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Research Article

Effect of drying temperature on antioxidant and antimicrobial activities of coffee pulp extract

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Abstract

The aim of this study was to investigate the effect of tray drying on antioxidant and antimicrobial activities of coffee pulp extract (CPE). Coffee pulp (CP) was dried at 70 ℃, 80 ℃ and 90 ℃ using tray dryer until the moisture content of sample reached 13 %. Then, the CP was extracted with water to obtain CPE70, CPE80 and CPE90, respectively. Bioactive compounds, antioxidant and antimicrobial activities against some pathogenic bacteria of the CPEs were determined and compared to those of the freeze dried sample (CPEFD). It was found that total phenolic content of the CPE90 (2.46 ± 0.02 gallic acid equivalent (GAE) /100 g CPE db) was higher than that of the CPEFD (2.19 ± 0.04 GAE/ 100 g CPE db), CPE70 (1.78 ± 0.02 GAE/ 100 g CPE db) and CPE80 (1.73 ± 0.02 GAE/100 g CPE db), respectively. The antioxidant activity of CPE90 (1,303.44 ± 56.09 g trolox equivalent (TE)/ 100 g CPE db) was higher than that of the CPEFD (732.69 ± 30.28 g TE /100 g CPE db), CPE80 (688.24 ± 41.10 g TE /100 g CPE db) and CPE70 (548.36 ± 31.93 g TE/ 100 g CPE db), respectively. At 200 mg/ml of CPE solution, it was found that antimicrobial activity of the CPE90 exhibited the highest inhibitory effect against *Staphylococcus aureus*, followed by CPEFD, CPE80 and CPE70, respectively.

Keywords: inhibitory effect, pathogenic bacteria, total phenolic compounds

Introduction

Chiang Rai is the most Arabica coffee (Coffea arabica L.) cultivation area in Thailand, particularly, Doi Chang contributing about 67 % of Arabica coffee production in Thailand (Pandey et al., 2000). Coffee pulp (CP) is a byproduct of coffee processing accounting for approximately 55 % of the whole coffee. It was reported to have bioactive compounds with antioxidant and antimicrobial activities (Murthy & Naidu, 2012). Drying is one of the methods used to reduce agricultural material weight, hence facilitating and reducing the transportation cost. However, optimum drying temperature must be applied to minimize the loss of bioactive compound content in the agricultural material, in order to maintain their functionalities, bioactivities, such as antioxidant, antimicrobial activities, etc. Several works reported that low temperature might have less effect on those properties, but the drying temperature should be high enough to inactivate the enzymes responsible for reducing bioactive compounds and bioactivity of the extract (Wojdylo et al., 2007; Shahidi & Naczk, 2003). Punbusayakul & Setha

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(2014) reported that sun drying method could retain the bioactive compounds which contributed to the higher antioxidant activity of the CP extract (CPE) compared to tray drying at 60 °C and higher temperature was recommended to inhibit the enzymes responsible for reducing those bioactive compounds as well as the antioxidant activity.

Limited works have been conducted on drying temperature effect on antioxidant and antimicrobial activities of the CPE (Punbusayakul & Setha, 2014). This research, therefore, was aimed to investigate the effect of the tray drying temperature (70 °C, 80 °C and 90 °C) on antioxidant and antimicrobial activities of CPE.

Materials and methods

Chemicals and reagents

All reagents were analytical grade. Folin–Ciocalteu's phenol reagent, gallic acid (≥ 99 %) and methanol (HPLC–grade) were purchased from Fluka (Buchs, Switzerland). Trolox ((±)–6–Hydroxy-2, 5, 7, 8–tetra–methylchromane–2–carboxylic acid) and DPPH (2, 2–diphenyl–1–picryhydrazyl) were purchased from Aldrich (Steinheim, Germany). Caffeine and chlorogenic acid standards were purchased from Sigma–Aldrich (USA). All tested microorganisms (Staphylococcus aureus; TISTR 1466, Salmonella typhimurium; TISTR 292, Escherichia coli; TISTR 780, Bacillus subtilis; TISTR 008) were purchased from Microbiological Resources Center, Thailand Institute of Scientific and Technological Research (TISTR), except Pseudomonas aeruginosa ATCC 27853 which was purchased from DMST culture collection, Department of Medical Sciences Thailand.

Coffee pulp (CP)

The CP was obtained from Doi Chaang Original Co. Ltd., Chiang Rai, Thailand in February 2014. The obtained CP was frozen at -20 °C until further used.

The CP was thawed to room temperature and directly freeze dried or dried at 70 °C, 80 °C and 90 °C using a tray dryer until the moisture content was ≤ 13 %. The dried CP was mixed with distilled water a ratio at 1:2 and blended for 3 min. Then, the mixture was filtered through the cotton wool. Subsequently, the filtrate was evaporated under reduced pressure in order to get the CP crude extract (Punbusayakul and Setha, 2014). The extract was then diluted in a certain concentration in order to be used for the determination of bioactive compounds, antioxidant and antimicrobial activities.

Determination of total phenolic compound (TPC)

The TPC was determined as described by Singleton and Roosi. (1965) with modifications. Gallic acid (10, 20, 40, 60, 80 and 100 µg/ ml) was used as a standard. Folin-Ciocalteu's reagent (10 % v/v, 2.5 ml) and sodium carbonate (7.5 % w/v, 2.0 ml) were added to 0.5 ml of the diluted sample or standard and vortexed. Then, the solution was left at room temperature for 60 min before the absorbance was determined at 765 nm. Total phenolic content of the sample was expressed as grams gallic acid (GAE)/ 100 grams CPE (dry basic (db)).

Determination of caffeine (CF) and chlorogenic acid (CGA) content

The CF and the CGA in the CPE were determined by the UV-spectrophotometry method described by Belay et al. (2008) and Belay & Gholap (2009), respectively. The CPE solution was mixed with dichloromethane at the ratio of 1:1, and the mixture was stirred for 10 min. After

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that, the mixture was transferred to a separating funnel. The CGA was in the upper layer, while the CF was in the lower layer. The CPE solution was extracted three times each with 20 ml of dichloromethane. The lower layer (CF) was subjected for absorbance determination at 260 nm using dichloromethane as a blank. The absorbance of the upper layer (CGA) was determined at 324 nm using distilled water as a blank. Caffeine content and chlorogenic acid content of the sample was expressed as grams caffeine (CF) per 100 grams CPE (dried basic, db) and grams chlorogenic acid (CGA) per 100 grams CPE (dried basic, db), respectively.

Determination of total monomeric anthocyanin (TMA)

The TMA of the CP was determined using the pH-differential method (Lee et al., 2005). The absorbance of the two tested portions diluted with pH 1.0 buffer (potassium chloride, 0.025 M), and pH 4.5 buffer (sodium acetate, 0.4 M) was determined at 520 and 700 nm, respectively, using distilled water as a blank. The TMA was then calculated regarding to the amount of the sample used to prepare extract and expressed as milligrams C-3-G equivalents per 100 grams CPE (db).

Determination of DPPH scavenging activity (DPPH Assay)

The DPPH assay was determined by the method of Brand-Williams (1995). The CPE solution (50 µL) and DPPH methanolic solution (1950 µL) were transferred to a test tube. The mixture was thoroughly mixed and kept in the dark for 30 min. The absorbance was determined at the wavelength of 517 nm using methanol as a blank.

Determination of ferric reducing antioxidant power activity (FRAP Assay)

The FRAP assay was conducted according to Benzie & Strain (1996) with some modifications. The FRAP reagent was prepared by mixing 10 mM 2,4,6-tripyridy-s-triazine (TPTZ), 20 mM Iron (III) chloride ($FeCl_3$) and 300 mM acetate buffer (sodium acetate dissolved in distilled water and the pH was adjusted to 3.6 by acetic acid) at the ratio of 1:1:10. Then, the extract at an appropriate concentration (0.2 ml) was mixed with 1.8 ml of the FRAP reagent and mixed well. The mixture was incubated in a water bath at 50 °C for 30 min, then, the absorbance at the wavelength of 595 nm was determined using distilled water as a blank.

Determination of antimicrobial activity

The disc diffusion method was conducted according to the National Committee for Clinical and Laboratory Standards Institute (2006). Within 15 min after adjusting the turbidity of the bacterial suspension, a sterile cotton swab was dipped into the suspension and swabbed on Mueller-Hinton Agar (MHA). The CPE are diluted to 200 mg/ml. Then, the CPE (20 µL) was impregnated onto the sterile paper disc (6.0 mm diameter). After the disc was dried, it was placed onto the surface of the microbial lawn on MHA. The plate was incubated at 37 °C for 24 hrs. Then, the inhibition zone was determined (mm).

Statistical analysis

All tests were conducted in triplicate for verification of the results. Mean values comparison at p-value < 0.05 was considered significantly different.

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Results and discussion

Bioactive compounds of CPE

The TPC, CGA, CF and TMA content in the extracts are shown in Table 1. The results showed that tray drying at 90 °C could preserve most of bioactive compounds. All bioactive compounds of the CPE obtained from the CP dried at 90 °C (CPE90) were higher than those of the freeze dried (CPEFD) and the CP dried samples at the other temperatures, respectively. These may due to the oxidative and hydrolytic enzymes responsible for the bioactive compounds losses during drying are deactivated at the temperature, hence the bioactive compounds preserved (Wojdylo et al., 2007; Šarić et al., 2013). These results are consistent with Punbusayakul and Setha (2014) reported that using higher temperature could preserve the bioactive compounds in the CPE.

Table 1. Bioactive compounds of the CPE from the CP dried by tray drying (at 70, 80 and 90 °C) and freeze drying.

Conditions		Bioactive compounds			
		Total phenolic content (g GAE/ 100 g db)	Chlorogenic acid content (g/ 100 g db)	Caffeine content (g/ 100 g db)	Anthocyanin (C-3-G mg/ 100 g db)
Tray drying	70 °C	1.78± 0.02^{c}	0.29± 0.01^{d}	0.14± 0.01^{d}	0.24± 0.01^{c}
	80 °C	1.73± 0.02^{d}	0.42± 0.00^{c}	0.28± 0.03^{c}	0.27±0.01^{c}
	90 °C	2.46± 0.02^{a}	0.66± 0.01^{a}	0.60± 0.02^{a}	0.52±0.06^{a}
Freeze drying		2.19± 0.04^{b}	0.55± 0.03^{b}	0.34± 0.04^{b}	0.37±0.01^{b}

Note: Means with the same superscripts in the columns indicate no significant difference ($p < 0.05$), GAE = gallic acid equivalent, C-3-G = cyaniding-3-glucoside equivalent, CP = coffee pulp, CPE = coffee pulp extract

Antioxidant activity of CPE

The antioxidant activity, obtained from both DPPH and FRAP assays, increased with the increasing tray drying temperature. The CPE90 exhibited the highest antioxidant activity (1303.44 g TE/ 100 g CPE db; DPPH assay) followed by the CPE obtained from freeze drying, CPEFD (732.69 g TE/ 100 g CPE db), CP dried at 80 °C, CPE80 (688.24 g TE/ 100 g CPE db) and at 70 °C, CPE70 (548.36 g TE/ 100 g CPE db), respectively. The antioxidant activity obtained from the FRAP assay also shows the same tendency (Table 2). These results are consistent with the bioactive compounds of the CPE which suggested that exposure of the CP to the higher temperature is able to preserve the bioactivity of the CPE. The lower temperature might allow the enzymes to convert the active compounds to some other inactive derivatives before the enzymes have been inactivated or some compounds might be converted to the more active compounds as a result of the higher temperature exposure (Wojdylo et al, 2007; Šarić et al., 2013).

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Table 2. Antioxidant activities of the CPE from the CP dried by tray drying (at 70, 80 and 90 °C) and freeze drying.

Conditions		Antioxidant activity	
		DPPH assay (g TE/ 100 g db)	FRAP assay (g FE/ 100 g db)
Tray drying	70 °C	548.36± 31.93^{c}	1172.60± 60.37^{d}
	80 °C	688.24± 41.10^{b}	2066.61± 25.35^{c}
	90 °C	1303.44± 56.09^{a}	4334.21± 30.61^{a}
Freeze drying		732.69± 30.28^{b}	3168.44± 73.18^{b}

Note: Means with the same superscripts in the columns indicate no significant difference ($p < 0.05$), TE = trolox equivalent, FE = ferrous sulfate equivalent, CP = coffee pulp, CPE = coffee pulp extract

Antimicrobial activity of CPE

Table 3 shows that the antimicrobial activity of the CPE from all treatments against all the tested microorganisms, except *E. coli* TISTR 780 and *S. aureus* TISTR 1466, showed no significant difference ($p > 0.05$). The inhibitory effect against *E. coli* TISTR 780 of the CPE90 was higher than that of the CPEFD and the CPE80, but it was not significantly different from the CPE70. Whereas, the inhibitory effect against *S. aureus* TISTR 1466 of the CPE90 exhibited no significant difference from the freeze dried one, but it is evidently higher than that of the CPE80 and the CPE70, respectively. These results suggest that tray drying at 90 °C is an optimum temperature that could preserve the bioactive compounds, consequently maintaining the CPE antimicrobial activity.

Table 3. Inhibitory effect (inhibition zone, mm) of the CPE (200 mg/ mL) from the CP dried by tray drying (at 70, 80 and 90 ° C) and freeze drying against some food pathogens

Microorganisms	Tray drying			Freeze drying
	70 °C	80 °C	90 °C	
Gram- negative bacteria				
Pseudomonas aeruginosa ATCC 27853 ^{ns}	8.44± 0.19	8.65± 0.09	8.74± 0.21	8.63± 0.09
Escherichia coli TISTR 780	8.89± 0.69^{ab}	8.49± 0.16^{b}	9.45± 0.23^{a}	8.49± 0.16^{b}
Salmonella typhimurium TISTR 292 ^{ns}	8.33± 0.33	8.21± 0.10	8.40± 0.23	8.23± 0.07
Gram-positive bacteria				
Bacillus subtilis TISTR 008 ^{ns}	8.11± 0.38	8.33± 0.33	8.49± 0.17	8.30± 0.33
Staphylococcus aureus TISTR 1466	7.56± 0.19^{c}	7.90± 0.87^{b}	8.28± 0.25^{a}	7.97± 0.14^{ab}

Note: Means with the same superscripts in the rows indicate no significant difference ($p < 0.05$), ns = no significant diference, CP = coffee pulp, CPE = coffee pulp extract

Conclusion

All bioactive compounds and antioxidant activity of CPE could be preserved by tray drying at 90 °C as observed by the higher bioactive compounds and higher antioxidant activity compared to those of the freeze dried one. These results were consistent with the antimicrobial

activity of the CPE, exhibiting the higher antimicrobial activity of the CPE obtained from the CP dried at 90 °C by tray drying against some pathogens compared to that of the CPEFD.

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