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# Evaluation of Coffee Pulp Waste from Coffee Cultivation Areas in Indonesia as Iron Booster

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**Abstract**

The research aimed to reduce the amount of coffee pulp (CP) as a pollutant and turn it into Coffee Cherry Fluor (CCF) as a functional food. CCF is expected to serve a function as a non-heme iron source to treat anemia. Further, reusing CP should be able to boost the circulation of economy. Six CP samples from three Arabica coffee areas (highlands of Mount Batur, Mount Ijen, and Mount Arjuno) in Indonesia were compared to La Boite CCF, a commercial product made in Brazil. Variables observed were iron contents which is determined by Inductively Coupled Plasma Optical Emission Spectrometer, vitamin C contents as enhancers by Iodimetric titration, total phenol contents as inhibitors by Folin-Ciocalteu method, tannin contents by spectrophotometry method, lignin contents by Van Soest method, total plant count by ISO 4833-1, and antioxidant capacity by IC 50 in the DPPH scavenging assay. Sample disparity significance was statistically determined by ANOVA, followed by Tukey test at 95 %. Sample resemblance with La Boite CCF was tested using Principal Coordinates Analysis (PCoA) and then presented in Heat Map. Conclusively, Mengani CCF and Mengani CP Estate samples are similar to La Boite CCF regarding vitamin C and inhibitor contents. It is suggested to reduce the temperature of the artificial drying device in Mengani. Further research should cover heavy metal contents, proximate analysis, and the detail of amino acid contents as research observed variables; expanding this research *in vivo* to study non-heme Fe's bioavailability should also be conducted.

**Keywords:** Anemia booster, Coffee cherry flour, Economy circular, Environmentally friendly, Functional food, Herbal medicine, Waste to food, Waste management

## 1. Introduction

Coffee (*Coffea* L.) owns worldwide popularity – in early morning work calls, casual gatherings, or smalls meeting; it has often been a go-to drink loved by many people. As a commodity, coffee is responsible for the livelihoods of over 125 × $10^6$ people globally. According to Fairtrade Foundation, coffee is one of the most widely-traded tropical agricultural products, 80 % of which is produced by 25 × $10^6$ smallholders (Atabani *et al*., 2019; Chala *et al*., 2018; Gill, 2021a; Lestari *et al*., 2022; Nurul *et al*., 2022; Wachamo, 2017). Concurring with this opinion, several researchers have declared that coffee is the most traded commodity only second to edible oil (Blinová *et al*., 2017; Corro *et al*., 2014; Das and Venkatachalapathy, 2016; Ijanu *et al*., 2020; Padmapriya *et al*. 2013; Poole *et al*., 2017).

In large cities throughout the globe, coffee is more of a lifestyle than just a drink, which makes the coffee business essential for corporations (Felton, 2019; Gill, 2021a and b). The rank in the world's big-five coffee companies in 2021 is (fifth) by McCafe (owned by McDonald's Corporation), (fourth) by Costa Coffee (owned by The Coca-Cola Company), (third) by Tim Hortons (owned by Restaurant Brands International Inc.), (second) by Dunkin' Donuts (owned by Inspire Brands), and (first) by Starbucks Corporation (Gill, 2021b). The facts have proven that coffee positively influences the economy (Gil 2021a; Shumeta and D'Haese, 2018) and social (Felton, 2019; Viartasiwi and Trihartono, 2020) globally. Further benefits of drinking coffee for dental health (Dewanti *et al*., 2020; Purwanto *et al*., 2020) and physical health

(Bizzo *et al.,* 2015; DeMelo Pereira *et al.*, 2020; Susilowati *et al*., 2020; Wachamo *et al*., 2017) are also indicated.

Ironically, the concerns about poor waste management in local coffee producers is also a focus given by many researchers (Corro *et al*., 2014; Das and Venkatachalapathy, 2016; Genanaw *et al*. 2021; Geremu *et al*., 2016; Novita, 2012, 2016). Coffee Pulp (CP) and Coffee Husk (CH) contain toxic substances, *e.g*., caffeine, alkaloids, tannins and polyphenolics (Chala *et al*., 2018; Dzung *et al*., 2013; Ijanu *et al*., 2020; Padmapriya *et al*., 2013) that shall bring negative impact towards the environment (Beyene *et al*., 2012; Carmen *et al*., 2020; Genanaw *et al*., 2021; Dzung *et al*., 2013; Lestari *et al*., 2022; Novita, 2012).

To illustrate the wet coffee processing, the amount of 1 t green bean, 2 t CP, 22 730 L liquid waste containing 80 kg Biological Oxygen Demand (BOD), and 0.28 t silver skin waste are made out of 5.5 t coffee cherry (Novia, 2012). In those local production sites, solid CP and CH are simply piled up at roadsides and riverbanks, while liquid Waste goes down the drains directly heading to rivers (Novia 2012; Novia *et al*. 2021; Setyobudi *et al*., 2019, 2021a) – such treatments in handling so much pollutant are dire threats for soil and water bodies.

As a part of solving the problems above, several experts (Chala *et al*., 2018; Novita, 2012, 2016; Novita *et al*., 2021; Padmapriya *et al*., 2013, 2015; Setyobudi *et al*., 2021a, 2021b, Syarif *et al*. 2012) recommended biogas digester to process liquid coffee waste before discharge. Employing a biogas digester should result in double benefits: a renewable energy source for coffee production (*i.e*., drying, lighting, power) and organic solid and liquid fertilizers for coffee cultivation (Abdullah *et al*., 2020; Novia *et al.,* 2021; Setyobudi *et al*. 2021a, 2021b; Susanto *et al*., 2020). Various types of the digester are advised by certain experts: Mulato and Suharyanto (2010) utilized a floating drum in Indonesia; Centro Nacional de Investigación del Café (CENICAFE) developed a Modular Anaerobic Treatment System (MATS) for coffee farms in the Colombian mountains (Bermeo-Andrade *et al*., 2020); Hernández-Sarabia *et al.* (2021) proposed tubular, or bag biodigester (Taiwan type - PVC 1005 geomembrane) in Colombia; Bombardiere (2006) reported the use of thermophilic Continuous Stirred Tank Reactor (CSTR) in Mexico. Rattan *et al*. (2015) suggest Upflow Anaerobic Hybrid Reactor-continuous (UAHR) and intermittent aeration system. Expressly, some researchers agreed to choose Upflow Anaerobic Sludge Blanket (UASB) digester type (Ijanu *et al*., 2019; Sengupta *et al*., 2020); Bruno and Oliveira (2008) even emphasized how two-stage digester had better performance compared to the one-stage. On the other hand, Adinurani *et al*. (2015), and Hendroko *et al.* (2013) were also on the side of two-stage, considering the acidic characteristic of liquid coffee waste (Genanaw *et al*. 2021; Novita, 2012, 2016; Rattan *et al.* 2015).

With simple technology, another way out is turning CP and CH into coffee cherry tea or cascara (from Spanish "cáscara," meaning husk or skin and pulp of a coffee cherry). There is nothing new about this since traditional beverages from coffee cherries have been consumed in Yemen (called Qishr), Ethiopia (Hashana), Bolivia (Sultana), and Costa Rica (Cáscara) (Ota, 2018). Studies on cascara have also been conducted (Ariva *et al*., 2018; Arpi *et al*., 2018; Heeger *et al*., 2017; Muzaifa *et al*., 2021; Nafisah and Widyaningsih, 2018; Novita *et al*. 2021; Pua *et al*., 2021; Zeckel *et al*. 2020). However, Setyobudi *et al*. (2019 and 2021a) concluded its inefficiency in solving the problem since CP and CH solid waste remained after being steeped in hot water.

Andrew Fedak and Dan Belliveau proposed to turn CP and CH into Coffee Cherry Flour (CCF) (Cheatham, 2019; WIPO–PCT, 2014). Damat *et al*. (2019), Elba *et al.* (2017), Mindarti *et al.* (2020), Moreno *et al*., 2019, and Rosas-Sánchez *et al*. (2021) have discussed CCF's positive impacts on its fibre content, while Setyobudi *et al*. (2019 and 2021a) advised CCF to be an iron (Fe) source. The latter, after reviewing a considerable amount of data, discovered that CP and CH contained Fe between (4.3 to 50) mg 100 $g^{-1}$ (Anna *et al*., 2019; Avinash *et al*., 2017; Elias, 1979; Hermosa *et al*., 2014; Iriondo-DeHond *et al.* 2020; Kayhanian and Tchobanoglous , 2016; Marín-Tello *et al*., 2020, Setyobudi *et al.*, 2018, 2019, 2021a; Wich, 2015; Zupancic and Grilc, 2012). Setyobudi *et al.* (2019) have also summed up that the Fe contents in CP and CH are higher than in sweet leaf or star gooseberry [*Sauropus androgynus* L. (Merr)], leaf of cassava or manioc [*Manihot esculenta* (Crantz)], leaf of papaya or papaw (*Carica papaya* L.), Indian mustard or vegetable mustard (*Brassica juncea* L.), tomato (*Solanum lycopersicum* L.), and wild carrot [*Daucus carota* subsp. Sativus (Hoffm.) Schubl. & G. Martens.]; they are even higher than in Indonesia's currently most-studied haemoglobin boosters: drumstick tree (*Moringa oleifera* L.) of (5.57 to 6.28) mg 100 $g^{-1}$, date-palm (*Phoenix dactylifera* L.) of (4.06 to 7.06) mg 100 $g^{-1}$, and spinach (*Spinacia oleracea* L.) of (2.7 mg to 3.9 mg) 100 $g^{-1}$ (DKBM Indonesia, 2017; Hamzah and Jusuf, 2019; Rania *et al*., 2014; USDA, 2018).

Setyobudi *et al*. (2021a) determined that the CCF of arabica coffee in Kintamani, Bali, Indonesia, met the requirement better than Brazilian commercial products despite its relatively lower Fe content, presumably due to the Inceptisol soil (Asfimanto *et al*., 2013; Nurul *et al*., 2022) that contains low Fe (Nandini and Narendra, 2012).

Following up Setyobudi *et al.* (2021a), CP potentials as Fe-source CCF in three coffee areas in Indonesia, were examined in this research, aimed to (i) minimize CP and CH pollutant potentials, (ii) encourage prosperity for coffee farmers and producers, and (iii) provide Fe non-heme source for haemoglobin booster due to the relatively high chance of anemia (Milman, 2011; Muhammadong *et al*. 2021; Nurbadriyah, 2019), especially in Indonesia (Bukhari *et al*., 2020; Ellie *et al*., 2012; Nurbadriyah, 2019; Yuniastuti, 2014).

## 2. Materials and Methods

### *2.1. Sample collection*

Three dried coffee CP samples were obtained from three indigenous *Coffea arabica* L. areas in Indonesia: Ijen Farm at the side of Mount Ijen, Bondowoso, East Java (7°57'59.55 "S 114°01'14.37 "E), Karangploso Farm downhill Mount Arjuno, Malang, East Java (7°52'13.80" S 112°34'54.44" E), and Mengani Farm at the slope of Mount Batur, Mengani, Bali (8° 17' 16.63" S 115° 15'

0.61" E). Varied enormously in particle sizes (Figure 1), all the samples were collected within February and July 2018, then dried and homogeneously ground and sieved before analysis. The CCF commercial product serving as a comparator had been made in Brazil, acquired from La Boite, a store in Manhattan, 724 11th Avenue, New York, NY 10019, in May 2018.


**Figure 1**. The appearance of dried coffee pulps (A), Four coffee cherry flour produced from Mengani (B), Karang Ploso (C), Ijen (D), and the La Boitê commercial product (E).

### *2.2. Observed Variables and Statistical Analysis*

Sample materials are organized as follows:
(i) Ijen CP, (ii) Karangploso CP, (iii) La Boite CCF, (iv) Mengani CCF, (v), Mengani CP-Est., (vi) Mengani CP-SP, (vii) Mengani CP-Hay.

**Notes:**

- CP = coffee pulp
- CCF = coffee cherry flour
- Mengani CP-Est. refers to CP collected from the industrial-scale coffee processor owned by the Arabica Coffee Factory Estate of Mengani.
- Mengani CP-SP refers to CP collected from small-scale processors owned by farmers in Mengani.
- Mengani CP-Hay refers to CP collected from the storage room of the Arabica Coffee Factory Estate of Mengani, packed in plastic bags, and stored at room temperature for 15 mo.

Setyobudi *et al.* (2021a) have listed the Fe non-heme booster agents in CCF–covering vitamin (Vit.) C, Vit. A, amino acid, dan reducing sugars. The observed variables in this study were the amounts of Fe and enhancer agent (Vit. C), inhibitors agents (total phenol, tannin, and lignin), of 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay, and of total plate count (TPC). Using GLM-ANOVA to diverge the samples' statistical significance and employing Tukey test at 95 % scale, the data were presented in box plot and vertical bar chart (Adinurani, 2016; Mishra and Homa, 2019).

Two analysis steps were conducted to determine the samples' similarity to the comparator (La Boite's CCF). Firstly, the seven observed variables were summed up through Principal Coordinates Analysis (PCoA) – also known as Multidimensional Scaling (MDS) – using Dissimilarity Analysis and Representation for Windows (DARwin) 6.0.010 software (Nasibeh 2019; Perrier *et al.*, 2003; Perrier and Jacquemoud-Collet, 2006). Then the Neighbor-Joining and Hierarchical Clustering results were laid out in a Heat Map (DeBoer, 2015; Tomanek and Schröder, 2018; Zhao *et al.*, 2014) by activating the feature Style > Conditional Formatting > Color Scales on Microsoft Excel 2010. The vector values were established from 2 (the highest score, light yellow) to -2 (the lowest score, dark red).

### *2.3. Analysis of Fe*

To analyze the Fe content, an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) Varian-730-ES by Varian Inc. under Agilent Technologies (Palo Alto, USA) was exercised. A total of 5 g CCF per sample was treated with dry-ashing procedures involving hydrochloric acid dilution to extract the minerals then studied in duplicates and replicated at least twice (Xianden et *al.*, 2016).

### *2.4. Analysis of Vitamin C*

The Iodimetric method (AOAC, 1995) was used in Vit. C content analysis. A total of 5 g CCF per sample was diluted in a 100 mL marked flask. The dilutions were then filtered, and 25 mL of each was then mixed with a few drops of starch and quickly titrated with 0.01N sodium thiosulfate to blue color. Vit. C content was calculated on Equation (1):

$$\text{Vit C (mg 100 g}^{-1}) = (\text{V I2} \times 0.88 \times \text{NFp}) \times 100 \text{ W s} \quad (1)$$

Note: V I2 = Iodium volume (mL), NFp = Dilution factor, W s = Sample weight (g)

### *2.5. Analysis of Total Phenol*

Following the procedures detailed in Almey *et al.* (2010), a total of 5 g CCF per sample was extracted with 5 mL of 85 % methanol in a test tube, centrifuged/vortex mixed in 3 000 rpm (1 rpm = 1/60 Hz) for 15 min and filtered. Each filtrate was then diluted to 5 mL, 0.4 mL of which was added to 0.4 mL of Folin-Clocaften reagent in a test tube, vortex mixed, and rested for 6 min. Next, 4.2 mL of 5 % sodium carbonate solution ($Na_2CO_3$) was added to each before vortex mixed and incubated at room temperature for 90 min. Absorbance was computed on ג max = 760 nm using Merck Perkin Elmer Lambda 25 UV-V Spectrophotometer. Standard curve construction followed by diluting gallic acid in 85 % methanol of various concentrations ranging between (0 to 100) mg $L^{-1}$. Total phenol content was calculated on the linear regression equation of gallic acid as in Equation (2):

$$y = ax + b \quad (2)$$

### *2.6. Analysis of Tannin*

A total of 0.25 g CCF per sample was diluted in 20 mL of boiling aqua dest and filtered after a few minutes. Each filtrate was then diluted to 50 mL, 1 mL of which was added to 2 mL of 0.02 M $FeCl_3$ in 0.02 M HCl solution and 2 mL of 0.0015 M $K_3[Fe(CN)_6]$ in a 25 mL flask, then further diluted to its maximum limit. A blank solution was employed to check the absorbance, recorded on ג max = 744.6 nm using Hitachi U 2010 Spectrophotometer UV-V (Arapitsas, 2012).

### *2.7. Analysis of Lignin*

Finding (1 g) the Acid Detergent Fiber (ADF) and the Neutral Detergent Fiber (PDF) rates of the samples is essential in estimating the lignin contents. Next, each sintered glass containing ADF was put on a petri dish, soaked in 20 mL of 72 % $H_2SO_4$ (stir for thorough coverage), and let sit for 2 h. After being heated in an oven of 1 000 °C for 8 h and rested overnight, every sample was laid in an exicator for 30 men and then weighed (a g)

before reheating up to 5 000 °C for 2 h. Once cooled, it returned to the exicator for 30 min and then weighed (b g). Total lignin content was evaluated based on Equation (3) (Van Soest *et al*., 1991):

$$\text{Lignin Content} = \frac{a - b}{\text{Sample weighed (1 g)}} \times 100\ \% \quad (3)$$

### *2.8. Analysis of Antioxidants*

A total of 200 mg CCF per sample was suspended in 20 mL of 80 % (V $V^{-1}$) methanol and homogenized for 30 s, an amount of 1 mL of which was then mixed with 0.4 mL of hexane before homogenized for another 30 s. After centrifuging at 3 000 g for 10 min at 25 °C, the supernatant was discharged from the tube, followed by washing with hexane twice. The methanol extract was then filtered with 0.45 m millipore mesh before being used for the DPPH (2,2- diphenyl-1-picrylhydrazyl) scavenging assay.

Antioxidant activity was measured by obtaining the IC 50 (50 % inhibitory concentration) in the DPPH scavenging assay. The sample extract was dissolved to produce solutions with concentrations between 100 mg $L^{-1}$ to 1 300 mg $L^{-1}$. A volume of 50 µL of 0.15 mM DPPH was mixed with 30 µL of the sample, then incubated in darkness for 30 min before the absorbance was measured using a spectrophotometer at a wavelength of 517 nm. As a control, 4.9 mL of DPPH solution was mixed with 0.1 mL of methanol, and the absorbance was measured at the same wavelength. Inhibition percentage was calculated based on Equation (4) (Xie and Schaich, 2014):

$$\%\ \text{inhibitation} = \frac{Control\ absorbance - Sample\ absorbance}{Control\ absorbance} \times 100\ \% \quad (4)$$

### *2.9. Analysis of Total Plate Count (TPC)*

A total of 25 g CCF per sample was added to 225 mL of Butterfield's Phosphate Buffered solution and homogenized for 2 min, an amount of 1 mL was mixed in 9 mL of distilled water to obtain dilutions $10^{-2}$, $10^{-3}$, and $10^{-4}$ sequentially. Next, 1 mL of each suspension was inoculated to a petri dish containing liquid agar and incubated at 37 °C for 2 d. TPC was noted using Colony Counter (ISO, 2013; Yunita *et al.*, 2015).

## 3. Result and Discussion

### *3.1. Iron (Fe) Content.*

Iron (Fe) is only a particularly essential micronutrient in red blood cells forming during the haemoglobin synthesis process (Achmad, 2000; Elstrott *et al*., 2020; Ogawa *et al*., 2020); Fe is only obtainable externally from food or supplement. Found in all types of bodily cells, this substance also plays a vital role in various biochemical reactions. Fe is also found in enzymes involved in electron distribution (cytochrome), oxygen activation, and oxygen distribution (haemoglobin and cytochrome) (Musallam and Taher, 2018; Seriki *et al*., 2017). Further, Fe is required in forming lymphocyte cells to build immunity, while transferrin and lactoferrin – proteins that absorb and deliver iron throughout the body – also work to prevent infection (Kell *et al*., 2020; Winarsi, 2007).

The non-heme Fe contents in CCF and CP from three coffee cultivation areas compared to one in La Boite CCF are presented in Figure 2.

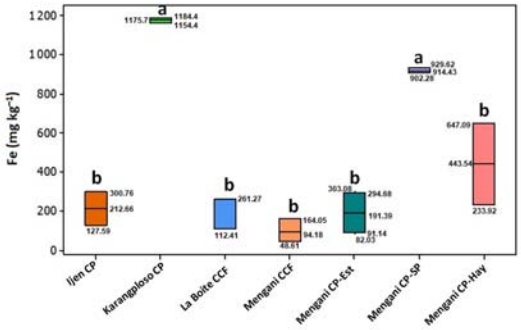


**Figure 2**. Box-plot of Fe contents in samples from three coffee cultivation areas.

Tukey test sorted the seven samples into two groups: (i) Karangploso CP and Mengani CP-SP, while (ii) Ijen CP, La Boite CCF, Mengani CCF, Mengani CP-Est, and Mengani CP-Hay. Relevant to the purpose of finding the highest Fe content was group (i), considering human body absorption towards non-heme Fe is relatively low – between 5 % and 10 % – compared to heme Fe which goes between 10 % and 30 % (Jumadi 2020; Nurbadriyah, 2019; Setyobudi *et al*., 2019, 2021a; Yuniastuti, 2014).

However, the authors were uncertain about the validity of Fe contents in group (i), *i.e.* [(91.43 to 117.47) mg 100 $kg^{-1}$]. Setyobudi *et al*. (2019, 2021a) reviewed 12 references to find out that the highest Fe content in CP and CH should only be 50 mg 100 $kg^{-1}$ (Kayhanian *et al*., 2016). The two samples in the group (i) were presumably contaminated by iron and rust flakes from worn-out mini pulper equipment in small-scale coffee processors. Such Fe contaminants may be formed since small-scale coffee processors typically use little water in the wet process. Hendarto (personal communication, 2020), and Sariadi (2012) admitted that the water used was of (7 to 9) $m^3$ $t^{-1}$ cherry. Assari (2021, personal communication) even verified that Kopilos, a small coffee processor in Karangploso, unused water in the pulping process. The facts defy Yahmadi (1972) information that the traditional wet process should involve water of (16 to 18) $m^3$ $t^{-1}$ cherry, and the Ministry of Agriculture of Indonesia – *Menteri Pertanian* (2012) data that one required (10 to 30) $m^3$ $t^{-1}$ cherry, and Dadi *et al*., (2018) finding of (15 to 20) $m^3$ $t^{-1}$ cherry. Arabica Coffee Factory Estate of Mengani used water of (16 to 25) $m^3$ $t^{-1}$ cherry in its pulping process (Hendarto, 2020, personal communication).

The water utilization in the wet process pollutes rivers and soils (Campos *et al*., 2021; Ijanu *et al*., 2020; Novita, 2012, 2016). Novia (2012, 2016) and Syarif *et al*. (2012) recommended wet-process technology based on clean production by reducing the amount of water up to 3 $m^3$ $t^{-1}$ cherry to cut the pollution down, resulting in the increase of environmentally disadvantageous BOD and COD formed in liquid waste. This situation requires appropriate technology to reduce the amount of BOD and COD without additional water which is applicable to small-scale coffee processors (Campos *et al*., 2021; Fereja *et al*., 2020; Gururaj *et al*. 2021; Ijamu *et al*. 2000).

As for Fe pollutant, it must be cut off due to the harms. Fe content of higher than 20 mg 100 $kg^{-1}$ can cause stomachache, constipation, nausea, vomiting, and loss of consciousness towards the human. Too much iron can trigger hemochromatosis (Jumadi 2020, Naruki *et*

*al*. 2010). Disorders like hemosiderosis or hemochromatosis are due to progressive iron deposition in the liver, pancreas, and joints (Arora and Kapoor, 2012; Naruki *et al*., 2010; Yuniastuti, 2014). Fenton reaction – where an electron of Ferro ion is transferred to HOOH molecule to create ferri ion and hydroxyl radical destructive towards cell protein (Naruki *et al*., 2010; Yuniastuti, 2014) – is next in the chain.

Figure 2 demonstrates how Fe contents in La Boite CCF and Mengani CCF are similar to those reported in Setyobudi *et al*. (2021a). Mengani CCF has the lowest Fe content of all samples for two probable reasons. (i) Inceptisol soil of Mount Batur, Bali, contains low iron (Nandini and Narendra, 2012), and (ii) CP drying process in Mengani Est. employs an artificial heater at 80 °C for 15 h (Setyobudi *et al*., 2021a). Arista *et al*. (2018) report on how boiling and steaming significantly decreased the Fe in beetroot (*Beta vulgaris* L.var Rubra), as well as Syarfaini *et al*. (2017); Wahyani and Rahmawati (2021) results on the Fe decreased due to heating and long processing time in sweet potato (*Ipomoea batatas* L.) and sorghum (*Sorghum bicolor* (L.) - based cookies, supported the latter cause. It also shows that CP-Hay stored in plastic bags at room temperature for 18 mo contains more Fe than the other four fresh samples; this data is convenient for maintaining CCF production even during off-seasons. Another point is that precisely in Mengani, Bali, four different rates of Fe are recorded – getting a consistent amount of certain content has been one of the obstructions faced by the herbal medicine industry.

Further revealed in Figure 2 that Fe contents in the group (ii)'s CP are generally equal to the control and therefore fitting for Fe-source CCF. The Fe values of [(9.42 to 44.35) mg 100 $kg^{-1}$] are higher than what is contained in sweet leaf, leaf of cassava, leaf of papaya, vegetable mustard, and wild carrot (Setyobudi *et al.,* 2019) and also higher than the currently most-studied haemoglobin-booster ingredients *i.e.,* drumstick tree, date-palm, and spinach [(2.7 to 7.06) mg 100 $kg^{-1}$] (DKBM Indonesia, 2017; Hamzah and Jusuf, 2019; Rania *et al*., 2014; USDA, 2018).

### *3.2. Vitamin C Content.*

Vit. C (ascorbic acid) as an enhancer works by reducing non-heme's Ferri ($Fe^{3+}$) into Ferro ($Fe^{2+}$) in the small intestine to make it 2 to 4 times easier to absorb. In addition to the role mentioned above, it also helps prevent haemosiderin from forming and distributing transferrin in plasma to ferritin in the liver. Focusing on transfers, it carries iron to all body parts, including the marrow, where haemoglobin is produced (Agusmayanti *et al*., 2020; Conner *et al*., 2012; Jumadi, 2020; Wulandari, 2015).

Not only Vit. C, other kinds of acid – *e.g*., citric acid, malic acid, and tartaric acid – also have the ability to enhance iron, as reported by Yuniastuti (2014), Jumadi (2020), and Susiloningtyas (2012). CP dan CH was said to contain citric acid (Shankaranand and Lonsane 1994). Ginz *et al.* (2020) noted that sucrose and reduction sugar were the main precursors to these acids, of which contents in CP had been confirmed by Setyobudi *et al*. (2021a). The Vit. C contents in CCF and CP from three coffee plantations compared to one in La Boite CCF are detailed in Figure 3.

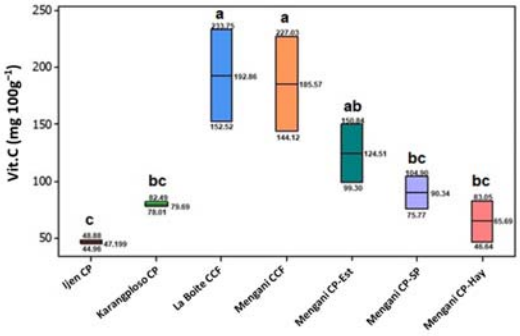


**Figure 3.** Box-plot of Vitamin C contents in samples from three coffee cultivations areas

Referring to the Tukey result, Vit. C content in Mengani CCF is equal to one in control, putting it in the first and second places of all samples – Mengani CP-Est is in the third, while Ijen CP is in the last. It has been elaborated in Setyobudi *et al*. (2021a) that Vit. C in Mengani CCF (and Mengani CP-Est) was lower than in La Boite CCF due to the drying method (equipment drying at 80 °C for 15 h). Some researchers on cascara (dried CP and CH for herbal drink, the basic component of CCF) suggested convection oven drying at 45 °C for 32 h (Ariva *et al*., 2020) or sun-drying at 30 °C to 35 °C for 2 wk to 3 wk (Nafisah and Widyaningsih, 2018). Almatsier (2009) explained that Vit. C started to degrade at approximately 35 °C, yet argued by Rahmadi and Bohari (2018) that it should be 50 °C.

Although the figure puts Karangploso CP, Mengani CP-Est, and Mengani CP-Hay on equal footing, the Vit, is convincing that plastic-bagged CP-Hay stored at room temperature reduces the Vit. C content is consistent with Jumadi (2020) statement that Vit. C reduction occurred in food storing.

The high Fe contents in Karangploso CP and Mengani CP-SP (Figure 2) are diverged by their Vit. C contents (Figure 3). It is apparent that poor control over the drying process in small-scale coffee processors is the problem. Despite meeting the requirements (Alakali *et al*., 2015; Ariva *et al.,* 2020; Nafisah and Widyaningsih, 2018; Rahmadi and Bohari, 2018; Setyobudi *et al*. 2021a) of sun-drying on tropical drying floors at 22.67 °C to 37.90 °C (Jaisyurahman *et al*. 2019; Setiawan *et al*., 2014), human resource needed to toss CP or CH every 1 h to 2 h his likely unavailable. To top it up, inconsistent sunlight related to ultraviolet and infrared exposures further oxidize Vit. C (Zhou *et al*., 2016). Vit. C reduction was also reported in the drying process of drumstick tree leaves (Gernah and Sengev, 2011; Mbah *et al*., 2012).

The low Vit. C content in Ijen CP (4. 72 mg 100 $kg^{-1}$) emphasizes the difficulty small-scale coffee processors face in maintaining it. At the same time, Sukartiningsih (2011) declared that the cascara of fresh before-ripe Arabica coffee in Ijen contains Vit. C of (275.7 to 651.2) mg 100 $g^{-1}$.

### *3.3. Total Phenol Content.*

To sustain, plants perform primary as well as secondary metabolism. In addition to food, primary metabolites – such as carbohydrates, protein, fat, vitamin, and mineral – also act as precursors of the secondary metabolism for self-defense against biotic and abiotic harms in a plant's habitat (Erb and Kliebenstein, 2020;

Goyal *et al*., 2012; Mazid *et al.* 2011; Vladimir-Knežević *et al*., 2012). Basically, pericarp, exocarp, and mesocarp of coffee beans, CP, and CH exist to protect the beans; therefore, secondary metabolites should be found there.

Esquivel and Jimenez (2012) and Martínez and Clifford (2000) confirmed it with their findings, stating four main polyphenolic compounds – flavan-3-ol, hydroxycinnamic acid, flavonoid, and anthocyanin – were identified in Arabica coffee cherry. Ramirez-Coronel *et al*., (2004) and Ramirez-Martinez (1988) also noted the presence of phenolic compounds – chlorogenic acid (5-caffeoylquinic acid), epicatechin, is chlorogenic acid I, II, and III, catechin, rutin, and protocatechuic acid – in the coffee pulp.

Gillooly *et al*., (1984); Jumadi (2020); Lesjak and Srai, (2019); Susiloningtyas (2012); Wahyani and Rahmawati (2021); and Yuniastuti (2014) agreed that phenol compounds were Fe inhibitors. Although Phenol consists of three hydroxyl groups bonded to triple valence iron in chelation, which can reduce iron bioavailability (Lesjak and Srai, 2019; Wahyani and Rahmawati, 2021; Yuniastuti, 2014), phenolic compounds are known to be beneficial to treat cardiovascular diseases, colon cancer, liver disorders, and diabetes (Pandey and Rizvi, 2009). Other researchers also pointed out phenolic compounds' capacity to combat various diseases associated with oxidative stress (Arts and Hollman, 2005; Lesjak and Srai, 2019) and exhibit antioxidant, anti-inflammatory, and anti-clastogenic activities (Lambert *et al*., 2005). More researchers (*e.g*., de Melo Pereira *et al*., 2020; de la Rosa *et al*., 2019; Dorsey and Jones, 2017; Geremu *et al*., 2016; Huang *et al*., 2010; Ifadah *et al.* 2021; Lestari *et al*., 2022; Vladimir-Knežević *et al*., 2012) encouraged the use of phenolic compounds to maintain health. The total phenol contents in CCF and CP from three coffee cultivation areas compared to one in La Boite CCF are depicted in Figure 4.

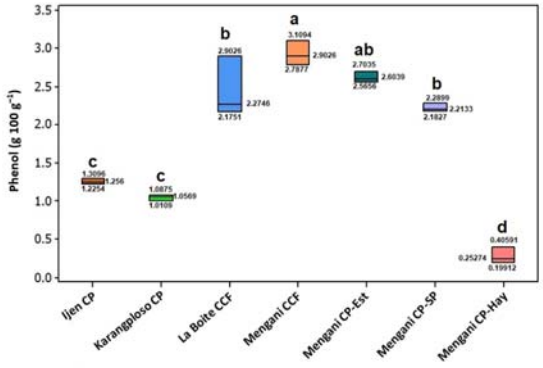


**Figure 4.** Box-plot of total Phenol contents in samples from three coffee cultivation areas

Tukey test sorted the seven samples into three groups: (i) Mengani CCF with the highest content, followed respectively by (ii) La Boite CCF, Mengani CP-Est, and Mengani CP-SP; Ijen CP and Karangploso CP; while (iii) Mengani CP-Hay with the lowest content. The result shows that plastic-bagged CP-Hay stored at room temperature reduces Vit. C content (see Figure 3) but also phenol compounds. The second group may be the best Fe source regarding inhibitor presence for iron absorption, but the first group is better concerning phenol benefits. The degrading amount of total Phenol in CCF due to cooking is another point since heating triggers the enzymatic oxidation process (Shabri dan Rohdiana, 2016). Diniyah and Lee (2020) and Xu and Chang (2008) reported total phenol damage due to soaking, steaming, and boiling. Nurhayati *et al*. (2021) recorded chlorogenic acid damage after heating. Ifadah *et al*. (2021), Mulyawanti (2020) noted anthocyanin damage. All those findings are consistent with the data on temperature limit of 50 °C (Budilaksana and Andaka, 2016; Hayati *et al.* 2012; Kwartiningsih *et al*., 2016; Sudarmi *et al.* 2015).

### *3.4. Tannin Content.*

Several researchers (Schmidl and Labuza, 2000; Susiloningtyas, 2012; Yuniastuti, 2014) trusted Tannin as an iron absorption inhibitor. However, Arpi *et al.* (2018), Jumadi (2020), and Lesjak and Srai (2019) disregarded its importance, especially when Vit. C was there. Other researchers (Esquivel and Jimenez, 2012; Kumari and Jain, 2012; Woldesenbet *et al*., 2015) even regarded Tannin anti-nutrient since when bounding with protein, it forms insoluble complex compounds that block the protein's digestive ability (Chung *et al*., 1998; Suarni, 2009).

Suarni and Subagio (2013) and Suarni and Firmansyah (2007) considered Tannin a unique nutrient with both positive and negative effects. A phenolic compound, Tannin is a natural antioxidant that bounds free radicals (Suarni and Subagio, 2013; Suarni and Firmansyah, 2007; Tandon and Rai, 2007) that can act as an astringent, anti-diarrhoea, anti-microbial activities (Chung *et al*. 1998; Hagerman, 2002; Malaggia *et al*. 2012), anti-carcinogen and anti-mutagen (Chung *et al*., 1998). It can also reduce the incidence of several human diseases such as cardiovascular diseases, colon cancer, liver disorders, and diabetes (Rasouli *et al*., 2017; Tandon and Rai, 2007). Another quality is exerting other physiological effects, such as accelerating blood clotting, reducing blood pressure, decreasing the serum lipid level, producing liver necrosis, and modulating immune responses (Chung *et al*., 1998; Tandon and Rai, 2007).

The total Tannin contents in CCF and CP from three coffee cultivation areas compared to one in La Boite CCF is presented in Figure 5.

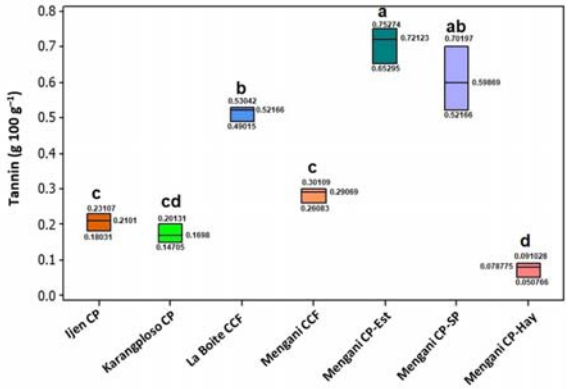


**Figure 5.** Box-plot of Tannin contents in samples from three coffee cultivation areas.

Tukey test sorted the seven samples into three groups: Mengani CP-Est, Mengani CP-SP, and La Boite CCF in the first; Ijen CP dan Mengani CCF were in the second; while Karangploso CP dan Mengani CP-Hay were in the third. Mengani CP-Est contains the highest Tannin, followed by Mengani CP-SP. Mengani CP-Hay is of the lowest, which matches the lowest total Phenol content in Figure 4.

The Tannin content in control is relatively the same as in Mengani CP-SP but higher than in Mengani CCF. Such discrepancy in the same farming area has been problematic in herbal medicine.

### *3.5. Lignin Content.*

While some researchers agreed that fibre acts as a Fe inhibitor (Cook *et al*., 1983; Fernandez and Phillips, 1982; Gillooly *et al*., 1984; Jumadi, 2000; Reinhold *et al*., 1981; Suarni and Firmansyah, 2016; Rufaizah, 2011; Wahyani and Rahmawati 2021; Yuniastuti, 2014), but Schmidl dan Labuza (2000) deduced that its effect on iron absorption is relatively lower than tannin dan phytic acid. Monnier *et al.* (1980) found that pectin inhibited the absorption of inorganic iron, not cellulose. Yet, Fernandez and Phillips (1982) reported that lignin and psyllium mucilage had a pronounced capacity to bind ferrous iron in vitro, whereas cellulose and pectin were much less potent. Although support has been on lignin's side (Plalt and Clydesdale, 1987; Reinhold *et al*., 1981).

The total lignin contents in CCF and CP from three coffee cultivation areas compared to one in La Boite CCF is depicted in Figure 6.

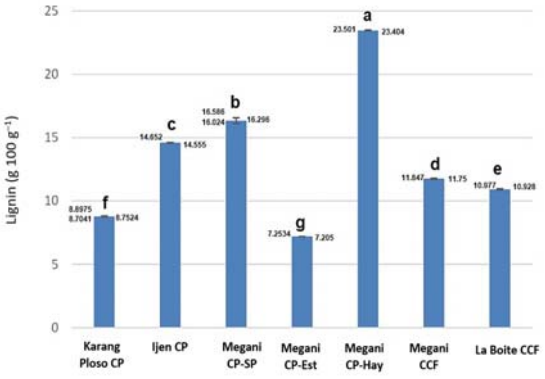


**Figure 6.** Bar-chart of Lignin from samples from three coffee plantations

In Figure 6, the highest lignin content is recorded by Mengani CP-Hay, whereas the lowest is by Mengani CP-Est. The control's content is in the lowest third, less than one of Mengani CCF.

Lignin may be an iron inhibitor, but Cook *et al*. (1983), Jumadi (2020), Kelsay *et al.* (1979), and Reinhold *et al.* (1981) said that Vit. C. (ascorbic acid) dan citric acid should be able to compensate for their weakness. It is vital since high dietary fibre intake helps diminish the chances of heart disease and obesity while lowering high blood pressure, maintaining ideal sugar content in the blood, and preventing colon cancer. Furthermore, in the case of cardiovascular conditions (coronary heart), fibre binds bile acids to reduce cholesterol in the blood (Damat *et al*., 2019; Elba *et al.* 2017; Mindarti *et al.* 2020; Wahyani and Rahmawati 2021). Furthermore, as insoluble dietary fibre, lignin thickens food mass in the digestive system, and it is adequate to prevent digestive ailments such as hemorrhoids, diverticulosis, and colon cancer (Damat *et al*., 2019; Mindarti *et al.* 2020). Astawan and Wresdiyati (2004), Dreher (2018), Fung-Jou *et al*. (1998), Harbone (1996), Huang *et al.* (2010), Kritchevsky and Bonfield (2012), Soliman (2019), Suarni and Firmansyah (2007), Veronese *et al*. (2018), and Yahia *et al*. (2019) were therefore in unison that lignin is an antioxidant compound.

### *3.6. Antioxidant Capacity*

Three plant bioactive compounds found in CP – total Phenol, Tannin, and Lignin – have been discussed in the previous sub-sections. The antioxidant capacity of the DPPH scavenging assay outcome is revealed in Figure 7 to complement.

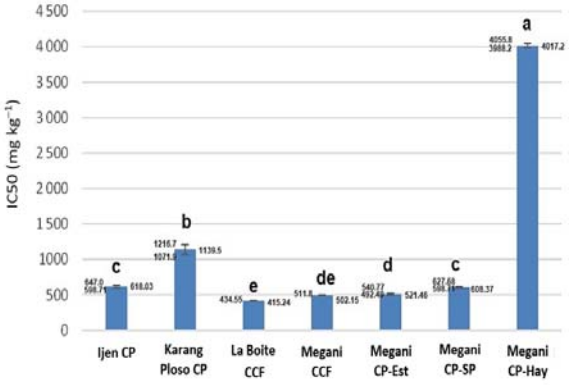


**Figure 7**. Bar-chart of IC-50 in samples from three coffee cultivation areas.

The figure generally sorts the seven samples into four groups. The lowest ratio, Mengani CP-Hay, is in the first. Karangploso CP is in the second. Ijen CP and Mengani CP-SP are in the third. The highest ratios – Mengani CP-Est, Mengani CCF, and La Boite CCF – are fourth. It goes along with Nurhayati *et al.* (2021) finding that the higher the total phenol rate is, the higher the antioxidant activity will be. Mengani CCF comes with the highest total phenol rate (followed by La Boite CCF, Mengani CP-Est, and Mengani CP-SP, respectively) in Figure 4, akin to Fig. 7, where La Boite CCF has the highest IC-50 (followed by Mengani CCF, Mengani CP-Est, and Mengani CP-SP respectively). As of the lowest total phenol content in Figure 4, Mengani CP-Hay also holds the lowest IC-50 result in Figure 7.

Figure 7 is key to the double purpose of this research – in addition to non-heme Fe, natural antioxidant contents in CP are also revealed. Since synthetic antioxidants (such as butylated hydroxytoluene and butylated hydroxyanisole) have recently been reported to be harmful to human health, the search for effective and non-toxic natural compounds with natural antioxidant activity should be feasible (Amarowicz *et al*. 2012; Felter *et al*., 2021; Xu *et al*., 2021). Figure 7 also supports the findings of Damat *et al.,* (2019), Lestari *et al*., (2022); and Moreno *et al*., (2019) that CP has a positive effect on antioxidant capacity.

### *3.7. Total Plate Count*

Total Plate Count (TPC) is a means to find the hygienic rates of CP as a CCF source, which result is disclosed in Figure 8.

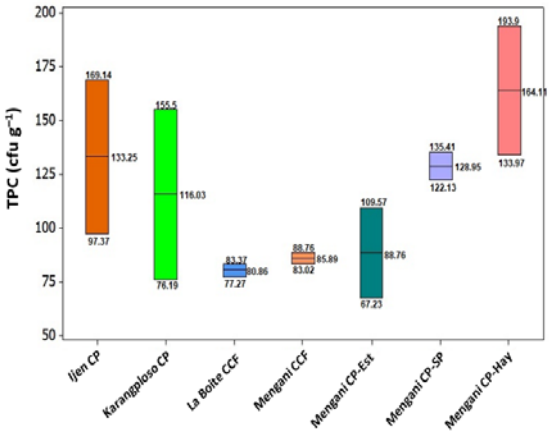


**Figure 8**. Box plot TPC in samples from three coffee cultivation areas.

Referring to the figure, the highest rate of TPC is in Mengani CP-Hay. Apparently, plastic-bagged CP-Hay stored at room temperature does not protect the goods from organism contamination. The following highest rates – meaning the least hygienic ones – go to Ijen CP, Karangploso CP, and Mengani CP-SP, respectively. The data illustrates that small coffee processor units are currently incapable of applying hygienic work. Due to the low TPC rate, the cleanest product is La Boite CCF, followed by Mengani CCF and Mengani CP-Est, respectively.

### *3.8. Principal Coordinates Analysis (PCoA) and Heat Map*

The authors summed up the results of seven observed variables (Figure 2 to Figure 8) applied to six different CP samples to find the most similar one to the control. Employing PCoA (MDS) through DARwin 6.0.010 software program (Perrier and Jacquemoud-Collet, 2006), the dendrogram was then visualized in the form of a Heat Map and included in Figure 9.

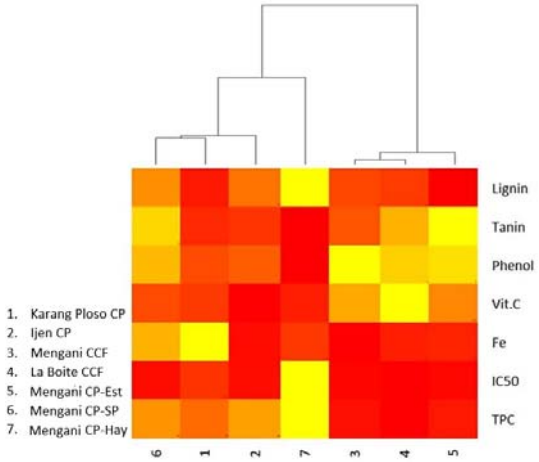


**Figure 9**. Heat Map of seven observed variable results of seven CP samples

Three groups are formed based on the objects' Euclidean distance: Mengani CP-Hay is in the first; Karangploso CP, Ijen CP, and Mengani CP are in the second; Mengani CP-Est, Mengani CCF, and La Boite CCF are in the third. Since Mengani CP-Est and Mengani CCF are in the same group as La Boite CCF, this means that they are the most similar samples to the control.

## 4. Conclusion and Recommendation

Two samples with the highest degrees of similarity to the control (La Boite CCF) are Mengani CCF and Mengani CP-Est. The authors recommend improving the drying system for Mengani CCF and Mengani CP-Est since high temperatures presumably damaged some observed variables. Furthermore, further investigation on Fe contaminant in Karangploso CP and Mengani CP-SP samples should also be conducted to prevent it from happening.

Following up on this research, the authors suggest applying other observed variables to the samples mentioned above, such as heavy metal content, proximate analysis, and detailed amino acid content. This *in vitro* research should also expand too as *in vivo*.

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