative properties

3.1.1 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

Table 1 illustrates SCG's 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities extracted using the pectinase-assisted technique at different periods, namely 0 min, 20 mins, 40 mins, 60 mins, 80 mins, and 100 mins. The 60 mins extraction showed the highest radical inhibition of DPPH compared with samples of 0 min, 20 mins, 40 mins, 60 mins, 80 mins, and 100 mins, respectively. Compared with other

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Table 1. FRAP assay, DPPH inhibition, SRSA method, Catalase test and peroxidase value of the SCG extracted at different periods using pectinase-assisted technique.

Samples	DPPH radical inhibition (%)	Ferric reducing antioxidant power (FRAP) assay (μM $FeSO_4$/mL)	SRSA method (%)	Catalase H_2O_2 radical inhibition (%)	Peroxidase guaiacol inhibition (%)
0 min	76.03 ± 5.11^{c}	76.47 ± 7.98^{bc}	31.65 ± 5.00^{cd}	43.07 ± 9.56^{c}	69.92 ± 5.37^{abc}
20 mins	78.34 ± 9.54^{c}	69.99 ± 2.01^{c}	26.34 ± 4.04^{de}	45.24 ± 9.30^{c}	67.45 ± 5.15^{abc}
40 mins	81.55 ± 2.18^{bc}	80.1 ± 2.87^{b}	30.71 ± 3.33^{cd}	50.01 ± 5.27^{c}	73.76 ± 7.18^{ab}
60 mins	84.23 ± 9.01^{abc}	80.72 ± 5.56^{b}	32.83 ± 6.75^{c}	76.65 ± 5.52^{b}	75.01 ± 7.67^{a}
80 mins	84.17 ± 7.01^{abc}	76.87 ± 3.35^{bc}	26.37 ± 2.07^{de}	52.08 ± 7.12^{c}	59.57 ± 3.61^{c}
100 mins	82.10 ± 8.08^{bc}	74.58 ± 5.52^{bc}	22.12 ± 1.41^{e}	51.71 ± 9.71^{c}	63.29 ± 4.42^{bc}
α-tocopherol	93.67 ± 1.41^{a}	137.61 ± 4.17^{a}	59.49 ± 1.62^{b}	89.41 ± 1.32^{a}	48.54 ± 7.00^{d}
β-carotene	91.51 ± 2.45^{ab}	55.82 ± 4.42^{d}	95.02 ± 1.49^{a}	90.64 ± 1.39^{a}	77.02 ± 8.25^{a}

Values represent the mean±standard deviation. Values with the same superscript letters within the sample column are not significantly different (P<0.05). α-tocopherol and β-carotene are used as the positive standards to compare the antioxidative activities in the samples

samples, the 60 mins extraction possessed more potent antioxidant compounds. The antioxidant capacity of the extracts is strongly related to the solvent, the techniques and the period of extraction, mainly due to the different antioxidant potential of compounds with different polarities (Boeing *et al*., 2014). This research suggested that in pectinase-assisted extraction, the time determines the power of inhibition in DPPH.

3.1.2 Ferric reducing antioxidant power (FRAP) assay

Table 1 also shows that the ferric reduction of antioxidant power (FRAP) assay was found to be greater than β-carotene for the sample extracted at each of the different time periods. Yashin *et al*. (2013), quoted the FRAP value of coffee to be 129.4 mmol/mL. They also showed that the FRAP value in SCG samples to be 45.5–60.3%, which was nearly half compared to the DPPH radical scavenging activity retention (95%). However, the study also found that the FRAP value was lower than the DPPH radical scavenging activity in pectinase-assisted extraction. This indicates that the major components contributing to DPPH and FRAP were different (Yilmaz and Akgun, 2008).

3.1.3 Superoxide radical scavenging assay (SRSA)

The samples displayed a low percentage of superoxide radical scavenging assay (SRSA), comparable to that of α-tocopherol and β-carotene, with a percentage of 59.50±1.62% and 95.02±1.50%, respectively (Table 1). The 60 mins extraction showed that there was no significant difference from 0 min extraction and 40 mins extraction (with a percentage of 32.83±6.75% for 60 mins extraction, 31.65±5.00% for 0 min extraction and 30.71±3.34% for 40 mins extraction). Although superoxide anions are weak oxidants, they give rise to a generation of powerful and dangerous hydroxyl radicals, as well as singlet oxygen, both of which contribute to oxidative stress (Naskar *et al*., 2010). The higher scavenging activity might be explained by the fact that sugar moieties did not contribute to the SRSA. Since α-tocopherol exhibited the highest pyrogallol inhibition, Traber and Atkinson (2007) claimed that the α-tocopherol form is the most important lipid-soluble antioxidant and that it protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction. This removes the free radical intermediates and prevents the propagation reaction from continuing. Priftis *et al*. (2018) stated that coffee's specific superoxide scavenging radical activity ranged from 8.68 up to 17.73 Units per mg coffee (corresponding to IC_{50} values from 61.67 to 138.40 μg/mL) based on the activity of Brazilian coffee extracts, a Green and Roasted samples.

3.1.4 Catalase test

Our results show that Catalase's maximum H_2O_2 radical scavenging activity inhibition reaction (76.65±5.52 %) was shown for an extraction period of 60 min, which was comparable to α-tocopherol and β-carotene. This could be due to the fact that the standards can lower the rate of oxidation by different mechanisms. Thus, having a higher ability to catalyse the decomposition of hydrogen peroxide to water and oxygen than the extraction time of SCG sample. These reactive oxygen species are eliminated by an elaborate antioxidant defence system consisting of enzymes such as catalase, superoxide dismutase, glutathione peroxidase, and numerous non-enzymatic antioxidants, including vitamins A, C and E, glutathione, ubiquinone, and flavonoids (Urso and Clarkson, 2003). The catalase enzymes are an efficient mechanism in the cellular detoxification process, participating in the removal process of free radicals. Thus, the reduction of catalase activity reduces the ability to prevent oxidative damage.

3.1.5 Peroxidase test

Our results show that the majority of the samples

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have shown high antioxidant activity with a high percentage of inhibition ranging from 59.57 to 75.01%. The highest antioxidant activity on H_2O_2 radical scavenging activity inhibition was expressed in the 60 min sample and β-carotene. Previous reports have shown that the stimulation of guaiacol peroxidase activity and expression of new isoenzymes in different plant species in response to various influences such as the treatment with SO_2 (Lepeduš *et al.*, 2004). Both catalase and peroxidase are heme enzymes that catalase reactions of hydrogen peroxide. In catalase, the enzymatic reaction is the disproportionation of hydrogen peroxide. The function of the enzyme appears to be the prevention of any build-up of potentially dangerous oxidants. Peroxidase reacts by mechanisms similar to catalase, but the reaction catalysed is the oxidation of a wide variety of organic and inorganic substrates by hydrogen peroxide (Shivakumar *et al.*, 2017). In this analysis of SCG with different timing of extraction in pectinase, the peroxidase test has higher antioxidant activity compared to the catalase test.

3.2 Total phenolic content

The bioactivity of phenolics may be related to their antioxidant behaviour, which is attributed to their ability to chelate metals, inhibit lipoxygenase and scavenge free radicals. Phenolic compounds are also known as ubiquitous secondary metabolites in plants (Rebaya *et al.*, 2015). Table 2 indicates the highest total phenolic content from 60 mins extraction (267.17±26.69 mg TAE/ mL sample). The data also show that the 20 mins extraction has no significant difference ($p < 0.05$) to the majority of samples excluding 60 mins and 100 mins extraction. The 100 mins extraction had the lowest overall phenolic content (202.10±9.93 mg TAE/mL). The study suggests that the optimum efficiency for SCG extraction is 60 mins. This shows that as the value reduced slightly when the extraction time was after 60 min. Longer enzyme treatment time could generate high levels of hydroxyl radicals in the presence of oxygen that leads to a negative impact on the purity of polyphenol. Furthermore, longer enzyme treatment time affects polyphenol purity by releasing an amount of reduced sugar and insoluble protein in the mass extract (Pasrija and Anandharamakrishnan, 2015). Hai *et al*. (2016) found that the treatment time was 60 mins for the total polyphenol content reached at 85.05 mg GAE/g from old tea leaves which were extracted with enzyme-assisted-extraction. Conversely, Zuorro and Lavecchia (2013), mention that 90% recovery of the phenolic compound of SCG collected at coffee bars and coffee capsules were 17.75 mg GAE/g and 21.56 mg GAE/g respectively within the 2 hrs extraction time. Yet, they used a solvent-extraction technique and operating under mild temperature conditions.

Table 2. Total phenolic content (TPC) and total flavonoid content (TFC) of the SCG extracted at different periods using pectinase-assisted technique.

Samples	TPC (mg GAE/g sample)	TFC (mg QE)/g sample
0 min	62.52±6.77^{b}	40.47 ±1.46^{d}
20 mins	65.34±5.03^{b}	50.65 ±2.48^{b}
40 mins	60.60±3.92^{bc}	56.25 ±1.44^{a}
60 mins	76.17±6.92^{a}	58.30 ±2.35^{a}
80 mins	62.24±5.87^{bc}	46.66±3.25^{c}
100 mins	57.24±4.98^{c}	31.13±1.69^{e}

Values represent the mean±standard deviation. Values with the same superscript letters within the sample column are not significantly different ($P<0.05$).

3.3 Total flavonoid content (TFC)

In this study, the total flavonoid content (TFC) was found to be abundant in the 60 mins extraction, however, it was no significantly different ($p < 0.05$) from the 40 mins extraction (Table 2). It is interesting to note that the TFC was continuously increased from 0 min to 60 mins extraction and decreased after 80 mins extraction. The data also depicted that SCG at 100 mins extraction had the least TFC with an amount of 31.13±1.70 mg QE/g sample. These values were in line with those reported previously for SCG and are higher than those found for other agroindustrial waste such as grace pomace, carrot peels and apple peels, which were below 15 mg GAE g^{-1} dry matter (Panusa *et al*., 2013). This supports the suitability of SCG as a source of potent antioxidants such as flavonoids. Functional hydroxyl groups in flavonoids mediate their antioxidant effects by scavenging free radicals and/or by chelating metal ions.

3.4 Determination of individual flavonoid using High-Performance Liquid Chromatography (HPLC)

Table 3 shows that most of the samples contain all the flavonoids tested except for the 20 min sample, which was missing the myricetin. Catechin was found in the highest concentration in every sample of SCG ranging from 390 to 1742 µg/L. Each of the samples contained rutin, myricetin, kaempferol, gallic acid, catechin, epigallocatechin gallate, quercetin and p-coumaric acid (Figure 1). Table 3 also shows that the 60 min sample had the highest concentration of flavonoid in the majority of standards used while the 40 min sample showed the lowest concentration of flavonoid with reading for 60 mins and 40 mins in catechin standard of 1741.658 µg/L and 389.852 µg/L, respectively. The data show that the 60 mins sample contains the highest flavonoid content amongst the samples, in both total flavonoid content antioxidant quantity and the identification of individual flavonoids by HPLC.

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Table 3. Concentrations of identified flavonoids in SCG extracted at different periods using pectinase-assisted technique via HPLC.

Flavonoid compound	The concentration of the individual flavonoid in every sample (μg/100 g)				
	20 mins	40 mins	60 mins	80 mins	100 mins
Catechin	130.71±1.21^{bc}	389.85±4.44^{b}	741.65±5.23^{a}	663.10±7.54^{ab}	278.39±6.93^{b}
Epigallocatechin Gallate	16.64±4.73^{de}	20.0±2.38^{de}	23.67±4.90^{de}	97.88±10.22^{c}	65.70±55.54^{cd}
Gallic Acid	1.02±0.23^{ef}	0.33±0.18^{f}	1.48±0.15^{ef}	2.02±0.19^{ef}	0.95±0.07^{f}
Kaempferol	0.53±0.01^{f}	0.62±0.02^{f}	1.81±0.04^{ef}	1.68±0.02^{ef}	1.06±0.01^{ef}
Myricetin	10.00±1.13^{def}	22.47±1.98^{de}	38.17±5.55^{cde}	34.76±3.15^{cde}	15.65±2.02^{de}
p-Coumaric Acid	11.37±2.15^{def}	13.65±1.96^{def}	13.93±1.43^{def}	12.19±1.65^{def}	7.97±8.14^{ef}
Quercetin	2.04±0.08^{ef}	3.58±0.09^{ef}	6.45±0.09^{ef}	5.80±0.08^{ef}	3.79±0.08^{ef}
Rutin	13.38±1.74^{def}	5.16±0.97^{ef}	16.4±1.89^{de}	15.58±2.26^{de}	10.20±2.10^{def}

Values represent the mean±standard deviation. Values with the same superscript letters within the sample column are not significantly different ($P<0.05$).

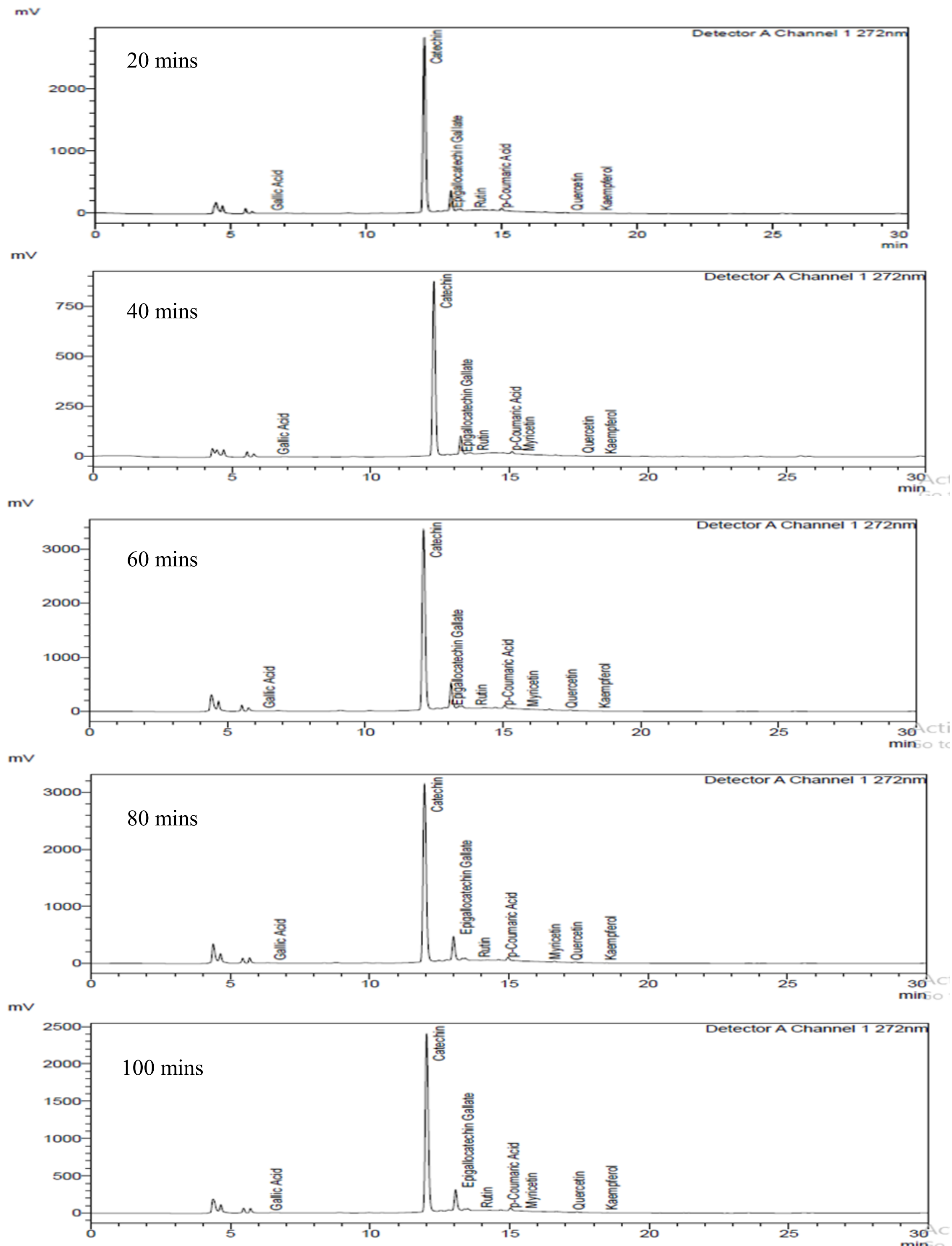

Figure 1. HPLC chromatograms of SCG extracted at different periods using pectinase-assisted technique.

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Palomino García *et al*. (2015), quoted that flavonoids in husk fermentation of Robusta coffee with a content per gram of sample of chlorogenic acid (132.50 μg/g sample), caffeic acid (28.27 μg/g sample) and rutin (8.26 μg/g sample). Chlorogenic acid was the most abundant phenolic acid in coffee beans (Madhava Naidu *et al*., 2008), which could explain its abundance in the by-products.

4. Conclusion

The SCG extracted for 60 mins using a pectinase-assisted technique showed higher antioxidant activity over other extraction times used for the analysis of DPPH, SRSA, catalase, FRA, and peroxidase. The application of HPLC had identified several individual flavonoids such as quercetin, kaempferol, rutin, gallic acid, catechin, epigallocatechin, p-coumaric acid and myricetin for all extraction times. Furthermore, the flavonoid content is highly responsible for the antioxidant activity of SCG, which could serve as good food preservation as well as in the pharmaceutical industry.

Conflict of interest

The authors declare no conflict of interest.

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