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Extraction Processes for Chlorogenic acid from Fruits of Arabica Coffee (*Coffea arabica* L.)

Food Science

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ABSTRACT

This research is evaluating extraction process of chlorogenic acid from fruits of arabica coffee (*Coffea arabica* L.). To determine optimum extraction process for chlorogenic acid from arabica coffee fruits various solvents (water, ethanol) and methods of extraction (shaking, reflux, and microwave) were compared. The highest contents of chlorogenic acid were obtained from 50% ethanol solvent extraction among all the solvents used. When extraction methods were compared with 50% ethanol, contents of chlorogenic acid were measured as follows: 2.13 ± 0.13 mg/mL at 20 min by reflux extraction with 80 mesh-powder, 0.98 ± 0.02 mg/mL at 40 min by shaking extraction with 80 mesh-powder, and 1.69 ± 0.03 mg/mL at 130 sec by microwave extraction with 120 mesh-powder. With reflux extraction for 30 min with 80 mesh, the lowest free radical scavenging activity was achieved (IC50: 1.32 ± 0.01 mg/mL). Chlorogenic acid (CGA) contents and IC50 value showed significantly inversed correlation.

KEYWORDS:

Coffea arabica L., extraction, chlorogenic acid, radical scavenging activity, IC50

Introduction

Coffee tree is commonly grown for its beans as unique ingredients for beverages [1]. Coffee plants belong to the family Rubiaceae. They are green shrubs with funnel-shaped flowers that develop into a pulpy fruit known as 'cherry', which contains two seeds otherwise called coffee beans. Coffee grows wild in Africa and Madagascar, and the genus includes a large number of species. Only three of them, namely, *Coffea arabica* L., *Coffea canephora* (robusta), and *Coffea liberica*, have been used successfully in commercial cultivation [2]. Coffee is consumed because of its desirable bitter taste and for its "medicinal" benefits. The effect of coffee on human physiology varies from person to person and also on the quality and quantity of coffee consumed. The caffeine content of green coffee bean varies according to the species; canephora (robusta) coffee contains about 2.2%, arabica about 1.2%, and the hybrid 'arabusta' 1.72%. Environmental and agricultural factors appear to have a minimal effect on caffeine content. During roasting there is no significant loss in terms of caffeine. A typical cup of regular coffee contains 70 to 140 mg of caffeine, depending on preparation, blend, and cup size [3].

Antioxidants may be of great benefit in improving the quality of life by helping to prevent or postpone the onset of degenerative diseases. Recently research in Norway has shown the role of coffee as a source of antioxidants in diet. Phenolic compounds in coffee are known to have antioxidant activity in which the prevalent one is hydroxy cinnamic acid and the major component of this class is caffeic acid, which occurs in food mainly as esters called chlorogenic acid [4]. The antioxidant and antineoplastic properties of coffee extracts are reported [5] [6]. Coffee is the major source of chlorogenic acid (CGA) in human diet, daily intake by coffee drinkers being 0.5-1.0 g whereas coffee abstainers typically ingest < 100 mg/day [7].

The main constituents of coffee bean are caffeine, chlorogenic acid, caffeic acid, ferulic acid, and its related compounds [1]. Chlorogenic acid, contained in coffee in a considerable amount [8], is secondary metabolites of great economic interest in coffee because of their degradation into phenolic compounds when the beans are roasted, responsible for coffee bitterness [9]. The antioxidant effect of green coffee bean extracts are attributed to chlorogenic acid [10]. Many investigators have reported that chlorogenic acid shows antioxidant activities [10] and inhibitory effects on chemical-induced carcinoenesis in vitro and in vivo [11]. Suzuki *et al*. (2002) [12] suggested that the antihypertensive action of green coffee bean extracts were due to the fact that green coffee bean extracts contain chlorogenic acid as a major phenolic compound.

The objectives of this study were to determine the process and conditions for chlorogenic acid extraction from arabica coffee fruits comparing different solvents (water and ethanol) and extraction methods (shaking, reflux, and microwave). The results obtained from this study will be used for basis of large scale process for chlorogenic acid extraction from arabica coffee fruits.

Materials and methods

Materials

Arabica coffee fruits were collected from Mae Taeng District, Chiang mai province, Thailand. Mature and immature arabica coffee fruits (red berry and green berry) were sun-dried as well as green beans from mature arabica coffee fruits. Dried ones were ground using pin mill with 40, 80, and 120 mesh sieve.

Proximate composition

Analysis of moisture, ash, crude fat, and protein contents were carried out according to AOAC official methods [13].

Extraction with different solvents

To determine suitable solvent, green bean (GB), red berry (R), and green berry (G) powder were extracted with different solvents (water, ethanol) in a shaking incubator at 25℃ for 24 hours. The solvent was then removed by filtration and collected. Fresh solvent was then added to residues to extract further. Shaking extractions were repeated 3 times and resulting filtrates were collected for the chlorogenic acid analysis.

Extraction methods

Different extraction methods such as shaking, reflux, and microwave-assisted extraction were used to determine the effectiveness of extraction type on the extraction of chlorogenic acid from arabica coffee fruits. For each extraction method, about 1g each of green bean (GB) powder (40 mesh) was mixed with 20 mL of 50% ethanol. Shaking and reflux extraction were carried out for 40min. Samples were taken at 10min interval.

In case of microwave-assisted extraction, microwave (LG Electrical Equipment, Korea) was used and the extraction was carried out every 10 sec with 20 sec resting. This process was repeated 4 times. For the samples taken from three different extraction, they were filtered under vacuum. Resulting filtrates were collected for the chlorogenic acid analysis.

Radical scavenging activity assay

The antioxidant activity of the extract was evaluated by measuring the ability of the extract to scavenge the free radical DPPH in vitro. The assay method was modified from that described in other reports [14]. For the purpose of comparing the antioxidant activity in various extracts, concentration (mg green bean powder/ml solvent) of sample producing 50% reduction of the radical absorbance (IC_{50}) was used as an index. each extract was diluted in series with ethanol and 200 μL of each diluted extract was added to 800 μL of DPPH (2,2-Diphenyl-1-picryl-hydrazyl, Sigma-D9132) solution. The solutions were mixed using a vortex and the mixture was then incubated for 30 min in darkness at room temperature, after which the absorbance was measured at the wavelength of 517 nm using ethanol as a reference. The IC_{50} values were determined using linear regression of radical scavenging activity (RSA) versus the concentration of green bean powder. The values of RSA were calculated using the following equation:

RSA(%) = 1−(Sample absorbance/Blank absorbance)×100

Analysis of variance and comparison of means were performed on these antioxidant results to compare the activity between the different methods [15].

Chlorogenic acid analysis

Extracts of arabica coffee fruit powder were filtrated 0.45 µm membrane and injected into the HPLC system. An Agilent 1100 liquid chromatograph system (Dionex HPLC, Ultramate 3000, USA) consisting of a quaternary pump, an autosampler and a photodiode array detector coupled with Agilent Chemstation was used. Separations were carried out with a Gemini C18 reversed-phase column (250mm×4.6mm, 5m, Phenomenex Sciences Instrument Co., Ltd., USA). The mobile phase consisted of 0.03% trifluoroacetic acid in water (A) and acetonitrile (B). The gradient program was as follows: 0~25 min, linear gradient 10% B. Chromatography was performed at 25 ℃. The flow rate was 1.0 ml/min and aliquots of 20 µL were injected. The UV detection wavelength was set at 330 nm and absorption spectra of compounds were recorded between 200 and 800 nm. The compounds were identified by comparing their retention times and UV spectra with those of the markers [16].

Statistical analysis

The data were represented as mean±SD. The statistical analysis was performed with SAS program (version 8.02). The values among groups were evaluated by one-way analysis of variance (ANOVA) followed by post-hoc Duncan's multiple range tests (p<0.05).

Results and discussion

Proximate composition

Proximate compositions of dried fruits of *Coffea arabica* L. (green bean (GB), red berry (R), and green berry (G)) were investigated [Table 1]. Moisture content of GB, R, and G were 11.34, 7.87, and 8.80%, respectively. In case of ash contents, R and G showed higher values than that of GB. However, GB contained higher protein content than R and G. In case of fat content, G showed remarkably high value whereas R contained high carbohydrate. As coffee berry became mature, fat content decreased whereas carbohydrate content increased.

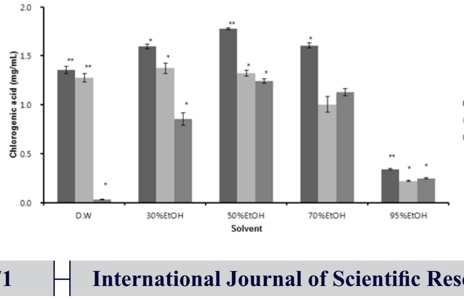
Table 1. Proximate composition of dried fruits of *Coffea arabica* L.

	GB*	R*	G*
Moisture (%)	11.34±0.0541)	7.87±0.021	8.80±0.130
Ash (%)	4.02±0.019	4.90±0.047	5.07±0.009
Protein (%)	13.48±0.096	10.81±0.094	10.96±0.046
Fat (%)	5.91±0.437	4.80±0.091	7.17±1.276
Carbohydrate (%)	65.25±0.468	71.58±0.024	67.95±1.434

GB: Green bean, R: Red berry, and G: Green berry
1) Mean±SD

Solvent types

The type of solvent (water and ethanol) did significantly influence the extraction of chlorogenic acid [Figure 1]. Distilled water extraction of G resulted in poor chlorogenic acid yield (0.03 mg/mL); in contrast, GB and R showed chlorogenic acid content of 1.35 and 1.28 mg/mL, respectively. With all the ethanol extractant containing different ratios of water, green beam showed higher chlorogenic acid content than those of green and red berry. In 50% ethanol extraction, green bean (GB) exhibited the highest chlorogenic acid contents, indicating that pericarp of coffee bean contained less chlorogenic acid as compared to green bean. Removal of pericarp will increase extraction yield of chlorogenic acid. Interestingly, all samples showed poor extraction yield with 95% ethanol. Therefore, GB and 50% ethanol were selected for further study.

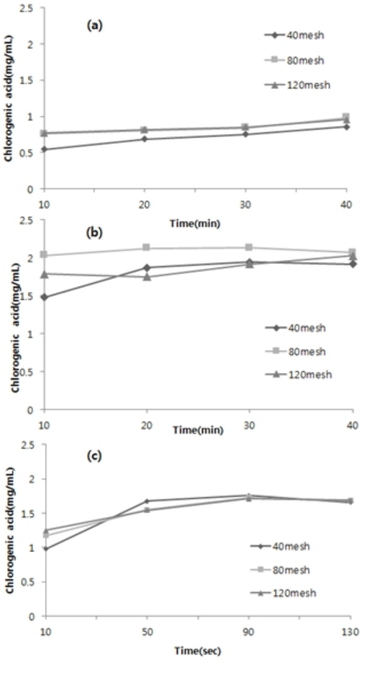


Fiure 1 Effect of ethanol concentrations on the yields of chlorogenic acid from arabica coffee fruit powder using shaking extraction method (GB: Green bean, R: Red berry, and G: Green berry).

* Values with different superscripts differ significantly by Duncan's multiple range test at $P < 0.05$
** Values with different superscripts differ significantly by Duncan's multiple range test at $P < 0.01$

Effect of extraction methods

In case of shaking extraction, extraction yield was slowly increased with extraction time extended (Figure 2(a)). Particle size of 80 and 120 mesh showed almost identical extraction yield throughout extraction. Low extraction yield was observed with particle size of 40 mesh. It was concluded that optimum condition for reflux extraction method should be particle size of 80 mesh and extraction time of 20 min. Unlike the shaking extraction result, reflux extraction showed different pattern (Figure 2(b)). With 80 mesh sample, higher chlorogenic acid yield was achieved than those of 40 and 120 mesh samples. Only 20min extraction was required to reach the maximum yield of chlorogenic acid (2.23 %) with reflux method [Table 2]. To save extraction time, microwave extraction was also evaluated (Figure 2(c)). Each 10 sec microwave heating was employed for each repetition. Interestingly, microwave extraction was not affected significantly by the particle size of samples. Even the maximum chlorogenic acid yield of microwave extraction was slightly lower than that of reflux extraction, microwave extraction should be better method because it required very short extraction time. Less thermal damage would be resulted with shorter heating time.



Figrue 2 Changes in chlorogenic acid contents during shaking (a), reflux (b), and microwave (c) extractions of Arabica green beans as affected by particle size.

Radical scavenging activity

Samples from shaking extraction showed poor radical scavenging activity as expressed by IC_{50} values [Figure 3(a)]. Sample of 40mesh showed higher IC_{50} values. It was coincided with the poor extraction yield of chlorogenic acid as in Figure 2(a). Reflux extraction resulted in the same pattern as shaking extraction; however, IC_{50} values of reflux extraction was significantly lower than those of shaking extraction, indicating reflux extraction contained more antioxidant. Like these two conventional extraction methods, microwave assisted extraction showed the similar trends [Figure 3(c)].

Table 2 summarized the maximum extraction yields of chlorogenic acid and lowest IC_{50} values from green bean extracts. Highest yield of chlorogenic acid and lowest IC_{50} value were obtained from reflux method followed by microwave extraction.

Table 2 The extraction yields and IC_{50} values of chlorogenic acid from GB (Arabica green beans) using different extraction methods

	Yield of chlorogenic acid (%)	IC_{50} values (mg/mL)
Shaking	$1.03\pm0.05^{1)c}$	2.58 ± 0.05^{c}
Reflux	2.23 ± 0.10^{a}	1.32 ± 0.01^{a}
Microwave	1.81 ± 0.05^{b}	1.55 ± 0.04^{b}

1)Values are expressed as mean±SD(n=3). Values in the same column with different superscripts are significantly at $P<0.05$.

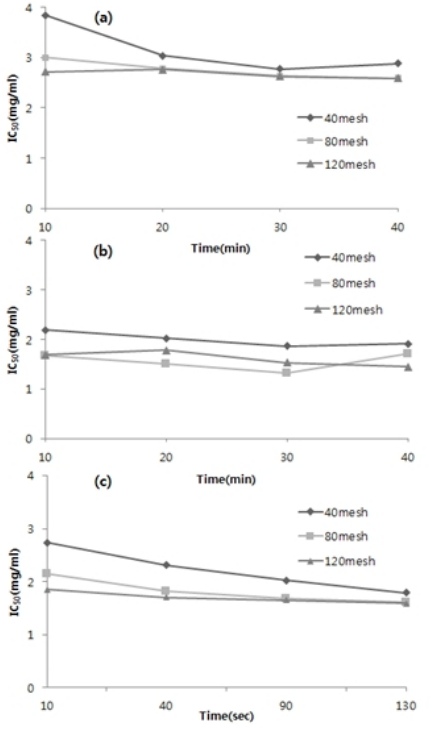


Figure 3 Changes in IC_{50} values (DPPH) contents during shaking (a), reflux (b), and microwave (c) extractions of Arabica green beans as affected by particle size.

Conclusions

We found that green bean of *Coffea arabica* L. contained the highest chlorogenic acid when compared with red berry and green berry; The best solvent for chlorogenic extraction were 50% ethanol solution. Even it did not effectively extract chlorogenic acid, shaking process is recommended for on-farm extraction method because of easy operation and low cost. On the other hand, reflux process is recommended for large scale process.

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