

**Title :** Antioxidant and antibacterial activity of fermented cold brew coffee extracted by cold drip method

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## ABSTRACT

Cold brew coffee has been considered for the rapid growth in coffee market around the world due to its harmony flavor. Fermentation of coffee using microorganisms is proved to enhance new flavors and taste in cold brew coffee but only few studies described the biological properties. Thus, we aimed to examine antioxidant activity, total phenolic content (TPC) and anti-foodborne bacterial of fermented cold brew coffee in this study. In addition, changing of biological properties was investigated during storage at 0, 30, 60, and 90 days. Three fermented cold brew coffee, including *Lactiplantibacillus plantarum* JT-PN39 (HLW), *L. plantarum* JT-PN39 combined with *Yarrowia lipolytica* (MOB), and *L. plantarum* JT-PN39 combined with *Pichia kluyveri* (GOD), were extracted by cold drip method. The commercial coffees (STB, SOD and HIL) were extracted by similar method and compared for their biological properties at initial storage until day 90 and the results showed that fermented cold brew coffee exhibited significantly higher TPC than commercial cold brew coffee with the values of  $35.5 \pm 0.11$  mg GAE/g (HLW),  $52.68 \pm 0.18$  mg GAE/g (MOB), and  $58.72 \pm 0.68$  mg GAE/g (GOD). Consequently, determination of the storage effect on biological property changes, showed a decreasing trends at two folds lower from day 0 until day 90. Investigation of antioxidant activity revealed higher activity than commercial ones at  $48.40 \pm 6.59\%$  (HLW),  $65.44 \pm 2.52\%$  (MOB), and  $75.88 \pm 5.03\%$  (GOD). Similar results for TPC, storage gave the negative effect on decreasing the activity from initial extraction until day 90. Anti-foodborne bacterial property was evaluated in fermented coffee grounds after extraction using *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Listeria monocytogenes*, and *Staphylococcus aureus*. Inhibitory effect was detected in *B. cereus* and *S. aureus* by agar well diffusion method and the MIC value of 62.5 mg/mL was able to kill those bacteria. In contrast, commercial cold brew coffees were not able to inhibit the bacteria on solid media. Spent coffee grounds from fermented cold brew extraction were dried and evaluated their properties by extraction with water and 75% ethanol. Water extraction revealed higher TPC than 75% ethanol extraction with the values of  $17.85 \pm 0.88$  mg GAE/g (HLW),  $10.51 \pm 0.90$  mg GAE/g (MOB), and  $5.85 \pm 0.53$  mg GAE/g (GOD). In addition, commercial coffee grounds were also examination and showed significant lower content than fermented coffee. Investigation of antioxidant activity water extraction revealed higher activity than 75% extraction with values of  $38.01 \pm 6.90\%$  (HLW),  $21.12 \pm 5.09\%$  (MOB), and  $12.67 \pm 5.71\%$  (GOD). A similar trend was observed with the TPC, commercial coffee grounds also exhibited lower significant content than fermented coffee. However, antibacterial property of coffee grounds was detected in 75% ethanol extraction but not by water. The inhibitory effect was found in *B. cereus*, *L. monocytogenes*, and *S. aureus* with the MIC value of 25 mg/mL. Studies on the extraction of coffee with water and 75% ethanol revealed water extraction had higher TPC and antioxidant activity than 75% ethanol extraction. However, 75% ethanol extraction showed better antibacterial efficacy. These findings suggested that fermented cold brew coffee and its spent coffee grounds have higher potential for health benefits than non-fermented or classical fermented coffee in the market. Spent coffee grounds, which are considered as waste, may able to extract and develop as the functional products, that promising antibacterial, antioxidant, and phenolic properties.