The influences of storage temperature and time on decocted Robusta coffee leaves tea

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Abstract. Coffee leaves tea has attracted researchers to utilize the pruning waste as a refreshing functional drink due to its phenolic compounds and other antioxidants. Meanwhile, the tannins also contributed to astringency as an important sensorial attribute for tea. All these components were changing over time. Thus, an evaluation of its changes in coffee leaves tea during storage is required. This research used robusta and liberica coffee leaves that were stored at temperature of 10 and 25° for a storage time of 24 days. Completely Randomized Block Design was assigned to investigate the main factors. As result, it was found that storage at 10°C could maintain the functional quality up of the leaves for up to 2 weeks. Meanwhile, 25°C of storage was acceptable for only less than 5 days. During storage, green flavor and aroma, astringency and bitter taste change proportionally to the decrease of phenolic content and tannin levels. In conclusion, the storage temperature and time on decocted robusta coffee leaves tea might influence the quality of leaves.

1. Introduction
As an increase in coffee production, the coffee leaf as waste was also elevated in number. In addition, the pruning process was also carried out on old leaves and but it was needed to control the pest of coffee leaves, as well as to avoid loss in humidity, facilitate airflow so that the pollination process might run well [1]. As consequence, this pruning process provides a high amount of coffee leaf waste. In general, coffee leaves were generally used as compost, animal feed or allowed to dry. As a waste product, thus the increase in the use of these coffee leaves as tea was then developed due to the potent use for the increase in sensory properties of drink [2].

The coffee leaf waste remained inconsistent and uncontrollable [3]. So it was necessary to keep the quality since the use might provide benefits for antioxidant resources and functional drinks such as herbal tea coffee leaves. However, the temperature and storage time may reduce its quality. In addition, the choice of decoction technique was used because it was able to extract the chemical compounds
contained in coffee tea leaves better, this was because the cooking temperature of the decoction technique was higher than the infusion technique of 950-1000°C for 2-3 minutes [2].

As previously explained, storage could change the characteristics of coffee leaf tea of antioxidant levels [3], this study was aimed at analyzing chemical and sensory profile of coffee leaf tea against temperature and storage time. The method of the spectrum and acceptance test were used in this test that might determine the level of liking and acceptance of coffee leaf tea at detected storage time and temperature.

2. Materials and Methods

2.1 Material
Oven, controlled stove, UV-Vis spectrophotometer, cuvette, vortex, pipette, pH meter, measuring flask, erlenmeyer, funnel separator, collecting flask, whatman paper filter paper, test tube, beaker glass, and thermometer were used in this experiments.

2.2 Sample
Old robusta and liberica coffee leaves from Ampelgading, East Java were used in this study. As analysis phenol was done in this investigation, gallic acid, folin ciocalteau, Na₂CO₃, tannic acid, methanol and DPPH were used. An organoleptic test was done in a standard organoleptic laboratory using standard procedure in the laboratory.

2.3 Total phenolic analysis
The spectrophotometrical method was done to analyse total phenolic [4]. The sample was boiled at 90°C for 2.5 minutes, then filtered using Whatman filter and followed by dilution using aquadest. Folin ciocalteau reagent was then mixed with this diluted sample and the blue-greenish. Finally, Na₂CO₃ was mixed and followed by UV-Vis detection at 764 nm [5].

2.4 Tannin analysis
Phenol analysis was performed using the spectrophotometric method [4]. The sample was brewed at 90°C for 2.5 minutes. Then the sample was filtered using filter paper. After filtering, dilution was carried out using distilled water, then reacted with folin ciocalteau which forms a turquoise color. After that, Na₂CO₃ was added and measured using a UV-Vis spectrophotometer at 764 nm.

2.5 Antioxidant activity analysis
Antioxidant analysis using the IC₅₀ method. The sample was brewed at 90°C for 2.5 minutes. Then the sample was filtered using filter paper. After filtering, dilution was carried using methanol. Then reaction process with DPPH was done which formed a purple-reddish color. After that, measurement using a UV-Vis spectrophotometer at 517 nm was conducted.

2.6 Moisture content analysis
Water content analysis was carried out on coffee leaves that were dried using the oven method. The principle of this method was to measure the moisture content of material by drying the material to a constant weight in an oven using a temperature of 100-105°C where the calculation was based on the differences in moisture content between the initial and the final weight [5].

2.7 Sensory evaluation
Panelists in this study were limited in the range of age 19-22 years old and have passed the selection process that had been carried out to choose the best knowledge in a coffee drink. This was based on a previous researcher [6]. This range of age was the best for evaluation [7]. As driven by Australian Standard 2542.1.1-1995, it was suggested that the selection of panelists should be chosen from local employees, laboratory assistants, employees and people around the company. Therefore, this study
applied to students from the Faculty of Agricultural Technology, Universitas Brawijaya. The selection was carried out by interviews, taste recognition, and triangle tests.

3. Results and Discussions

3.1 Raw material characteristics
Old robusta leaves were used in this research since this type of leaf was good sensory compared to other types [2]. The leaves as samples were taken from the third to fifth from tips of the plant. The leaves were sorted and washed, then the leaves were chopped to a size of approximately 1 cm. The next process was fermentation for 8-10 hours to produce oolong tea. Then the leaves were dried using a cabinet dryer for 4 hours at a temperature of 60°C. The coffee tea leaf powder was then sifted by 20 mesh, and brewed by the decoction method at 92°C for 2 minutes to maximize the extraction process of bioactive compounds [2]. The dried leaves were then mashed in a blender. The following are the characteristics of the raw materials Table 1.

Table 1. Raw material characteristics.

| Parameter                     | Amount | Unit |
|-------------------------------|--------|------|
| Moisture Content              | 6.2    | %    |
| pH                            | 7.3    |      |
| Color                         | 52.5, 17.9, 35.5 | L, a, b |
|                               | 162, 117, 73 | R, G, B |
| Color Visualisation           |        |      |
| Brix                          | 1.2    | Brix |
| Antioxidant Activity          | 34.5   | ppm  |
| Total Phenolic Content        | 2,354.3| μg/g |
| Tannin                        | 23.7   | μg/g |

3.2 Total phenolic content analysis
Phenol is one of the substances that play a role in forming perceptions of bitter tastes, aromas and flavors of green and woody [7]. In this study, phenol levels in both treatments decreased both at room temperature and cold temperature (Fig. 1). According to previous researchers [7], phenol was able to provide health effects with levels of 1000 μg/g/day for 4-6 months continuous intake and might reduced blood pressure. So it is recommended to consume coffee leaf tea before the 12th day of storage.

In oolong tea that is not fully oxidized for enzyme inactivation, there are still several active enzymes, for example phenylalanine ammonia lyase (PAL) enzyme which is main enzyme in plant metabolism that catalyzes reactions in the biosynthesis of L-phenylalanine and phenylpropionate framework [8]. In stressful conditions such as when plants are injured, this enzyme will be active, PAL enzyme will be activated to convert phenol into anthocyanins so that may affect chemical, physical and sensory parameters [8]. According to another researcher [8], the type of might affected the performance of the PAL enzyme. In plants that still have a source of phenylalanine, PAL catalyzes phenylalanine deamination and produces trans-cinnamic acid, which is converted into p-coumaric acid by oxidative catalization involving the enzyme cytochrome P450, and then converted to Coumaroyl CoA. Coumaroyl CoA is the basic ingredient of flavones with anthocyanin and pro-anthocyanin end products. In the tea
harvesting process, the tea tissues are injured resulting in the activation of the PAL enzyme, but after the drying and fermentation process, the enzymes are drastically reduced and this causes the phenylpropanoid pathway to run slowly. After drying, the leaves do not have enough phenylalanine sources so the PAL enzyme breaks down flavonons (including phenols). According to [9] the decrease in cold temperatures is due to the activation of the PAL enzyme during the injury period, at this time the plant still has leaf tissue which is a source of phenylalanine so that the formation of flavonones occurs. However, when the tea is dried and brewed, there is no tea leaf tissue that contains phenylalanine to form flavonones, but there is still PAL enzyme in tea that is stored at cold temperatures when it is brewed, so the pathway continues.

![Figure 1](image_url)

**Figure 1.** Total phenolic content (A) and tannin content (B) at cold (10°C) and room (25°C) storage temperature.

While the cause of the decrease in room temperature is due to the PAL enzyme which is easily damaged at room temperature, according to [10] blanching at 95°C for one minute can inhibit PAL enzyme activity by 63%. Meanwhile, in the storage period with a temperature of 270C for three days in mandarin fusion, the increase in PAL enzymes did not experience a drastic increase after picking. In addition, this is due to the degradation of phenols by microbes. This is because phenol is an antimicrobial agent [11]. Microbial growth was indicated by the presence of fog on the tea on the fifth day, causing 80% rejection of the panelists. In plants, fungi are the main destroyer that can degrade phenolic compounds by oxidizing phenolic compounds so that phenol becomes damaged when there is too much fungal contamination [11].

3.3 Tannin analysis

Tannin is one of the substances that play a role in the formation of bitter taste perception, and mouthfeel astringent [12]. Tannins give the impression of a bitter taste, and an astringent mouthfeel. In this study, tannin levels in both treatments decreased both at room temperature and cold. Dissolved tannins are compounds that are responsible for the perception of astringent taste. At cold temperatures, tannins will be polymerized by acetaldehyde contained in plants to form oxidized tannin compounds that are insoluble, so that the astringent taste can be reduced. In oolong tea that is not fully oxidized for enzyme inactivation, there are still several active enzymes, one of which is Phenylalanine Ammonia Lyase (PAL) enzyme which is the main enzyme in plant metabolism which catalyzes reactions in the biosynthesis of L-phenylalanine into natural products based on the phenylpropane framework [8]. In stressful conditions such as cold, this enzyme will be active, Phenylalanine Ammonia Lyase (PAL) enzyme will be activated to convert tannin into pro-anthocyanin so that it affects the chemical, physical and sensory parameters [9]. According to [9], the type of tea will affect the performance of the PAL enzyme. According to [9], the type of tea will affect the performance of the PAL enzyme. In plants that still have a source of phenylalanine, PAL will catalyze phenylalanine deamination and produce trans-
cinnamic acid, which will then be converted into p-coumaric acid by oxidative catalization with the enzyme cytochrome P450, and converted to Coumaroyl CoA. Coumaroyl CoA is the basic ingredient of flavones with anthocyanin and pro-anthocyanin end products. In the tea harvesting process, many of the tea tissues are injured, resulting in the activation of the PAL enzyme, but after the drying and fermentation process, the enzymes are drastically reduced and this causes the Phenylpropanoid Pathway to run slowly. After drying, the leaves do not have sufficient phenylalanine sources so that the PAL enzyme breaks down tannins into proanthocyanidins which can be seen in the pathway [9].

Meanwhile, microbial contamination might reduce phenol due to an anti-microbial activity. Microbial growth was indicated by the presence of fog on the tea on the fifth day, causing 80% rejection of the panelists. In plants, fungi are the main destroyer that can degrade phenolic compounds with the laccase enzyme which is able to oxidize phenolic compounds so that tannins become damaged when there is too much fungal contamination [11].

3.4 Sensory analysis
3.4.1 Bitter taste
Tannins are responsible for the appearance of the perception of astringent taste. At cold temperatures, tannins will be polymerized by acetaldehyde in plants to form oxidized tannin that is insoluble and this may contribute to the reduction in astringent taste. In oolong tea that is not fully oxidized for enzyme inactivation, there are still several active enzymes, for example, phenylalanine ammonia lyase (PAL) which is the main enzyme in plant metabolism which catalyzes reactions in the biosynthesis of L-phenylalanine into natural products based on the phenylpropopane framework [8]. In stressful conditions such as cold, this enzyme will be active, PAL will be activated to convert tannin into pro-anthocyanin so that it affects chemical, physical and sensory parameters [9].

![Figure 2. Sensory attributes at cold and room temperature.](image)

According to the previous report [9], the type of tea will affect the performance of the PAL. Furthermore, the type of tea will affect the performance of the PAL enzyme [9]. In plants that still have a source of phenylalanine, PAL will catalyze phenylalanine deamination and produce trans-cinnamic acid, which will then be converted into p-coumaric acid by oxidative catalization with the enzyme cytochrome P450, and converted to Coumaroyl CoA. Coumaroyl CoA is the basic ingredient of flavones with anthocyanin and pro-anthocyanin end products. In the tea harvesting process, many of the tea tissues are injured, resulting in the activation of the PAL enzyme, but after the drying and fermentation
process, the enzymes are drastically reduced due to phenylpropanoid pathway to run slowly. After drying, the leaves do not have sufficient phenylalanine sources so that the PAL enzyme breaks down tannins into proanthocyanidins which can be seen in the pathway [9].

Meanwhile, the cause of the decrease of phenolic compounds at room temperature is likely due to microbial contamination. This is because phenol may serve as an anti-microbial agent [9]. Microbial growth was indicated by the presence of a white appearance on the tea on the fifth day, causing 80% rejection of the panelists. In plants, fungi are the main destroyer that can degrade phenolic compounds with the laccase enzyme which is able to oxidize phenolic compounds that may contribute to the damage of tannins by fungal contamination [11].

3.4.2 Green aroma

The aroma of green is the perception of smells like vegetables or the first leaves that are felt by the panelists during the first sip. One of the most recognizable aromas in tea is the green aroma [1] which is caused by the alcohol content bound in phenol [3]. The main constituents of green aroma and aroma are trans-2-hexenol and cis-3-hexenol. Author [11] wrote the phenol components that form green scents such as ethyl acetate, butanol, 1-pentane-3-ol, amyl alcohol, octanal, 2-methyl-6-heptane-6-one, nonanal, deanal, and L-menthol. The aroma and aroma of green are quite difficult to find in processed foods but are often found in fresh products. One of the causes of this high perception of taste in a plant-based product is the stress level of the product which produces more phenol [1]. Research has been carried out on onions in India, that the process of pulverizing onions at the chopping stage affects the perception of green aroma and aroma. In tea products, slicing and rolling fresh leaves will cause a high level of stress, thereby increasing the perception of green aroma and aroma in plants [1].

At room temperature (25°C) for five days, 80% of the researchers refused on the eighth day, with a decreased green scent. This reduction was calculated using the general linear model and showed a significantly different result every day. So it can be interpreted that a significant decrease in green aroma on days one to eight. The decrease is caused by phenol degraded by microbes that can happen rapidly at room temperature.

3.4.3 Astringency

According to another investigator [13], mouthfeel astringent in a material is connected to the content of tannin. This is directly proportional to those experiments, where tannin levels decreased, causing a decrease in mouthfeel astringent at cold temperatures (10°C). Meanwhile, at room temperature, mouthfeel astringent was not significantly different even though the phenol content was also decreased. This was because phenol at room temperature remained through enzymatic pathways so that it still provided mouthfeel astringent perception. The loss of mouthfeel astringent perception in tea due to PAL enzyme which changed flavonoids (including phenols) into a simpler form for example anthocyanins. While at room temperature, a decrease in phenol content is thought to be due to microbial contamination. This also occurs in wines where the decrease in phenol levels in improper storage does not affect the perception of astringent and astringent mouthfeel although the phenol and tannin levels decrease due to microbial contamination [14]. In addition, PAL enzyme activation is more active when plants are stressed such as being injured and treated in cold temperatures [9].

3.4.4 Green flavor

This flavor is the perception of taste like vegetables or the first leaves that are felt by the panelists during the first sip. One of the most well-known flavors in tea is the green flavor which is caused by the alcohol content bound in phenol [3]. The main constituents of green aroma and flavor are trans-2-hexenol and cis-3-hexenol. Another investigator [14] found that the phenol components that produced green scents such as ethyl acetate, butanol, 1-pentane-3-ol, amyl alcohol, octanal, 2-methyl-6-heptane-6-one, nonanal, deanal, and L-menthol. Green flavor and aroma are quite difficult to find in processed foods but are often found in fresh products due to the reduction in phenol content [1]. This may contribute to the decline in the perception of green flavor. According to [9] dan [15] brewing technique can also have
potential in the performance of PAL enzymes. The higher the brewing temperature or heat exposure, the more damaging the PAL enzyme that may change the perception of flavor and aroma of green.

4. Conclusions

The high quality of coffee leaves as tea was considered to possess high antioxidant and acceptable sensory potential by consumers. The storage temperature and time on decocted robusta coffee leaves tea might influence the quality of leaves. Based on our investigation, antioxidants compounds in the leaves as tea such as phenols and tannins might be preserved in its quality when it was stored maximally on the 12th day.

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