

Abstract

Cold brew coffee is a preferred choice among health-conscious consumers due to its cold extraction process. By utilizing cold water with longer extraction time, thereby the coffee is preserving bioactive compounds more effectively than hot extraction. However, there are several protocols of cold brew extraction that yield the different biological activities. In this study, we aimed to analyze the effects of the extraction method on antioxidant activity, total phenolic content (TPC), and antibacterial properties in cold brew coffee and spent coffee grounds after extraction. Three arabica coffee that fermented with *Lactiplantibacillus plantarum* JT-PN39 (LP), *L. plantarum* JT-PN39 combined with *Pichia kluyveri* (LPI), and *L. plantarum* JT-PN39 combined with *Yarrowia lipolytica* (LYar) were extracted using cold drip and immersion method. Antioxidant activity and TPC of cold brew coffee were assessed by ABTS⁺ and Folin-Ciocalteu assay, respectively. The results indicated that cold drip extraction exhibited significantly higher antioxidant activity and TPC than immersion with a statistically significant difference values of LP, LPI, and LYar. The radical scavenging values for cold drip extraction were 48.39 ± 6.60%, 75.88 ± 5.04%, and 65.44 ± 2.52%, respectively, while those from immersion were 15.84 ± 1.58%, 40.12 ± 4.84%, and 24.81 ± 1.58%, respectively. Similarly, TPC values for cold drip extraction were 35.50 ± 0.11, 58.72 ± 0.69, and 52.68 ± 0.18 mg GAE/g, respectively, while those from immersion were 10.34 ± 0.37, 18.66 ± 0.76, and 12.39 ± 0.09 mg GAE/g, respectively. Antibacterial property was tested against foodborne pathogens, including *Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, using agar well diffusion. Cold drip extraction inhibited *B. cereus* and *S. aureus*, whereas none of the bacteria were inhibited by immersion. Consequently, determination of the relationship between cold drip extraction and storage effect on biological property changes were observed at 0, 30, 60, and 90 days. Both antioxidant activity and TPC significantly decreased from fresh extraction with 2 times and 3 times lower at day 90. The antibacterial property against *B. cereus* initially showed MIC of 62.5 mg/mL but declined throughout the storage period. In contrast, the inhibition of *S. aureus* remained stable at MIC 62.5 mg/mL throughout the storage period. Biological properties of three spent coffee grounds after cold drip extraction were evaluated by water and 75% ethanol extraction. Water extraction demonstrated significantly higher antioxidant activity with values of 38.01 ± 6.90%, 12.67 ± 5.71%, and 21.12 ± 5.09%. Similarly, TPC exhibited values of 17.85 ± 0.87, 5.85 ± 0.53, and 10.51 ± 0.90 mg GAE/g, whereas none of the bacteria were inhibited. However, 75% ethanol extraction exhibited the activity against *B. cereus*, *L. monocytogenes*, and *S. aureus* at MIC 25 mg/mL. These findings indicated that the extraction method for cold brew coffee preparation significantly influenced the solubility of bioactive compounds and will be applied for industrial extraction scale. Moreover, the spent coffee grounds after cold drip extraction have the potency for further extraction of high value compounds which are able to supply in food or cosmetic industries.

Objectives

- To study the effects of different extraction methods on biological properties, including antioxidant activity, total phenolic content, and antibacterial properties, of cold brew coffee.
- To study the biological properties, including antioxidant activity, total phenolic content, and antibacterial properties, of spent coffee grounds after extraction.

Methodology

1. Cold brew coffee extraction

1.1 Cold drip

The method uses a cold drip coffee maker. Cold water slowly drips through the coffee grounds.

1.2 Immersion

The method involves placing the coffee grounds in a coffee filter in water inside a sealed container before filtering the coffee.

2. Spent coffee grounds extraction

2.1 Water

The method involves using water by boiling it, then filtering the extract.

2.2 75% Ethanol

The method involves using 75% ethanol by using a rotary shaker, then filtering the extract.

3. Antioxidant activity



The ABTS⁺ solution at 734 nm, with an absorbance of 0.700 ± 0.02, is a free radical for testing with the samples.

The absorbance is measured at 734 nm to calculate the % radical scavenging of the samples.

4. Total phenolic content

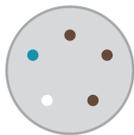


A 10% Folin-Ciocalteu phenol reagent and a 7.5% Na₂CO₃ solution are used to measure the phenolic content in the samples.

The absorbance is measured at 765 nm to calculate the phenolic content in mg GAE/g dry weight of the samples.

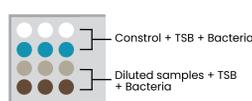
5. Antibacterial properties

5.1 Agar well diffusion



MHA is used for *B. cereus*, *E. coli*, *P. aeruginosa*, and *S. aureus*, while TSA is used for *L. monocytogenes* in the inhibition zone test of the samples.

5.2 MIC



Select the bacteria that showed inhibition in the agar well diffusion method to test the minimum inhibitory concentration on a 96-well plate using TSB and the bacteria with an inhibition zone for testing with the samples.

5.3 MBC



Select the concentration of samples that can inhibit bacteria based on the MIC to test for the minimum bactericidal concentration on agar, using the same agar as in the agar well diffusion method.

Positive control: gentamicin 200 µg/ml Negative control: water / 75% ethanol

Results

1. Antioxidant activity and total phenolic content

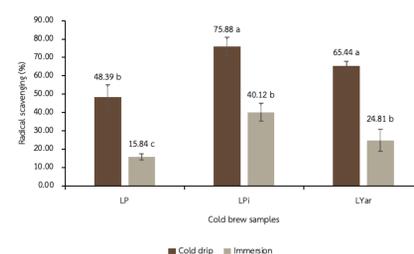


Figure 1. Comparison of % radical scavenging of cold drip coffee and immersion coffee at 250 mg/mL.

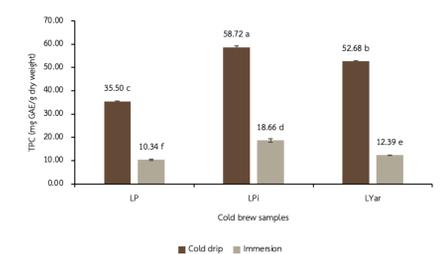


Figure 2. Comparison of TPC of cold drip coffee and immersion coffee at 250 mg/mL.

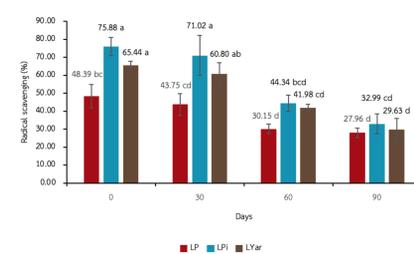


Figure 3. The % radical scavenging of cold drip coffee at 0, 30, 60, and 90 days.

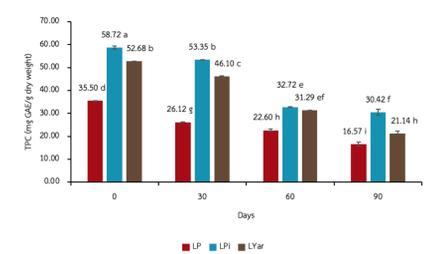


Figure 4. TPC of cold drip coffee at 0, 30, 60, and 90 days.

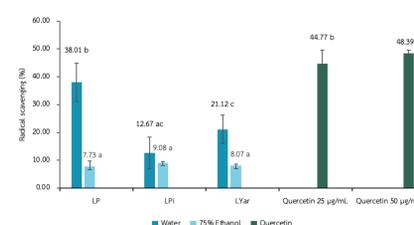


Figure 5. Comparison of % radical scavenging of water extract and 75% ethanol extract at 100 mg/mL.

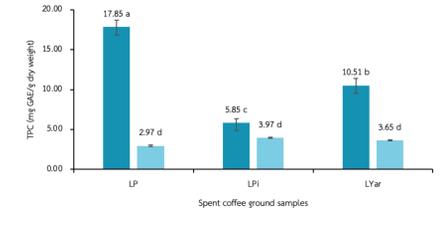


Figure 6. Comparison of TPC of water extract and 75% ethanol extract at 100 mg/mL.

2. Antibacterial properties

Cold brew samples	Inhibition zone (mm)				
	<i>B. cereus</i>	<i>E. coli</i>	<i>L. monocytogenes</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
Cold drip					
LP	13.22 ± 0.77	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	15.78 ± 0.51
LPI	13.11 ± 0.69	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	14.22 ± 1.07
LYar	12.44 ± 0.77	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	15.33 ± 1.00
Gentamicin 200 µg/mL	20.00 ± 1.00	28.67 ± 0.58	25.33 ± 0.58	25.33 ± 0.58	25.67 ± 0.58
Water	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Immersion					
LP	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
LPI	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
LYar	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Gentamicin 200 µg/mL	28.33 ± 0.58	27.67 ± 0.58	26.33 ± 0.58	26.33 ± 0.58	26.33 ± 0.58
Water	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 1. Inhibition zone of cold drip coffee and immersion coffee at 250 mg/mL.

Cold drip spent coffee ground samples	Inhibition zone (mm)				
	<i>B. cereus</i>	<i>E. coli</i>	<i>L. monocytogenes</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
Water					
LP	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
LPI	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
LYar	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Gentamicin 200 µg/mL	24.67 ± 0.58	20.33 ± 0.58	24.33 ± 1.15	20.67 ± 0.58	25.33 ± 0.58
Water	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
75% Ethanol					
LP	13.89 ± 0.77	0.00 ± 0.00	10.11 ± 0.69	0.00 ± 0.00	11.11 ± 0.38
LPI	11.89 ± 2.69	0.00 ± 0.00	10.78 ± 1.35	0.00 ± 0.00	10.22 ± 0.38
LYar	12.11 ± 1.26	0.00 ± 0.00	9.00 ± 0.33	0.00 ± 0.00	9.33 ± 1.15
Gentamicin 200 µg/mL	24.67 ± 0.58	20.33 ± 0.58	24.33 ± 1.15	20.67 ± 0.58	25.33 ± 0.58
75% Ethanol	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 2. Inhibition zone of water extract and 75% ethanol extract at 100 mg/mL.

Cold drip coffee samples	Minimum inhibitory concentration (mg/mL)	
	<i>B. cereus</i>	<i>S. aureus</i>
7 days		
LP	62.5	62.5
LPI	62.5	62.5
LYar	62.5	62.5
30 days		
LP	62.5	250
LPI	62.5	250
LYar	62.5	250
60 days		
LP	62.5	250
LPI	125	250
LYar	125	250
90 days		
LP	-	62.5
LPI	62.5	250
LYar	62.5	250

Table 3. MIC of cold drip coffee at 0, 30, 60, and 90 days.

Cold drip coffee samples	Minimum bactericidal concentration (mg/mL)		
	<i>B. cereus</i>	<i>L. monocytogenes</i>	<i>S. aureus</i>
7 days			
LP	250	250	250
LPI	250	250	250
LYar	250	250	250
30 days			
LP	250	250	250
LPI	250	250	250
LYar	250	250	250
60 days			
LP	250	250	250
LPI	250	250	250
LYar	250	250	250
90 days			
LP	250	250	250
LPI	250	250	250
LYar	250	250	250

Table 4. MBC of cold drip coffee at 0, 30, 60, and 90 days.

75% Ethanol extract samples	Minimum inhibitory concentration (mg/mL)		
	<i>B. cereus</i>	<i>L. monocytogenes</i>	<i>S. aureus</i>
LP	25	25	25
LPI	25	25	25
LYar	25	25	25

Table 5. MIC of 75% ethanol extract.

75% Ethanol extract samples	Minimum bactericidal concentration (mg/mL)		
	<i>B. cereus</i>	<i>L. monocytogenes</i>	<i>S. aureus</i>
LP	50	50	50
LPI	50	50	50
LYar	50	50	50

Table 6. MBC of 75% ethanol extract.

Conclusion

The extraction method influences the biological properties of cold-brewed coffee, and storage time also affects the changes in these properties. Additionally, the spent coffee grounds after extraction have the potential for further use.