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Effect of Processing on Green and Black Tea DPPH Radical Scavenging Activity, IC_{50} Value, Total Polyphenols, Catechin and Epigallocatechin Gallate content

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Abstract. Green tea and black tea are rich in antioxidants that have a beneficial effect on human health. The antioxidants in green tea and black tea are mainly polyphenols including catechin and its derivative epigallocatechingallate. The DPPH radical scavenging activity, IC_{50} value, total polyphenols, catechin and epigallocatechingallate content were analyzed throughout green and black tea processing. The DPPH radical scavenging activity, IC_{50} value, total polyphenols, catechin and epigallocatechingallate decreased during processing. The study confirms that the processing influences the antioxidant activity of green and black tea.

1. Introduction

Tea from the shoots and young leaves of *Camellia sinensis* which can be processed in different ways to make beverages that have certain characteristics. Amongs these green tea and black tea are the most popular. Green tea is processed by picking, withering at high temperatures for enzyme inactivation, rolling (reducing the size) then first drying and second drying. Black tea is picked, wilted at room temperature, rolled, wet-sorted, enzymatic oxidized and dried. Green tea and black tea both contain catechins; epicatechin (EC), gallocatechin (GC), gallocatechingallate(GCG) and epigallocatechingallate (EGCG) with epigallocatechingallate being the most abundant [1,2]. Significant chemical components in green tea are catechin, epicatechin, epigallocatechin, epigallocatechingallate, chlorophyll, quercetin, myricetin and other chemicals that provide fragrance [3]. Because of these components, green tea and black tea are deemed to have positive health effects such as antioxidants, anticancer and as a treatment for oxidative stress [4].

In green tea processing, the heating process is used in the withering process and in two drying stages. In the processing of black tea, heating only occurs out at the end when drying [5]. Polyphenol components found in tea have different heat stabilities so any heating can result in a decrease in these components. Catechin, in particular, is a phenolic compound that is unstable to heat [6]. Oxidation also results in a decrease in polyphenol content in tea. Oxidation can occur when reducing the size of the tea during the rolling process.

Catechin is an antioxidant compound found in green tea and black tea, but has a lower ability as an antioxidant compared to epigallocatechingallate. which is the strongest anti-oxidant amongst its derivatives [7]. This study looks at changes in antioxidant activity and the content of catechin and EGCG at each stage of the processing of green tea and black tea.

2. Materials and Method

2.1. Raw material

Tea leaves from PT. Mitra Kerinci, a plantation in South Solok, West Sumatra, Indonesia were used.

2.2. Chemicals

These included 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Ethanol, Methanol, Buffer potassium chloride, sodium acetate buffer, HCl, Folinciocalteu, Na_2CO_3, Gallic acid, sodium hydroxide, DMSO, phosphoric acid, tetrahydrofuran, acetonitrile, catechin, epicatechin and epigallocatechingallate standards from Sigma Aldrich Company, Germany.

2.3. Green tea processing

Young tea leaves were picked and then withered using a Rotary Panner (RP), then rolled using the Open Top Roller (OTR). A first drying used Endless Chain Pressure (ECP) and the second drying used a Ball Tea (BT) machine. After each processing stage samples were analyzed after drying to a moisture content of 4%.

2.4. Black tea processing

Young tea leaves were picked and then withered using a Withering Trough (WT), After 70% withering, the leaves were rolling with an Open Top Roller (OTR), followed by wet sorting using DIBN, then oxidized enzymatically and finally dried using the Two-Stage Dryer (TSD). After each processing stage samples were analyzed after drying to a moisture content of 4%.

2.5. DPPH assay[8]

One gram samples were added to 10 mL of either water, methanol or ethanol then homogenized. The crude extract samples were mixed with 3.9 ml of methanol and 1 ml of a DPPH solution (1mM in methanol) in a test-tube and the absorbance measured at 517 nm after 30 minutes incubation.
DPPH radical scavenging activity was calculated as follows:

$$\text{DPPH radical scavenging activity} = [\,1 - (A_{517}\,(\text{sample}) / A_{517}\,(\text{blank})\,] \times 100\ \%$$

2.6. Total phenolic content

The total phenolic content of the samples was determined using the Folin Ciocalteu colorimetric method as described by Tumbarski et al (2019) [9]. One g samples were put in 10 mL of methanol and then put into a homogenizer for 15 minutes. One mL of the resulting crude extract was added to 2 mL of distilled water and 1 mL Follin- Ciocalteu reagent and homogenized for 5 minutes. One mL of 5% Na_2CO_3 was added and the mixture incubated 2 h at room temperature. The absorbance was then measured at 725 nm. Phenolic content was calculated using gallic acid (3,4,5-trihydroxy benzoic acid (C_6H_2 (OH_3) CO_2H))as the standard and expressed as mg gallic acid equivalents (GAE)/100 g dry weight (DW).

2.7. Catechin and EGCG [10]

Cathecin content was determined using a HPLC series system with a high-pressure gradient pump, an autosampler, a column oven (Prominence HPLC 20A Shimadzu), and a reverse-phase chromatographic column (agilent Zorbax SB-C18 column) coupled to a UV- visible detector. The eluents were (A) water with 0,2% of phosphoric acid and 1% of tetrahydrofuran, and (B) acetonitrile with 1 % of tetrahydrofuran. The separation was achieved under a step gradient polarity system that was from 5 to 25 % B for 20 min at a flow rate of 0,8 ml/min and the detection wavelength was at 230 nm.

3. Results and Discussion

3.1. DPPH Radical Scavenging Activity

DPPH radical scavenging activity measurement is a way to predict antioxidant activity in a plant or food product. It is easily carried out with chemicals that are readily available and takes a relatively short time. Changes in DPPH radical scavenging activity in the processing stages of green tea and black tea can be seen in Figure 1 and Figure 2.

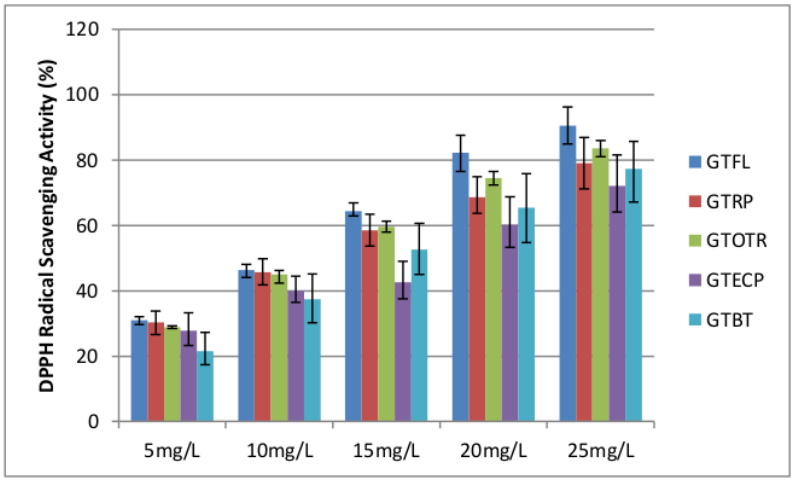

Fig.1. DPPH Radical Scavenging Activity of Green Tea Processing

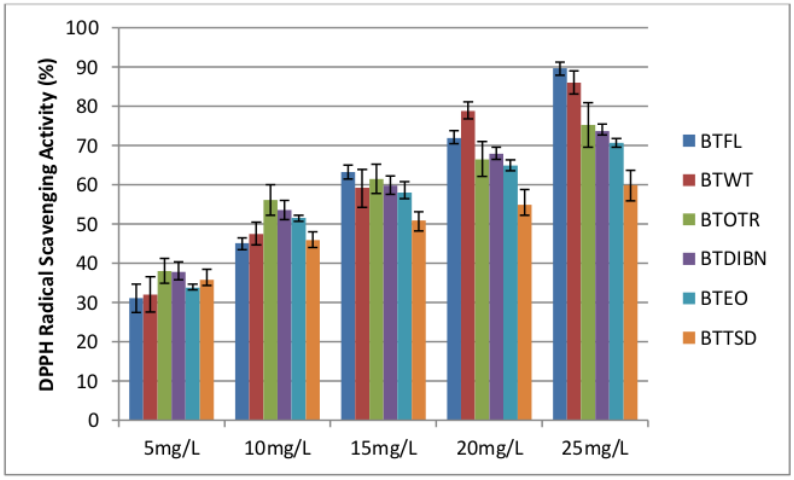

Fig.2. DPPH Radical Scavenging Activity of Black Tea Processing

The value of DPPH radical scavenging activity is strongly influenced by the concentration of antioxidants. The higher the concentration of antioxidants, the higher the activity. From Figure 1 and Figure 2 it can be seen that as processing proceeds there is a decrease in the DPPH radical scavenging activity.

For green tea, one process that leads to decrease in DPPH radical scavenging activity is the enzyme inactivation which uses a temperature of 90 ° C because the polyphenol component is unstable at this temperature. This also affects the color, after the withering process, the color of the leaf which was originally dark green turns to yellowish color because chlorophyll has begun to be destroyed. Chlorophyll is also an antioxidant [11]. Rolling usingOpen Top roller (OTR) could decrease the DPPH radical scavenging activity due to oxidation. After leaving the rotarypanner, the tea leaves are carried about 20 meters by conveyor to the OTR where they are reduced in size so that the larger the particle size which causes oxidation to occur more easily. The DPPH radical scavenging activity continues to decrease with each processing step. The first drying of green tea ain the ECP is at 110-135 ° C for 25 minutes and final drying using BT at 80 ° C for 10 hours.

Antioxidants in black tea include, in addition to catechins and their derivatives, theaflavin and thearubigin, the end result of enzymatic oxidation, which also give color to black tea brew [12]. The aroma component also acts as an antioxidant in black tea. Carotenoids, fatty acids, glycosides, and amino acids/sugar are precursors for the formation of aroma molecules in tea and the amount of these is affected by the processing. Some examples of aroma components with amino acid/carbohydrate precursors are (E) -2-Hexenal, Hexanal, Hexanoic acid, (Z) -3-Hexen-1-ol, (E) -Linalool oxide (furanoid) and Phenylacetaldehyde. Each of these components is more abundant in black tea compared to green tea [13].

Decreased antioxidant activity is also seen in each black tea processing step. The decrease can be caused by oxidation, such as in the OTR machine. The wet sorting process also slightly decreases the antioxidant content of tea because in this process the sifted tea shoots have more contact with air in the presence of particles that are already small in size, having passed through a sieve with 6-10 circular holes per square inch. Sifting ensures uniform particle size and during this process, oxidation and chlorophyll destruction by chlorophyllase can beginAs can be seen in Figure 2, the greatest decrease in DPPH radical scavenging activity occurs during drying. This is because an inlet temperature of 100 ° C for 30 minutes is used.

3.2. IC_{50} Value and Total Polyphenol content during Tea Processing

IC_{50} and total polyphenol content at each stage of green tea processing are shown in Table 1. Thevalues for black tea are in Table 2.

Table 1. IC_{50} value and Total Polyphenol of Green Tea Processing

	IC_{50} (ppm)	Total Polyphenol (mg/g)
GTFL	11.32±2.30	794.31±5.33
GTRP	12.70±1.85	642.00±7.32
GTOTR	12.00±2.56	628.73±7.89
GTECP	14.91±3.42	610.65±8.43
GTBT	17.62±1.76	500.85±3.89

GTFR : Green Tea fresh Leaves
GTRP : Green Tea Rotary Panner
GTOTR : Green Tea Open Top Roller
GTECP : Green Tea Endless Chain Pressure
GTBT : Green Tea Black Tea

As with DPPH Radical Scavenging Activity, the IC_{50} value and total polyphenol also decreased with each processing step of green tea and black tea. From Table 1 it can be seen that the total decrease in polyphenols in green tea was 293.46 mg / g or around 37%. In black tea, the decrease in catechin is more drastic at around 747.11 mg / g or about 76%. The decrease in the total polyphenol content is thought to be due to the temperature used. Other polyphenols found in tea besides catechin include gallic, trans-cinnamic, caffeic, ferulic and coumaric acids in differing amounts depending on the processing process [14].

Table 2. IC_{50} value and Total Polyphenol of Black Tea Processing

	IC_{50} (ppm)	Total Polyphenol (mg/g)
BTFL	10.81±5.23	985.46±7.24
BTWT	11.82±2.64	680.85±6.76
BTOTR	34.17±4.30	356.42±4.50
BTDIBN	36.47±4.34	253.54±5.93
BTEO	33.07±5.21	237.77±3.87
BTTSD	53.66±4.57	238.35±6.57

BTFL : Green Tea Fresh Levaes
BTWT : Black Tea Withering Through
BTOTR : Black Tea Open Top Roller
BTDIBN : Black Tea Double Indian Breaker Netsortider
BTEO : Black Tea Enzyme Oxydation
BTTSD : Black Tea Two Stage Dryer

3.3. Catechin and EGCG content during Green Tea and Black Tea Processing
These values are presented in Table 3 and Table 4.

Table 3. Catechin and EGCG of Green Tea Processing

	Catechin (μg/mg)	EGCG (μg/mg)
GTFL	156.41±22.39	525.13±90.00
GTRP	137.79±21.71	417.9±17.59
GTOTR	112.63±4.80	384.54±87.09
GTECP	90.97±13.19	305.87±27.62
GTBT	76.57±12.53	246.1±45.20

Table 4. Catechin and EGCG of Black Tea Processing

	Catechin (μg/mg)	EGCG (μg/mg)
BTFL	193.03±17.36	521.44±19.80
BTWT	175.03±16.08	465.45±18.41
BTOTR	90.76±9.89	8.01±1.12
BTIDBN	74.73±17.20	5.3±3.12
BTEO	71.13±12.65	3.67±0.47
BTTSD	68.74±10.55	2.28±1.50

Catechin is the most abundant polyphenol found in green tea and black tea. The black tea processing destroys more of this catechin than the green tea process because, along with the damage by heat and oxidation, the enzymatic oxidation process changes catechin to theaflavin and thearubigin [15]. In green tea processing, these oxidating enzymes are inactivated during the withering process. EGCG is degraded during processing to EGC. Catechin in tea can be transformed into an epimer form at high temperatures (200 ° C for 5 minutes) [16]. In this study, green tea heating occurred in two stages, where the first stage with ECP with a temperature of 110-135 °C for 25 minutes and BT with a temperature of

80 ° C but for 10 hours. Black tea is heated only at 100 ° C, but for 30 minutes, so a decrease may also occur even though the temperature does not reach 200 ° C because of the duration of the heating.

According to Ref. [17], catechin decreases dramatically during the enzymatic oxidation process of black tea while gallic acid increases during the processing of black tea which is influenced by epimerization and oxidative degradation [17]. The EGCG content in fresh tea is 30.9 ± 0.39 mg / g, in green tea 27.9 ± 0.24 mg / g but only 4.67 ± 1.79 in black tea. Carloni et al. (2013) also found the amount of catechin in green tea was higher than that of black tea while the theaflavin content was detected in green tea (< 0.01mM), but it was in much greater amounts in black tea (>0.1mM) [1].

4. Conclusion

Processing reduces the DPPH Radical Scavenging Activity value, IC50 value, total polyphenols and catechin and EGCG values of green tea and black tea. The polyphenol content decreased more in black tea than in green tea. This suggests that green tea offers superior antioxidant benefits to consumers.

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