Changes of chemical contents during the withering process of white tea

H Maulana¹, M I Prawira-Atmaja¹, Shabri¹, N Hamdini², J Alyanisa², S Harianto² and D Rohdiana¹

¹Department of Postharvest & Engineering, Research Institute for Tea and Cinchona, Gambung, Kab. Bandung, West Java, Indonesia.
²Departement Teknologi Industri Pangan, Fakultas, Teknologi Industri Pertanian, Universitas Padjajaran, Jatinangor, Sumedang, West Java, Indonesia.

Email: iqbal.prawira@ritc.id; hilman.maulana@ritc.id

Abstract. White tea is produced from the bud of Camellia sinensis, which is processed with minimum processing. Withering is an important step in processing white tea. Withering in white tea processing utilizes sunlight to obtain moisture content properly. This study aims to determine the chemical changes of tea buds during the processing of white tea. Observations were carried out on the withering of 4, 21, 45, 69 and 93 hours. The parameters observed included total polyphenols, theaflavin, and thearubigin. The results showed that during the process of withering changes in total polyphenols, from 33% at 4 hours of withering, to 28% at 93 hours of withering. Theaflavin content decreases from 0.05% to 0.036% at 4 hours to 96 hours withering, respectively. This shows that enzymatic oxidation reactions still occur during the processing of white tea. Results suggest that withering for 96 hours on the processing of white tea is optimum. The present study also provides guidelines on application processing to produce a high quality of white tea.

Keywords: white tea, withering, total polyphenol, theaflavin

1. Introduction

All Type of tea is produced from the same origin of Camellia sinensis with two subspecies of var. Sinensis mainly from China, and var. Assamica from Assam. As mentioned by Engelhardt (2007) about white tea definition, first white tea defined by Fujian province tea leaves of subspecies and manufactured with minimal processing, the second was based on non-China countries producing white tea, that the bud or bud and first leaves plucked, followed by minimal processing defined the white tea [1]. Following the description of white tea manufacturing, after plucked, the tea leaves were sun withered and dried to keep the leaves relatively fresh, different with green tea or black tea manufacturing [2]. This paper will discuss the changes in the chemical of white tea produced from Camellia sinensis var. Assamica from Research Institute for Tea and Cinchona (RITC). The parameters observed included total polyphenols, theaflavin, and thearubigin.
2. Material and methods

2.1. Plants material and location
Apical bud of Camellia sinensis meticulously plucked by hand in the early morning (06.00 am). Apical bud plucked from February to April 2018. All research was carried out in Gambung tea plantation (latitude: 7° 08’ 37”N, longitude: 107° 30’ 54”R, altitude 1200 m) of RITC in West Java, Indonesia.

2.2. White tea manufacturing
Plucked apical bud was immediately brought to processing plant of white tea and placed on an aluminum tray ready for the withering process. The withering of the apical bud was processed by sunlight from 11:00 am to 03:00 pm, and continued overnight in the room, the processing steps were repeated for 4 days. Tea samples were taken every 4, 21, 45, 69, and 93 hours of the withering process. At the last step, dried in oven 60°C until the obtained moisture content of the product was 3-4%. The tea sample was packed in aluminum foil and store in a room with Relative humidity (RH) ≤ 60.

2.3. Determination of total polyphenol
2.3.1. Preparation and extraction. Samples were placed in a mortar and ground with a pastel finely. Add 2 g of sample into 30 ml boiled methanol 70%, heated up for 10 minutes and followed by maceration in oven 60 °C for 2 hours. The mixture was then sonicated for 30 min using a Sonicator (Bransonic-220 Inst., New York, USA). The extraction mixture was constantly kept cold by adding ice water into the sonicator vessel. The mixture was then passed through a filter with a Whatman No. 1 filter paper to obtain a clear extract. The residues and all glassware were then washed with 70% aqueous methanol and the total volume of the extract was made up to 50 ml in a volumetric flask. One milliliter of the extract was pipetted into a 25 mL volumetric flask and diluted with distilled water. The dilution extract was further used in determining total polyphenol. These preparation and extraction methods used, according to Prawidya-Atmaja et al [3].

2.3.2. Determination of total polyphenol. Determination of total polyphenol (TP) according to International standard ISO 14502-1:2005 [4] using the Folin-Ciocalteu method. One milliliter of dilution extract was reacted with 5 mL of Folin-Ciocalteau’s reagent (10%) for 5 min then, 4 mL of sodium carbonate 7.5% (37.5 g was diluted with 500 ml distilled water). The solution allowed to stand for 2 hours before spectrometric analysis. Total polyphenols content was measured at 740 nm using Varian carry win UV spectrophotometer. The number of total polyphenols was obtained from the standard curve equation of Gallic acid solution with a concentration range of 1-100 mg / L (ppm).

2.4. Determination of theaflavin and thearubigin
Total theaflavin (TF) and thearubigin (TR) were determined according to Robert and Smith's method [5] as described by Obanda et al [6]. A nine gram of samples tea infused with 375 ml boiling water into a vacuum flask. The flask was shaken for 10 minutes, the infusion filtered using cotton wool, and allowed to cool to room temperature. The cooled infusion was extracted using IBMK (iso-butyl methyl ketone) and gently shaken for 10 minutes. The layers were allowed to separate and a 4-ml portion of the IBMK layer was taken and made to 25 ml with methanol in a volumetric flask (Solution A). Two-milliliter portions of the aqueous layer were diluted to 10 ml with distilled water and then to 25 ml with methanol (Solution B).
Twenty-five milliliters of the remaining initial IBMK layer were taken in a separate flask and mixed with 25 ml of 2.5% aqueous sodium hydrogen carbonate. The mixture was vigorously shaken before the layers were allowed to separate and the aqueous layer discarded. A 4-ml portion of the washed IBMK layer was made to 25 ml with methanol (Solution C).
Two milliliters of a saturated oxalic acid aqueous solution and 6 ml of water were added to a 2-ml portion of the aqueous layer left from the first extraction with IBMK and diluted to 25 ml with
methanol (Solution D). The absorbencies $A_A$, $A_B$, $A_C$, $A_D$ of solutions A, B, C and D at 380 and 460 nm were obtained using a Varian Carry Win UV spectrophotometer with distilled water as the blank. The theaflavin and thearubigin contents were calculated as:

$$\%TF = 6.25 \times A_c \times F1 \quad (1)$$

$$\%TR = [(12.5A_D + 6.25(A_A - A_c)) \times F2] \quad (2)$$

Where $F1$ and $F2$ are factor conversion of spectrophotometric. The value of $F1=0.36$; and $F2=1.13$.

2.5. Statistical analysis
All data study was analyzed by using one-way ANOVA at a significance level of 95% followed by Duncan's Multiple Range Test (DMRT). All data analysis was performed using XLSTAT 2014.

3. Results and discussions
3.1. Changes of total polyphenol during sun withering
Our white tea samples were produced from GMB Clones Series from RTC. Even though the ANOVA and DMRT showed no significant signal for every point of times selected, we could view there were changes in total polyphenols during sun withering of white tea for every 4, 21, 45, 69, and 93 hours have a decreasing tendency, as showed in figure 1.

![Figure 1. Total polyphenol content during sun withering of white tea.](image)

Polyphenol was referred to as aromatic molecules with multiple hydroxyl compound group and the abundance of polyphenols in tea leaves naturally connected to tea chemistry. Also, polyphenols in tea are responsible for the formation of tea color, taste strength, and partly for flavor in the beverages [7]. The purpose of withering is to alter the respiration, metabolism, and oxidoreductive process that occur before plucking to be maintained after plucked. Changes during withering of tea leaves are physical changes, which lead to loss of moisture and cell permeability, and chemical changes, which influenced the tea aroma and other characteristics of made tea [8].

The polyphenols changes during the withering of the tea leaves in this paper suggested the decreasing value of the total polyphenol content [9]. The withering accompanied by oxidative and hydrolytic enzymes activation affecting the chemical compositions of the leaves, and in the course of withering total catechins in the freshly plucked decrease in the withered leaves [10] forming an oxidation product of theaflavins and thearubigin [11].
3.2. Formation of theaflavin and thearubigin during sun withering
Theaflavins and thearubigins changes also investigated in the leaves during white tea withering. Figure 2 indicated the decrease of theaflavins content and increasing of thearubigins content.

![Figure 2](image)

**Figure 2.** Changes of theaflavin (a) and thearubigin (b) during white tea withering.

From the perspective of the formation of theaflavin and thearubigin, the concentration of both substances was below the concentration of theaflavin and thearubigin formed in black tea, as in black tea average value for theaflavin is 1-2% and thearubigin is 7-20% [12,13]. Because there was no rolling in white tea production, as it was kept and manufactured as naturally as possible [2], the content of theaflavin and thearubigin could not reach the black tea value.

Figure 2 also showed that even though there was no rolling process, the formation of theaflavin and thearubigin occurred. The withering process could increase the concentration of the enzymes and activating the catechol oxidase of phenol oxidase [14,15] that lead to oxidize phenol compounds such as EGCG to the formation of theaflavin and thearubigin.

The increasing value of thearubigin following the decreasing of theaflavin, as the high activity of enzymes in the withering process, including polyphenol oxidase and peroxidase, transforming theaflavin to thearubigin over time [16–18] or theaflavin as a precursor for further oxidation to form thearubigin [19].

White tea processing first step is withering and did not undergo inactivation enzymes such as green tea, in this sense, the enzymes are active and the polyphenols subjected to slight and slow oxidation confirmed by the amount of theaflavin detected (figure 2) [20,21]. As the reaction did not stop, thearubigin identified in this paper suspected as a derivative from polyphenols, theaflavin, and other benztropolones, and from theasinensins [22].

4. Conclusion
The white tea that produced from withering a bud in 96 hours observation indicated the oxidation reaction still running after plucked. Despite the minimum processing step of white tea manufacturing, total polyphenols of the leaves are decreasing, whereas a small amount of theaflavin detected and reducing but thearubigin detected in the experiment are increasing.

References
[1] Hilal Y and Engelhardt U 2007 Characterisation of white tea - Comparison to green and black tea J. fur Verbraucherschutz und Leb. 2 414–21
[2] Jiang H-Y 2008 White Tea Its Manufacture, Chemistry, and Health Effects Tea and Tea Products Chemistry and Health-Promoting Properties ed C-T Ho, J-K Lin and F Shahidi (Boca Raton: CRC Press)
[3] Prawira-Atmaja M I, Shabri S, Khomaini H S, Maulana H, Harianto S and Rohdiana D 2018 Changes in chlorophyll and polyphenols content in Camellia sinensis var. sinensis at different stage of leaf maturity IOP Conf. Ser.: Earth Environ. Sci. 131 1–7
[4] Anon 2005 ISO 14502–1:2005 (E) Determination of substances characteristic of green and black tea- Part 1: Content of total polyphenols in tea-colorimetric method using Folin-Ciocalteu reagent Int. Stand. ISO

[5] Roberts E A H and Smith R F 1963 The Phenolic Substance of Manufactured Tea. IX.-The Spectrophotometric Evaluation of Tea Liquors J. Sci. Food Agric. 14 689–700

[6] Obanda M, Owuor P O, Mang’oka R and Kavoi M M 2004 Changes in thearubigin fractions and theaflavin levels due to variations in processing conditions and their influence on black tea liquor brightness and total colour Food Chem. 85 163–73

[7] Harbowy M E, Balentine D A, Davies A P and Cai Y 1997 Tea Chemistry CRC. Crit. Rev. Plant Sci. 16 415–80

[8] Tumlins K I and Mashingaidze A 1997 Influence of withering, including leaf handling, on the manufacturing and quality of black teas - A review Food Chem. 60 573–80

[9] Luo S J 1995 IV. Processing of tea Food Rev. Int. 11 409–34

[10] Mikhail A B and Nina I S 1980 The biochemistry and technology of tea manufacture vol 12

[11] Omiaidze N T, Mchedlishvili N I, Rodriguez-Lopez J N, Abutidze M O, Sadunishvili T A and Pruidze N G 2014 Biochemical processes at the stage of withering during black tea production Appl. Biochem. Microbiol. 50 394–7

[12] Roberts E 1958 The chemistry of tea manufacture J. Sci. Food Agric. 9 381–90

[13] Thanaraj S N S and Seshadri R 1990 Influence of polyphenol oxidase activity and polyphenol content of tea shoot on quality of black tea J. Sci. Food Agric. 51 57–69

[14] Deb S and Pou K R J 2016 A Review of Withering in the Processing of Black Tea J. Food Qual. 41 365–72

[15] Baruah D, Bhuyan L P and Hazarika M 2012 Impact of moisture loss and temperature on biochemical changes during withering stage of black tea processing on four Tocklai released clones Two a Bud 59 134–42

[16] Jiang Y, Hua J, Wang B, Yuan H and Ma H 2018 Effects of Variety, Season, and Region on Theaflavins Content of Fermented Chinese Congou Black Tea J. Food Qual. 2018

[17] Samanta T, Cheeni V, Das S, Roy A B, Ghosh B C and Mitra A 2015 Assessing biochemical changes during standardization of fermentation time and temperature for manufacturing quality black tea J. Food Sci. Technol. 52 2387–93

[18] Ngure F M, Wanyoko J K, Mahungu S M and Shitandi A A 2009 Catechins depletion patterns in relation to theaflavin and thearubigin formation Food Chem. 115 8–14

[19] Yassin G H, Koek J H and Kuhnert N 2015 Model system-based mechanistic studies of black tea thearubigin formation Food Chem. 180 272–9

[20] Carloni P, Tiano L, Padella L, Bacchetti T, Customu C, Kay A and Damiani E 2013 Antioxidant activity of white, green and black tea obtained from the same tea cultivar Food Res. Int. 53 900–8

[21] Dai W, Xie D, Lu M, Li P, Lv H, Yang C, Peng Q, Zhu Y, Guo L, Zhang Y, Tan J and Lin Z 2017 Characterization of white tea metabolome: Comparison against green and black tea by a nontargeted metabolomics approach Food Res. Int. 96 40–5

[22] Haslam E 2003 Thoughts on thearubigins Phytochemistry 64 61–73