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Effect of Cocoa Bean Fermentation Using Lactic Acid Bacteria and Yeast Starters on Flavonoid Formation and Antioxidant Activity

ANJA MERYANDINI[1,2*], IRVAN ANWAR[3], AND TITI CANDRA SUNARTI[4]

[1] *Department of Biology, Bogor Agricultural University, Jl. Raya Dramaga, Bogor, Jawa Barat, 16680, Indonesia;*
[2] *Biotech Center, Bogor Agricultural University, Jl. Kamper, Bogor Jawa Barat, Indonesia;*
[3] *Biotechnology Study Program, Graduate School, Jl. Raya Dramaga, Bogor, Jawa Barat, 16680, Indonesia;*
[4] *Department of Agroindustrial Technology, Jl. Raya Dramaga, Bogor, Jawa Barat, 16680, Indonesia.*

This study investigates the effect of fermentation using lactic acid bacteria and yeast as starters on the formation of flavonoid compounds and the antioxidant activity of cacao beans. The fermentation process were divided into 4 groups: F1: spontaneous fermentation, F2: fermentation using Lactic Acid Bacteria (LAB), F3: fermentation using yeast and F4: fermentation using LAB and yeast. The extraction process was done using ethanol. Flavonoid content was analysis using spectrophotometer assay. The antioxidant activity was analyzed by 1,1-difenil-2-pikrilhidrazil (DPPH) method. All ethanol extract samples of fermented cacao beans contained alkaloids, polyphenols, flavonoids, and tannins. The flavonoid compounds from ethanol extract of cacao beans in F1 is 4.35 ± 0.20 mg L^{-1}, F2 (5.64 ± 0.05), F3 (5.37 ± 0.17), and F4 (5.99 ± 0.23 mg L^{-1}). The antioxidant activity of cacao bean fermentation extracts using starter were increase compared to the spontaneous fermentation extract (F1). The antioxidant activity in F2 increased to 46.45 ± 2.00%, F3 (49.05 ± 0.58%), and F4 (50.33 ± 0.43%), while the antioxidant activity of F1 was 42.31 ± 0.66%. IC50 value as the ability of the extract to reduce 50% DPPH radical on the ethanol extract of cacao beans from spontaneous fermentation (F1) was 141.67 mg L^{-1}. The IC50 value of the fermented cacao bean extract with the addition of starter was obtained at F2 at 109.30 mg L^{-1}, F3 (97.51), and F4 is 88.15 mg L^{-1}.

Key words: antioxidant, DPPH, ethanol extract, Starter

Penelitian ini mengkaji pengaruh fermentasi menggunakan bakteri asam laktat dan khamir sebagai starter terhadap pembentukan senyawa flavonoid dan aktivitas antioksidan biji kakao. Proses fermentasi dibagi menjadi 4 kelompok: F1: fermentasi spontan, F2: fermentasi menggunakan Bakteri Asam Laktat (BAL), F3: fermentasi menggunakan khamir dan F4: fermentasi menggunakan BAL dan khamir. Proses ekstraksi dilakukan dengan menggunakan etanol. Kandungan flavonoid dianalisis menggunakan spektrofotometer. Aktivitas antioksidan dianalisis dengan metode 1,1-difenil-2-pikrilhidrazil (DPPH). Semua sampel ekstrak etanol biji kakao fermentasi mengandung alkaloid, polifenol, flavonoid, dan tanin. Senyawa flavonoid ekstrak etanol biji kakao pada F1 adalah 4,35 ± 0,20 mg L^{-1}, F2 (5,64 ± 0,05), F3 (5,37 ± 0,17), dan F4 (5,99 ± 0,23 mg L^{-1}). Aktivitas antioksidan ekstrak biji kakao fermentasi menggunakan starter meningkat dibandingkan dengan ekstrak fermentasi spontan (F1). Aktivitas antioksidan pada F2 meningkat menjadi 46,45 ± 2,00%, F3 (49,05 ± 0,58%), dan F4 (50,33 ± 0,43%), sedangkan aktivitas antioksidan F1 adalah 42,31 ± 0,66%. Nilai IC50 sebagai kemampuan ekstrak untuk mereduksi radikal DPPH 50% pada ekstrak etanol biji kakao hasil fermentasi spontan (F1) adalah 141,67 mg L^{-1}. Nilai IC50 ekstrak biji kakao fermentasi dengan penambahan starter diperoleh pada F2 sebesar 109,30 mg L^{-1}, F3 (97,51), dan F4 sebesar 88,15 mg L^{-1}.

Kata kunci: antioksidan, DPPH, ekstrak etanol, Starter

Chocolates are produced from cocoa beans powder. As the 3rd largest country in cocoa production after Ivory Coast and Ghana, Indonesia plays a significant role in cocoa powder production (BPS 2020). To obtain a good quality chocolate powder, fermentation of cocoa beans is one of the processes which can bring the right good aroma (chocolate flavor) due to chemical changes during fermentation. The chocolate flavor is a chemical change caused by microbial enzymatic activity. Cocoa fermentation is characterized by a succession of microbial activity from three groups of micro-organisms, namely yeast, lactic acid bacteria (LAB) and acetic acid bacteria (AAB), which produce good cocoa beans (de Vuyst and Wecks 2016).

Cocoa beans can be naturally fermented, but the fermentation process should not be excessive because it can lead to the lowly quality aroma and taste of cacao beans. These problems can be controlled by using microbes as a starter in the fermentation process. Lactic acid bacteria and yeasts are microbes that are widely used in the fermentation process. The addition of

*Corresponding author: Phone: +62-251-8622833; Fax: +62-251-8622833; E-mail: ameryandini@apps.ipb.ac.id

Table 1 Design of cacao beans fermentation experiment

Treatment	Duration of fermentation (hour)	Specific treatment
F_1	5 × 24	Spontaneous fermentation
F_2	5 × 24	Fermented by LAB culture
F_3	5 × 24	Fermented by yeast culture
F_4	5 × 24	Fermented by combination of LAB and yeast

bacteria *Lactobacillus plantarum, Acetobacter aceti* and yeast (*Saccharomyces cerevisiae*) in the fermentation of cacao beans made the processing time shorter (Sandhya *et al.* 2016).

The application of cocoa powder in food and beverage products in the community is increasing, but the knowledge about its efficacy in health is still very limited. Cocoa beans contain secondary metabolite compounds that have good antioxidant activity (Brito *et al*. 2016). The antioxidant activity in cacao beans is due to the compound of polyphenols. Polyphenol content will decrease along with the length of fermentation time (Goya *et al*. 2022). Given the importance of the chemical processes that occur during the fermentation of cacao beans and the importance of flavonoid compounds known to have antioxidant activity, so processing of cacao beans should be able to maintain the flavonoid content. One that can be done to maintain or increase the flavonoid content is the fermentation process with the addition of a starter.

Previous research has isolated lactic acid bacteria and yeasts from cacao beans. The isolates have characters as a starter in the cacao fermentation process which is resistant to acid conditions, high temperatures, and high ethanol content and are able to produce high acid. This study aims to analyze the effect of lactic acid bacteria and yeast as a starter in cocoa beans fermentation.

MATERIALS AND METHODS

Fermentation of Cacao Beans. Lactic acid bacteria (*Lactobacillus* sp. H 2.34) and yeast (isolate IP4) used in this experiment were from spontaneously fermented cacao beans in Parungkuda Sukabumi West Java. The fermentation was conducted in 1000 mL Erlenmeyer, each containing 500 grams of cocoa beans and inoculated with 106 CFU microbes/g cocoa beans, according to Meryandini *et al.* (2019). Cocoa beans used in this research are Forastero from Central Java Coffee and Cacao Research Center of Central Java. The design experiment is shown in Table 1.

Ethanol Extraction from Cocoa Beans. The fermented cocoa beans were washed with running water to remove the remaining pulp or impurities attached to the cocoa beans, then dried and ground. The supernatant was evaporated to obtain ethanol extract from fermented cocoa beans. The powder was sieved using a sieve (60 mesh), and 100 g of cocoa bean powder into a glass container with 300 mL of ethanol as solvent. Samples were incubated for 3x24 hours; every 1x24 hours the solvent was replaced with new ethanol.

Phytochemical Component of Ethanol Cacao Bean Extract.

a. Alkaloid content was determined using reagent Dragendorff (Raaman 2006). Formation of orange color indicates presence of alkaloid.
b. Polyphenol content was determined using $FeCl_3$ 5% (Raaman 2006). Formation of green blackish color indicates presence of polyphenol.
c. Flavonoid content was determined using concentrated HCl solution and Mg powder (Rusita and Suhartono 2016). Formation of red color indicates presence of flavonoid.
d. Tannin content was determined using NaCl 10% and gelatin 2% (Tiwari *et al*. 2011). White precipitation was formed, indicating presence of tannin.

Standard Curves of Quercetin. Quercetin compounds in ten series of concentrations of 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 mg L^{-1} was used for standard. Each quercetin concentration was taken at 0.5 mL and mixed with 2 mL of distilled water and 0.15 mL of 5% NaNO2, allowed to stand for 5 minutes. A total of 0.15 mL of AlCl3 10% was added to the solution, then allowed to stand for 5 minutes. The solution was reacted with 2 mL 4% NaOH, diluted with distilled water to a volume of 5 mL, and the absorbance was measured by UV-Vis spectrophotometer at λ 510 nm.

Quantification of Flavonoid Compound. The amount of flavonoid content was carried out by spectrophotometric methods using various standard concentrations of quercetin (Madaan *et al*. 2011). As much as 0.5 mL extract (100 mg L^{-1}) was mixed with 2

Table 2 The yield of extracts, water content and residual solvents of ethanol extract of fermented cacao beans

Treatment	The Yield extract (%)	The water content and residual solvent (%)
F_1	3.92±0.09[b]	7.67±0.12
F_2	4.62±0.13[a]	7.13±0.23
F_3	3.99±0.12[b]	7.27±0.31
F_4	4.39±0.16[a]	7.20±0.35

Different numbers in the table have significant differences of $p < 0.05$.
Value are means ± SD (n = 3)

A B C

Fig 1 (A) *Fermented*; (B) *Unfermented*; (C) *Nonfermented* Cocoa beans.

mL of distilled water and 0.15 mL $NaNO_2$ 5% then incubated for 5 min. After incubation, 0.15 mL $AlCl_3$ 10% was added to the solution and incubated for 5 minutes. The solution was mixed with 2 mL of 4% NaOH, diluted with distilled water to a volume of 5 mL and measured by spectrophotometry at λ 510 nm. The results were expressed as mg of quercetin equivalents per g of dry cocoa bean in comparison with the standard curve, which was developed under the same conditions.

Analysis of Antioxidant Activity by 1,1-difenil-2-pikrilhidrazil (DPPH) Method. Measuring antioxidant activity was according to Rahmi *et al.* (2016). Ethanol extract of fermented cacao beans was prepared in 100 mg mL^{-1}. Three ml of each sample was added to 2 mL DPPH 0.1 mM, incubated at 37°C for 30 min, and measured with spectrophotometry at λ 517 nm. The control solution was used 3 mL of methanol in 2 mL DPPH 0.1 mM. The percentage of inhibition is calculated based on the formula below:
DPPH scavenging activity (%) = [Abs control – Abs sample / Abs control] × 100% (Nishaa *et al.* 2012). The measuring for each extract was repeated 5 times.

Determination of IC50 value was done by ethanol extract of cacao beans prepared in 5 series concentrations: 50, 100, 150, 200, and 250 mg L^{-1}. Three mL samples were mixed with 2 mL DPPH 0.1 mM and incubated at 37°C for 30 minutes. The scavenging activity was measured spectrophotometrically by the decrease in absorbance at 517nm. Quercetin compound was used as a control with concentrations of 10, 15, 20, 25, and 30 mg L^{-1}.

Data Analysis. Data were tested statistically by diversity analysis of variances (ANOVA). If there was a significant difference, then proceed with Tukey methods range test with a significant level of 95% using *Software Statistical Analysis System* (SAS) Windows 9.

RESULTS AND DISCUSSION

Cacao Beans Fermentation. Various aspects can view the success of a cacao bean fermentation process, one of which is the dry cacao bean split test. The split test is intended to determine the success rate of bean fermentation with color changes (Fig 1). Good fermented cacao beans produce dark brown color and hollow bean texture. The highest fermented cacao beans produced by fermentation were with the addition of a combination of lactic and yeast acid bacteria with 83% of fermented beans, followed by addition of lactic acid bacteria (69%), addition of yeast (55%), and without any addition, only 15%. The fermentation process will impact chemical compounds such as secondary metabolites found in cacao beans (Meryandini *et al.* 2019).

Characteristic Ethanol Extract of Cacao Beans.

Yield, Water Content and Residual Solvents from Ethanol Extract of Cacao Beans. Obtained yield from the ethanol extract of cacao beans is shown in Table 2. The yield extracts from spontaneous

fermentation (F1) was 3.92 ± 0.09%. Adding a single starter or starter combination of lactic acid bacteria and yeast increased the yield value of the ethanol extract of cacao beans compared to F1. The F2 increased to 4.62 ± 0.13% or with a percentage of 15.2%, F3 (1.8%) and F4 10.7%. Utami *et al.* (2016) obtain 15 % polyphenol from cocoa bean shell fermented 120 hours, using aceton:water (70:30).

Water content and residual solvents from the ethanol extract of cacao beans are presented in Table 2. The water content and residual solvents from the ethanol extract of cacao beans from spontaneous fermentation were 7.67%. Water content and residual solvents with the addition of a starter contained water and residual solvents which were not significantly different from the extracts of spontaneous fermentation ($p > 0.137$), where the percentage of water content and residual solvent at F2 are 7.13%, F3 (7.27%), and F4 (7.20%). The increase in the yield value of the extract (F2, F3, and F4) indicates that it is not affected by the water content or the remaining solvents contained in the extract but can occur due to the fermentation process carried out.

Increasing the yield of ethanol extract of cacao beans during fermentation with the addition of starter (F2, F3, and F4) occurs due to the stretching of carbon bonds between the constituents of cacao bean cells which causes the components of the covalently bonded compounds to the cell wall to be quickly released. The presence of lactic acid bacteria and yeast in the fermentation contributes to converting compounds in simplicia cacao beans, especially compounds with high molecular weight. So that when the extraction process takes place, it is suspected that the binding of compounds causes more yield extract becomes high.

The ability of ethanol solvents to bind polar and nonpolar compounds is to withdraw compounds in cacao beans, such as fat, especially fat, with a short chain chemical structure because it is polar. The fat content in cacao beans is generally around 54% (Afoakwa 2010). Research by Ristanti *et al.* (2016) reported that the fat content of cacao beans from 12 regions in South Sulawesi was the highest at 38.21%, and the lowest was 20.73%. The high amount of fat in cacao beans causes fat extracted by ethanol solvents. So the extract yield was obtained in F1, F2, F3, and F4 containing secondary metabolites and other chemical compounds such as fat.

Phytochemical Components Ethanol Extract of Cacao Beans. The increased quality of fermented cocoa beans using lactic acid bacteria and yeast as a starter is caused by the formation of compounds that play a role in taste and aroma precursors of cocoa beans. Phytochemical component analysis was carried out to determine the presence of a phytochemical component in the tested extract. Qualitative determination can be seen from changes in color or formation of foam or sediment if the extract or sample is reacted with certain chemicals. The test results of phytochemical components of ethanol extract of cacao beans from various fermentation treatments are presented in Table 3. Phytochemical components of ethanol extract of fermented cacao beans with a single starter or starter combination of lactic and yeast acid bacteria include alkaloids, polyphenols, flavonoids, and tannins.

The alkaloid content of the cacao bean extract resulting from spontaneous fermentation (F1) contains moderate-intensity alkaloids. The results of F1 are directly proportional to the ethanol extract of fermented cacao beans with lactic acid bacteria (F2), which also contain moderate-intensity alkaloids. Samples of F3 and F4 contain alkaloids with strong intensity, which is thought to have increased alkaloids in the extract. Alkaloid compounds in cacao beans is theobromine which causes a bitter taste (Sandhya *et al.* 2016). During fermentation occurs, theobromine will be degraded (Aromolaran and Motilola 2018; Balc´azar-Zumaeta *et al.* 2023.)

Polyphenols are secondary metabolites with the highest content in cacao beans that play a role in producing good taste and aroma in cacao beans (Afoakwa *et al.* 2012). One of the polyphenol compounds in cacao beans is the flavonoid class. The content of polyphenols and flavonoids in F1 (ethanol extract of cacao beans from spontaneous fermentation) has moderate intensity. This can be due to the oxidation of polyphenols by polyphenol oxidase enzymes, diffusion to cotyledons than to the skin layer, polymerization, especially flavonoid compounds, and the formation of protein complexes.

Polyphenols and flavonoids in F2, F3, and F4 have strong intensity, meaning that there is an increase in the content of polyphenols and flavonoids in the extract compared to F1. The strong intensity of the sample can be due to the production of secondary metabolite produced by lactic acid bacteria and yeast at the stationary phase and affect the content of polyphenol or flavonoid compounds.

The content of tannin compounds in extracts fermented with the addition of starter (F2, F3, F4) has the same content intensity as in F1 (ethanol extract of

cacao beans resulting in spontaneous fermentation). These results indicate that the fermentation process with or without adding lactic acid and yeast acid starter to the tannin content did not change significantly. It is suspected that tannin compounds in cacao beans do not have a role in the formation of flavor or aroma in cacao beans.

The Content of Flavonoid Compounds Ethanol Extract of Cacao Beans. Flavonoids are a phytochemical component with the highest range of cacao beans polyphenols group (Chin *et al.* 2013). Flavonoid compounds play a role in forming the aroma and taste of cacao beans. They are known to have high antioxidant activity, so quantitative analysis of the content of flavonoids is carried out. The flavonoid content of cacao beans ethanol extract was determined by using the quercetin standard. The flavonoid content of the ethanol extract of cacao beans from fermentation is presented in Table 4.

The content of flavonoid compounds from 100 mg L^{-1} of ethanol extract of cacao beans on samples of spontaneous fermentation extract (F1) contained flavonoid compounds of 4.35 ± 0.20 mg L^{-1}. The flavonoid compounds' content from fermentation extracts with the addition of a single starter or combination starter proved to increase compared to F1. F2 increased by a percentage of 22.9%, F3 (18.9%), and F4 (27.4%).

Improvement to the quality of cocoa beans by the addition of lactic acid bacteria dan yeast in the fermentation process is corelated to the high flavonoid compounds in the cacao beans ethanol extract. In the fermentation process, polyphenol compounds undergo oxidation by polyphenol oxidase enzymes and play a role in changing the color of beans (Hoa *et al*. 2018, Fang *et al.* 2020).

Increasing of flavonoid compounds after fermentation can be due to the breakdown of the storage cells. Unfermented cocoa beans contain 12–18% polyphenols of the dry weight of beans on average. These compounds are stored in polyphenolic cells in unfermented beans. Sugars from pulp are converted to acids during fermentation. These acids move into the beans and lower their pH, which leads to breakdown of storage cells (Bariši´c *et al*. 2019**).**

Increasing the content of flavonoid compounds can also be caused by plant cultivars used. The type of cacao used in the study was Forestero cacao. Research by Hii *et al*. (2009) and Towaha (2014) explains that the content of polyphenol compounds from cacao types Forastero > Trinitario > Criollo are presented in Table 5. The country of origin of cacao beans can also affect the chemical content in cacao beans. A country's climate difference causes cacao beans to contain different secondary metabolites. The results of the study by Othman *et al.* (2007) reported that the highest polyphenol content of ethanol extract from cacao beans was produced by Malaysia > Indonesia (Sulawesi) > Ghana > Ivory Coast.

Antioxidant Activity Ethanol Extract of Cacao Beans. Antioxidant activity test in ethanol extract of cacao beans was carried out using 1,1-diphenyl-2-pikrilhydrazyl (DPPH) method. DPPH is a stable free radical. Parameters used to show the results of antioxidant activity by DPPH method are % inhibition of DPPH free radicals. The results of determination of DPPH % inhibition of ethanol extract of cacao beans are presented in Table 6.

Antioxidant activity (% inhibition) DPPH shows that in the concentration of 100 mg L^{-1} the ethanol extract of cacao beans is capable of inhibiting various DPPH free radicals. The antioxidant activity of spontaneous fermentation extract (F_1) was 42.31 ± 0.66%, F_2 (46.45 ± 2.00%), F_3 (49.05 ± 0.58%), and F4 at 50.33 ± 0.43%. Antioxidant activity of ethanol extract of cacao beans fermented with the addition of a single starter or combination starter (F_2, F_3, and F_4) increased when compared with the ethanol extract of cacao beans from spontaneous fermentation (F_1). Antioxidant activity in F_2 increased by 8.9%, F_3 (13.7%), and F_4 (15.9%). Dordevic *et al.* (2010), explained that the increase in antioxidant activity in the fermentation process can be due to degradation of the cell wall so that it frees or induces bioactive components from a material.

In general antioxidant activity is directly proportional to total flavonoids. This is because flavonoids' ability to capture free radicals is closely related to the presence of hydroxyl groups (Tosun *et al.* 2009). The correlation between flavonoid content and antioxidant activity in general illustrates the effect of flavonoid compounds on antioxidant activity produced by extract samples.

Antioxidant Activity of Ethanol Extract. The IC50 value is the concentration of the extract compound or an ingredient needed to reduce a free radical given as much as 50%. The smaller the IC50 value produced, the greater the ability of compounds, extracts, or ingredients to counteract free radicals.

The results of % inhibition of DPPH radicals were F1, F2, F3, and F4, which occurred at extract concentrations of 250, 200, 150, 100, and 50 mg L^{-1}

Table 3 The phytochemical component from ethanol extract of fermented cacao beans

Type of testing	Phytochemical content			
	F_1	F_2	F_3	F_4
Alkaloid	+	+	++	++
Polifenol	+	++	++	++
Flavonoid	+	++	++	++
Tanin	+	+	+	+

++ = positive with a strong intensity, + = positive with a moderate intensity

Table 4 The flavonoid compounds from cacao beans at a concentration of 100 mg L^{-1} of ethanol extract

Treatment	The flavonoid compounds from ethanol extract of cacao beans (mg L^{-1})
F_1	4.35±0.20^c
F_2	5.64±0.05ab
F_3	5.37±0.17b
F_4	5.99±0.23a

Different numbers in the table has significant differences of $p < 0.05$.
Value are means ± SD (n = 3)

Table 5 The content of polyphenols based on cultivars and the country of origin of cacao beans

Country	Cultivar	Total polyphenol content (mg g^{-1})
Pantai Gading	Forastero	81.5
Columbia	Forastero	81.4
Venezuela	Trinitario	64.3
Peru	Criollo	50.0
Dominika	Criollo	40.0
Malaysia	Forastero	71.4
Indonesia	Forastero	82.3

Resources: Hii *et al.* 2009; Tahowa 2014.

Table 6 Antioxidant activity (% inhibition) of DPPH from 100 mg L^{-1} ethanol extract of fermented cacao beans

Treatment	Antioxidant activity (% inhibition) ethanol extract of fermented cacao beans (%)
F_1	42.31±0.66^c
F_2	46.45±2.00^b
F_3	49.05±0.58^{ab}
F_4	50.33±0.43^a

Different numbers in the table have significant differences of $p < 0.05$.
Value are means ± SD (n = 3)

(Fig 1). This shows that the higher the concentration of ethanol extract in cacao beans, the higher the ability to reduce DPPH free radicals. These results are consistent with Emelda (2015) study which reported that antioxidant activity (% inhibition) will increase with increasing extract concentration, where at a concentration of 5 mg L^{-1} extract antioxidant activity was obtained at 32.49%, at a concentration of 10 mg L^{-1} (53.11%), 25 mg L^{-1} (83%), 50 mg L^{-1} (91%), 100 mg L^{-1} (94%), and 200 mg L^{-1} concentration of antioxidant activity by 93%.

The IC50 results of cacao bean ethanol extract is presented in Table 7. It is stated that the ethanol extract from fermentation with the addition of starter has a

Table 7 IC_{50} value of ethanol extract of cacao beans and quercetin

Treatment	IC_{50} value of ethanol extract of cacao beans and quercetin (mg L^{-1})
F_1	141.67
F_2	109.30
F_3	97.51
F_4	88.15
Quercetin	20.33

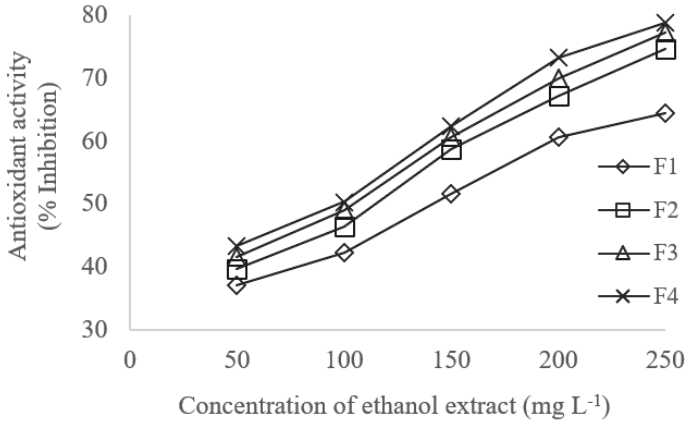

Fig 2 Radical DPPH inhibitory of various concentration

lower IC50 value compared to F1, which means the ability to extract by F2, F3, and F4 to capture DPPH free radicals is higher because it requires extracts with a lower concentration, where F2 is 109.30 mg L^{-1}, F3 (97.51 mg L^{-1}), F4 (88.15 mg L^{-1}). In contrast, F1 can capture 50% DPPH free radicals with a concentration of 141.67 mg L^{-1}.

IC50 value of all cacao bean ethanol extract from spontaneous fermentation extract (F1) and from fermentation results with the addition of lactic acid and yeast acid starter (F2, F3, and F4) compared to IC50 from quercetin compound (comparator), then the ability of ethanol extract cacao beans to inhibit 50% DPPH radicals lower than quercetin compounds. Quercetin reduced 50% of DPPH free radicals using only quercetin concentrations of 20.33 mg L^{-1}. The high ability of quercetin as an antioxidant because quercetin is known to have an OH group in its chemical structure that functions to capture DPPH radicals by donating H atoms so that radical DPPH becomes nonradical (Salamah and Widyasari 2015).

In conclusion, cacao bean fermentation process with or without the addition of lactic acid and yeast acid starter is proven to contain flavonoid compounds. The ethanol extract of cacao beans with the addition of a single or combination starter has a high content of flavonoids compared to cacao bean extract resulting from spontaneous fermentation. The increase of flavonoid compounds from ethanol extract of cacao beans fermented with the combination of starter also increased the antioxidant activity to be higher than the ethanol extract of cacao beans from spontaneous fermentation.

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