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Microwave – Assisted Extraction of Chlorogenic Acid from *Coffee liberica L*

Josephine Q. Borja [*,1]
Marylou M. Uy [1]
Jose Salvador Lim [1]
Matthew Edson Ong [1]
Alejandro Miguel Ros [1]
[1] *Chemical Engineering Department, Gokongwei College of Engineering, De La Salle University, 2401 Taft Avenue, Manila*
[*]*e-mail :* josephine.borja@dlsu.edu.ph

This study applied microwave-assisted extraction of chlorogenic acid (CGA) from *Coffee liberica L.* using ethanol as solvent. It sought to determine the effects of temperature, extraction time, solvent-to-solid ratio, and solvent concentration on the CGA yield expressed as gallic acid equivalent per litre (mg GAE L^{-1}). The values of these factors were varied at three levels each and experiments were implemented using the $L_9 3^4$ orthogonal array of the Taguchi design of experiment. Results showed that increasing the solvent-to-solid ratio from 2.5 to 7.5 mL g^{-1} decreased the yield significantly. Conversely, increasing the solvent concentration from 0.6 to 0.7 (v v^{-1}) increased the yield, but beyond this, lower yield was obtained. Likewise, yield increased when the extraction time was increased from 5 to 7 minutes but decreased subsequently when extraction was extended to 10 minutes. Temperature did not show significant effect on yield. Among the factors tested the solvent-to-solid ratio has the most significant effect on yield, followed by solvent concentration and extraction time while temperature had no significant effect. In the Taguchi design the highest yield of 304.90±0.58 mg GAE L^{-1} was obtained at 90°C, extraction time of 7 minutes, solvent-to-solid ratio of 2.5 mL g^{-1} and solvent concentration of 0.8 (v v^{-1}). Using the same extraction temperature and time and solvent-to-solid ratio but lower solvent concentration, the confirmatory run resulted is significantly higher yield of 854.35±3.35 mg GAE L^{-1}. Chlorogenic acid was identified in the extract at a concentration of 3152 mg L^{-1}. By applying Soxhlet extraction using the same solvent concentration and solvent-to-solid ratio at the same temperature as that of the confirmatory run the yield was significantly lower at 570.42±5.3 mg GAE L^{-1}.

Keywords : Polyphenols; chlorogenic acid, antioxidant, microwave-assisted extraction; Taguchi method

INTRODUCTION

The extraction and characterization of several active plant components such as polyphenols has been the subject of research by drugs and food manufacturers because of their antioxidant properties and their probable role in the prevention of various diseases. Several thousand molecules having polyphenol structures

have been found in edible plants (Manach *et al*. 2004). One of these is coffee, which is known to be rich in natural polyphenols such as chlorogenic acid (CGA) (Brezová *et al*. 2008; Mendonca, *et al*. 2008; Naidu, *et al*. 2008).

The conventional method of extracting active plant components is solid-liquid extraction, which employs the idea of selective solubility. Some solvents used were water (Sacchetti *et al*. 2009), aqueous ethanol (Pan *et al*. 2003; Kojić-Bucić *et al*. 2007), methanol (Priego-Capote *et al*. 2004; Pérez-Seradilla *et al*. 2007), and hexane (Pérez-Seradilla *et al*. 2007). The use of conventional technique has been shown to be time-consuming, thermally unsafe and the analysis of the constituents of the extracts is limited by the extraction step (Mandal *et al*. 2007). In addition, it requires the consumption of a significant amount of solvent and the long contact time of about eight to twenty four hours or longer (Naidu, *et al*. 2008).

An improvement to the conventional method of extraction is the microwave–assisted extraction (MAE) where microwave energy is used to heat solvents in contact with solid samples and to partition compounds of interest from the sample into the solvent (Pérez – Seradilla *et al*. 2007). The advantages of this method include fast extraction with less solvent used. In addition, heating occurs in a targeted and selective manner with practically no heat lost to the environment (Huie, 2002).

MAE is influenced by several factors. It requires elevated temperature to increase the capacity of the solvent to solubilize the analyte from the plant (Mandal *et al*. 2007), but prolonged extraction time may result in less amount of extract (Pan *et al*. 2003; Katsube *et al*. 2009) and degradation of active components (Hu *et al*. 2009). MAE also requires high solvent concentration to increase the mass transfer of the active constituents into the extracting solvent (Wang *et al*. 2006) but this decreases the partition coefficient resulting in lower yield. In addition, the solvent volume must be sufficient to ensure that the plant matrix is entirely immersed in the solvent, but excessive solvent causes inadequate stirring of the solvent by microwaves resulting in lower yield. The challenge is to select the right condition that will result in high extract yield and at the same time preventing the degradation of the active components.

This study applied microwave-assisted extraction of chlorogenic acid from *Coffee liberica L*. in the presence of ethanol as extracting solvent by varying the extraction temperature, extraction time, solvent-to-solid ratio and solvent concentration. The same material was extracted by Soxhlet method and the yield from the two methods were compared.

MATERIALS

Roasted *Coffe liberica L*. was obtained from Marlo Agricultural Corporation which came from one batch of harvest as certified by the supplier. This eliminates other factors related to cultivation and harvest that may have effect on the chemical components of the coffee. The coffee beans were ground by means of a ball mill and then sieved using Tyler standard screens. Samples that passed

Table 1. $L_9 3^4$ Taguchi Orthogonal Array

Run	Factors			
	Temperature °C	Extraction time, min	Solvent-to-solid ratio mL g^{-1}	Solvent concentration (v v^{-1})
1	50	5	2.5	0.6
2	50	7	5.0	0.7
3	50	10	7.5	0.8
4	70	5	5.0	0.8
5	70	7	7.5	0.6
6	70	10	2.5	0.7
7	90	5	7.5	0.7
8	90	7	2.5	0.8
9	90	10	5.0	0.6

through mesh 35 (0.422 mm) but retained on mesh 48 (0.295 mm) were collected, stored in a dry clean jute sack and placed in dry cool place until used for the experiment.

The solvent used for extraction was ethanol because of its relatively high dielectric constant suited for microwave absorption (Zhou and Liu, 2006). In addition, it has a good polarity, hence a good extracting solvent for polyphenols. For the determination of total phenolic content in the extract, Folin-Ciocalteau reagent (Fluka) and 7% aqueous solution of sodium carbonate (AR grade) were used. Analytical grade chlorogenic acid (Sigma Aldrich) was used as reference.

EXPERIMENTAL

Design of Experiment

In the extraction of the ground coffee beans, the factors considered were temperature, extraction time, solvent concentration, and solvent-to-solid ratio. Each factor was varied at three levels. Preliminary experiments were conducted to determine the appropriate values of the three levels in each factor. The values of factors used in the preliminary experiments were based on previous studies on MAE involving plant materials.

The combinations of factors and levels were obtained following the Taguchi $L_9 3^4$ orthogonal array design of experiment shown in Table 1. The response is the total phenolic yield expressed as gallic acid equivalence per litre (mg GAE L^{-1})

The Taguchi method is a technique where the influence of individual factors on the experiment output and interaction within the factors can be studied in the shortest possible number of experimental trials. In this case, nine different treatments were used and triplicate runs were done for each treatment.

Experimental Procedures

Ground roasted *Coffee liberica L* was mixed with ethanol with pre-determined

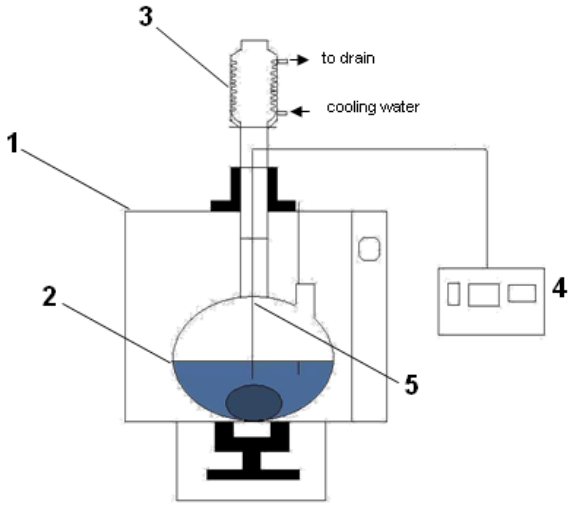


Fig. 1: Experimental set – up

1. **Microwave oven - a** hole was bored on the upper cavity to establish connection between the reactor inside, the temperature control system and the condenser.
2. **Reactor -** 250 ml spherical flask with two openings. The first opening served as port for the thermocouple while the other is for the condenser
3. **Condenser and cooling system**
4. **Control Terminal 320 -** used to set the microwave power, irradiation time and extraction temperature
5. **Thermocouple (ATC-300) and Temperature Control System -** connected to the thermocouple to keep the temperature at the desired set point

concentration and desired solvent-to-solid ratio. The mixture of coffee and solvent was irradiated by microwave at a constant microwave power input of 400 W and extraction was conducted at different temperatures and irradiation time. The extraction was done in a modified domestic microwave oven (Whirlpool). The experimental set-up is shown in figure 1. In all experimental runs, 2 g of ground coffee was used.

The extracts were concentrated by evaporating the solvent at 45°C for about 5 minutes using a rotary evaporator (Heidolph-Loborota, rotavap). The extracts were analyzed to determine the yield.

The data obtained were analyzed to determine the factors that significantly affected the yield. Also, main effects plot of the yield were constructed and the level of the factor that resulted in the highest yield was determined. These values were pooled and used in a confirmatory run following the same extraction procedures.

The ground roasted coffee was also extracted by Soxhlet extraction method using aqueous ethanol. The solvent concentration and solvent-to-solid ratio used were the same as the ones used in the confirmatory run. The yield obtained in this process was compared with that obtained in the confirmatory run.

Analytical Techniques

The yield in terms of total phenolic content of the concentrated extracts was determined using Folin-Ciocalteau method and the result was expressed as mg gallic acid equivalence per litre (mg GAE L^{-1}). Standard solutions of gallic acid of

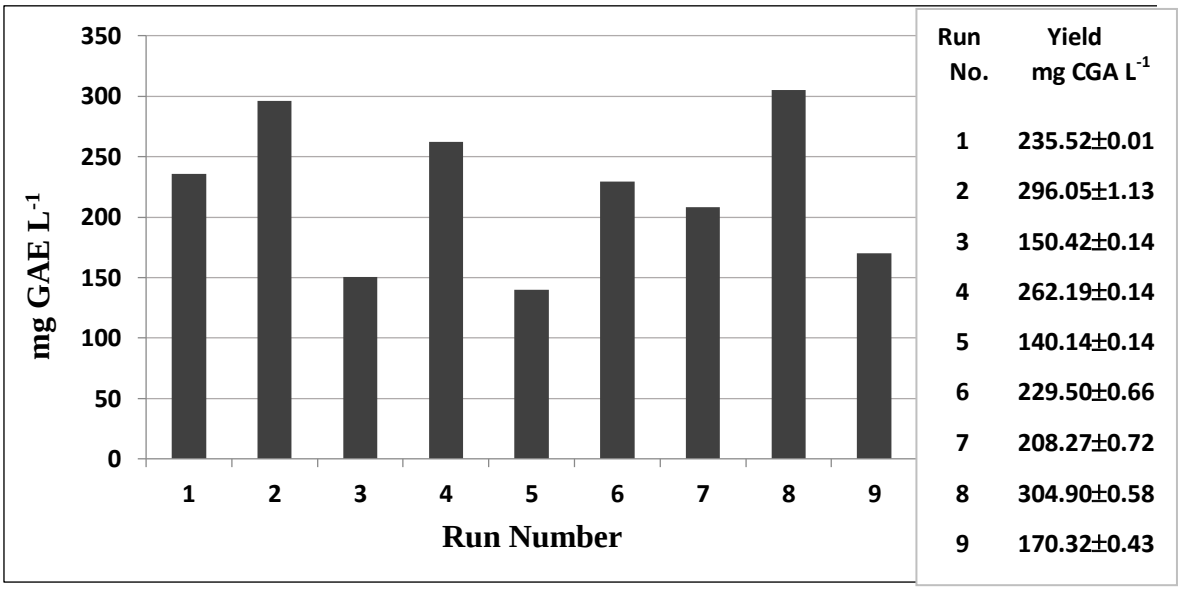
different concentrations were prepared and each was reacted with Folin-Ciocalteau reagent and 7% aqueous solution of sodium carbonate. The standards were incubated for 90 minutes after which the absorbance was measured at a wavelength of 765nm. Based on this, a calibration curve was prepared which served as reference for the extracts. To determine the total phenolic content of the extracts, the same procedure was followed but instead of gallic standards, this was replaced with the extract.

The extract from the confirmatory run was also analyzed using HPLC (Agilent 1200) to determine the presence of CGA and its concentration. The extract was first centrifuged to remove the solids and the supernatant liquid was injected to the column (Phenomenex Luna C18, 5u, 250 x 4.6mm) and eluted with a gradient elution of mobile phase A consisting of 5% acetonitrile in 0.035% trifluoroacetic acid (TFA) and B consisting of 80% acetonitrile in 0.025% TFA where the flow rate of B was increased from 10% to 20% in 10 minutes, to 50% in 20 minutes and maintained for 5 minutes before the next injection.

RESULTS AND DISCUSSION

The extract from coffee appeared to be dark brown and turbid due to the presence of suspended solid and has a strong smell of brewed coffee. The amount of extract varied as the extraction condition was changed. For the same solvent-to-solid ratio, solvent concentration and extraction time, the extract collected at 90°C was about half the extract obtained at 50°C. The same is true when the solvent-to-solid ratio was varied while maintaining the other parameters constant.

The yield obtained from each experimental run is illustrated in figure 2. It can be observed that the highest yield (304.90±0.58 mg GAE L^{-1}) was obtained in Run 8 where extraction took place at 90°C for 7 min using a solvent-to-solid ratio of 2.5 mL g^{-1} and solvent concentration of 0.8



Run No.	Yield mg CGA L^{-1}
1	235.52±0.01
2	296.05±1.13
3	150.42±0.14
4	262.19±0.14
5	140.14±0.14
6	229.50±0.66
7	208.27±0.72
8	304.90±0.58
9	170.32±0.43

Fig. 2: Yield in the nine experimental Runs

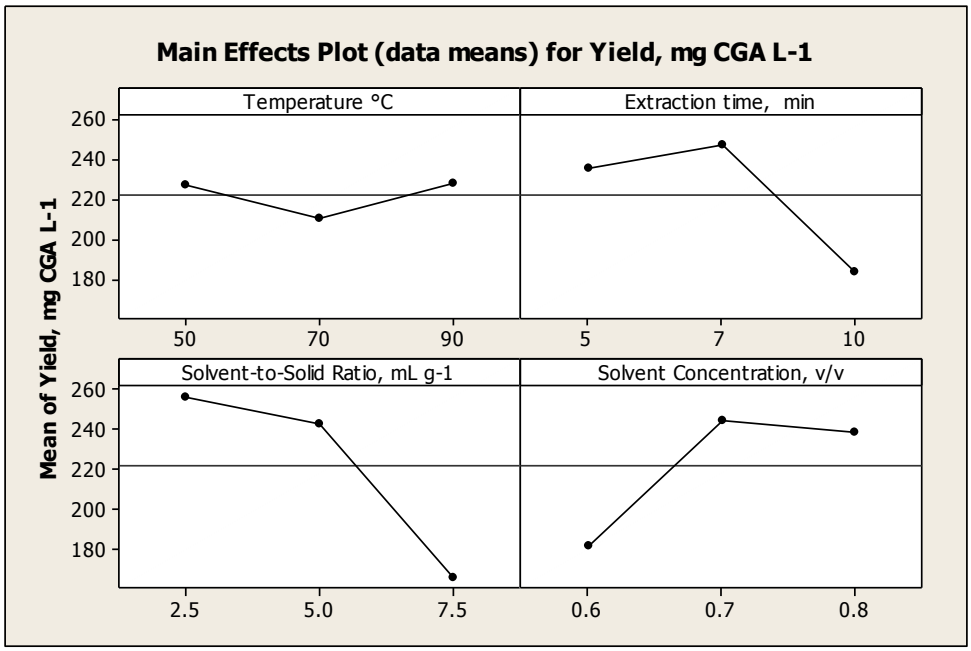


Fig. 3: Main effects plot of yield

(v v^{-1}). For the same extraction time at lower temperature and solvent concentration and twice the amount of solvent used, a lower yield of 296.05±1.13 mg GAE L^{-1} (Run 2) was obtained. On the other hand, the lowest yield (140.14±0.14) was obtained at the 70°C, same extraction time of 7 minutes but at a relatively higher solvent-to-solid ratio of 7.5 mL g^{-1} (3x higher) and lower solvent concentration of 0.6(v v^{-1}). The low yield could be attributed to the excessive amount of solvent used and low solvent concentration.

Main effects plot for yield was constructed and is shown in figure 3. It can be observed that within the temperature range tested, the yield was not appreciably affected by the temperature. The yield at 50°C and 90°C was almost the same. With regard to extraction time, the yield increased when irradiation time was increased from 5 to 7 minutes, but extending it beyond 7 minutes resulted in significant decrease in yield. Generally, by increasing the extraction time, the quantity of analyte extracted is increased, but degradation of active components may occur if material is overexposed.

Increasing the solvent-to-solid ratio from 2.5 to 5 mL g^{-1} resulted in 5.4% decrease in yield. Further increase of the ratio to 7.5 mL g^{-1} decreased the yield even more. This trend is consistent with that of Pan *et al*. (2003) in the extraction of phenolics from green tea leaves. Although conventional extraction methods require large amount of solvent, MAE greatly differs due to the inadequate stirring of the solvent in the MAE extraction process (Wang and Weller, 2006).

The increase in solvent concentration from 0.6 to 0.7 (v v^{-1}) resulted in the increase of yield by 34.4%. However, when the solvent concentration was further increased to 0.8 (v v^{-1}), the yield started to decrease. The initial increase in yield with increase in solvent concentration could be explained by the high driving force at the

start of extraction due to concentration gradient. However, as the concentration of the polyphenol in the extract increases, the concentration gradient decreases until equilibrium is reached which means that the concentration of the target compound in the solid material and in the solvent are the same. When this happens the partition coefficient of the analyte between the solid phase and aqueous solvent phase decreases and there will no longer be mass transfer of the active material from solid to the solvent (Gamse, 2002).

A multiple regression analysis at 5% significance level showed that the factor with the most significant effect was (in decreasing order): solvent-to-solid ratio, solvent concentration, extraction time and temperature.

Looking at the individual effect of the four parameters illustrated in the main effects plot, the highest yield was obtained at 90^{o}C, extraction time of 7 minutes, solvent-to-solid ratio of 2.5 mL g^{-1} and solvent concentration of 0.7 (vv^{-1}). By combining these values of the four factors in the confirmatory run a yield of 854.35±3.35 mg GAE L^{-1} was obtained which is significantly higher than the highest yield obtained in the treatments of the Taguchi design. The values of the factors in the confirmatory run was the same as Run 8 except the solvent concentration which is lower in the confirmatory run (0.7 v v^{-1}) than in Run 8 (0.8 v v^{-1}). The extract was analyzed by HPLC and chlorogenic acid was found to be present at a concentration of 3152 mg L^{-1}.

In the Soxhlet extraction of the ground coffee beans using the same temperature, solvent concentration and solvent-to-solid ratio used in the confirmatory run of MAE with extraction time of one hour, the yield obtained was 570.42±5.3 mg GAE L^{-1}. This is significantly lower than the yield obtained in MAE despite the longer extraction time of one hour compared to 7 minutes in the MAE. This suggests that the use of MAE is more effective in the extraction of polyphenols from coffee.

CONCLUSIONS

The use of MAE proved to be effective in extracting polyphenols from *Coffee liberica L*. Of the four factors tested, the solvent-to-solid ratio had the most significant positive effect on yield, whereas temperature had no significant effect. With regard to solvent concentration, high yield could be obtained only at concentration up to 0.7 (vv^{-1}). The same is true for the extraction time where the highest yield was obtained at 7 minutes irradiation time. Prolonging the extraction time beyond 7 minutes proved to have adverse effect on yield. In addition, using excessive amount of solvent has negative effect on yield. The highest yield of yield 304.90±0.58 mg GAE L^{-1} was obtained at 90^{o}C in 7 min of extraction using a solvent-to-solid ratio of 2.5 mL g^{-1} and solvent concentration of 0.7 (vv^{-1}). Other process parameters could be tested such as power input and type of solvent. The values of the parameters tested may also be varied to optimize the yield.

The yield obtained in the Soxhlet extraction was 570.42±5.3 mg GAE L^{-1}. For the same solvent concentration and solvent-to-solid ration and longer

extraction time of one hour, the yield was significantly lower than the yield of 854.35±3.35 mg GAE L^{-1} obtained in MAE. Thus, it can be concluded that MAE is more effective than the conventional method in extracting polyphenols from plant materials such as coffee.

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