# INFLUENCE OF CLONE AND DURATION OF WITHERING ON QUALITY OF BLACK TEA (*Camellia Sinensis* (L).O Kuntz)

**M.Sc. THESIS**

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**JANUARU, 2013**

**JIMMA UNIVERSITY**

# INFLUENCE OF CLONE AND DURATION OF WITHERING ON QUALITY OF BLACK TEA (*Camellia sinensis* (L).O Kuntz).

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**MASTER OF SCIENCE IN HORTICULTURE**
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By

**SEMIRA NASIR**

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# APPROVAL SHEET
# SCHOOL OF GRADUATE STUDIES
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As Thesis Research advisor, I hereby certify that I have read and evaluated this thesis prepared, under my guidance, by **Semira Nasir,** entitled **Influence of Clone and Duration of Withering on quality of Black Tea (*Camellia sinensis* L.).** I recommend that it be submitted as fulfilling the thesis requirement.

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As member of the Board of Examiners of the M.Sc. Thesis Open Defense Examination, We certify that we have read, evaluated the Thesis prepared by Semira Nasir and examined the candidate. We recommended that the Thesis be accepted as fulfilling the Thesis requirement for the Degree of Master of Science in Horticulture (Coffee, Spices and Tea Science).

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## DEDICATION

This thesis is dedicated to my beloved family especially my father Nasir Abba Gidi and my mother Fatuma Abba Jobir who paved me the way towards education and for nursing me with affection and love and for their wholehearted partnership in the success of my life.

# STATEMENT OF AUTHOR

First, I declare that this thesis is my work and that all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for M.Sc. degree in Horticulture at Jimma University College of Agriculture and Veterinary Medicine and is deposited at the university library. I solemnly declare that this thesis is not submitted to any other institutions anywhere for award of any academic degree, or certificate.

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# BIOGRAPHICAL SKETCH

The author, Semira Nasir, was born on June 10, 1989, at Kersa Woreda, Jimma zone of Oromiya Region. She attended her elementary school from 1995-2004 at Kersa woreda in Serbo town and she started her high school study in 2005 at Jimma Preparatory School and completed it in 2006.

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Soon after graduation she got employed in Oromiya Regional Agricultural Research Center at Sinana Agricultural Research Center (SARC) where she was assigned to work as junior researcher in horticulture department from 2009 - 2010. Then she got the opportunity to join Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) to pursue her postgraduate study in September 2011 in Horticulture Sciences; specializing in coffee, tea, and spices.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| ANOVA | Analysis of variance |
| CTC | Cut Tear Curl |
| CV | Coefficient of variance |
| LSD | Least Significant Difference |
| PPO | Polyphenol oxidase |
| RCBD | Randomized Complete block design |
| SAS | Statistical Analysis System |
| SNNPRS | Southern Nations, Nationalities and Peoples Regional State |
| TF | Theaflavins |
| TH | Thickness |
| TR | Thearubigins |
| TRA | Tea research agency |
| VFC | Volatile Flavor Components |

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## ABSTRACT

*At Wush wush five commercial Camellia sinensis var.assamica of tea clones are under production. Withering is the first processing step in the factory and is a process in which freshly plucked leaf is conditioned physically, as well as, chemically for subsequent processing stages. Indeed, withering is one of the most important tae production process steps and can be said to constitute the foundation for achieving quality in tea manufacture. This most important processing (withering) step is being done for uniform duration (18h) irrespective of the type of clone to be processed. Furthermore, there was no research done so far to optimize withering duration for these five different types of clones grown under Wush wush tea plantation and hence only subjective judgment is used by factory cup tasters to determine the optimum withering duration. Therefore, this study was initiated with the objective of investigating the influence of clone and duration of withering on black tea quality. The research was conducted at Wush wush and Jimma southern part of Ethiopia in the year 2011\12. The experiment consisted of five clones (11\4, BB\35, 11\56, 6\8 and 12\38) and five duration of withering laid out in 5x5 factorial arrangement in Randomize Complete Block Design (RCBD) with three replications. Data were first checked for all assumptions and subjected to the analysis of Variance (ANOVA) by using SAS version 9.2. Software and significant means separated using least significant difference (LSD) test. The result of the study showed that interaction of clone and duration of withering had significantly influenced the theaflavin(TF), thearubgine(TR), brightness(BR), thickness(TH), briskness(BS), strength (ST) and color (CL)and the highest (3.2) TF was obtained with 17h duration of clone 6\8 and lowest with 20h for clone 11\56 (1.8), while the highest (19.2%) TR at was recorded with 19h withering of clone 12\38 and lowest with 16h withering of clone 6\8 (11.1%). The maximum value (26.8) for BR was observed with 16h withering of clone 11\4 and the minimum (15.3) was noted from clone 11\56 which was withered for 20h. On the other hand, TH was maximum (2.67) when withering duration was between 16 and18h and lowest (1.3) at 20hr. The highest (2.67) BRS developed from 16hrs and 18hrs withering of clone 11\4 and 11\56 while the lowest (1.67) was from 20h withering in clone 6\8. The strength of the tea was at its maximum (4) ST when the withering duration was adjusted to 18h in clone 6\8 and lowest(2) in clone 6\8 and 11\56 withered for 16 and 20h. Moreover, the highest value (2.67) for CL was registered when tea leaves were withered for 20h in clone 6\8 and 12\38 and lowest (1.3) in clone 11\56 withered for 16h and 20hrs. It was generally observed that almost all clones gave good quality when the withering duration was fixed between 16 and 18 hours and both chemical and sensory analysis result showed that the optimum withering time for clone 11\4 and 12\38 are16h and BB\35 and 6\8 are show good quality at 17 hour and 11\56 18h. Therefore by considering these optimum withering hours for the different clones we can save time many man power and most important thing improving the quality of black tea.*



*Key words: Tea Clone, Withering, Theaflavine, Thearubgin*

# 1. INTRODUCTION

Tea is the second largest drink consumed in the world after water. Tea refers to the products of the leaves, leaf buds, and internodes of the *Camellia sinensis* or *Camellia assamica* plant prepared by different methods, such as daily preparation, infusions, decoctions etc. The plant itself is also considered as “tea” (Shapiro and Bruck, 2006).

In Ethiopia, tea is mostly grown in the highland dense forest regions where the land is fertile and thus the use of fertilizer is very minimal. Moreover, the availability of abundant and cheap labor in the country has made the use of manual weeding, instead of chemical weeding, possible. Because of this mostly organic cultivation, Ethiopian tea is increasingly sought for its aroma and natural flavors. This is confirmed by the "International Gold Star" award for quality recently given by B.D.I. in Madrid, Spain to one of the major Ethiopian tea exporters, Tea Production and Marketing Enterprise (Anon, 2011). Black tea is manufactured from the tender leaves and buds of the evergreen shrub *Camellia sinensis* (L.) O. Kuntze. Tea manufacturing consists of four stages, namely withering, rolling, fermentation and drying. Of these, withering is the first and most important step in tea manufacture and is the most expensive processing step in terms of time, money and energy. Withering is the most expensive process in terms of time, money and energy. Withering is done to make the leaf ready, physically and biochemical, for effective rolling, fermentation and drying processes (Muthumani and Kumar, 2006).

Withering results in an increased levels of amino acids, caffeine content, sugars and polyphenol oxidase activity (Tomins *et al*., 2010), changes in chlorophyll content (Tomlins and Mashingaidze, 1997), formation of precursors of volatile flavor compounds (Mahanta and Baruah, 2006) and an increase in cell membrane permeability (Sanderson, 1968). All these changes, except for cell membrane permeability, are independent of moisture loss during withering. An increase in cell membrane permeability, on the other hand is important in facilitating the mixing of substrates and enzymes involved in tea fermentation. Even though cell membrane permeability is not a chemical phenomenon, it is a prerequisite for the occurrence of the chemical reactions, which contribute to the final tea quality. If cell membrane permeability were to be achieved in a shorter period, tea factories would be able to handle greater amounts of leaf per day (Ramaswamy, 1989). The primary goal of any tea industry is to supply the market with safe and quality product. Quality of made tea is directly influenced by the taste and aroma of tea liquor (Sanderson, 1968). Good manufacturing practices are necessary to process good quality tea. Withering is very important in black tea processing because it consumes the highest amount of electrical energy and fairly large amount of thermal energy. In addition, uniformly withered tea leaves are essential to produce good quality end product (Botheju, *et al*, 2011).

In Ethiopian (Wush Wush) five commercial *Camellia sinensis var. assamica* of tea clones are under production. The green leaves are harvested at 10 to14 day's interval for export and 14 to18 day's interval for local market. The leaves are harvested all year around. However, during export processing, the factory treats all five clones with similar withering time 18hours. The company overcomes the quality problems by its experienced cup tasters. This subjective assessment of tea tasting and visual inspection is not enough to produce competitive product in the international market. So, biochemical parameters (colorimetric approach) have been used to detect the amount of theaflavin and thearubigin formed with different extent of withering, such experiment clearly help to select the highly withered and less withered bush and help to optimize withering time in the tea industry to produce quality tea. So Biochemical parameter is important to determine the optimum withering time for each clone according to Wush-Wush agro ecological conditions. This Biochemical study was done to find out whether withering duration or Clonal differences could affect the individual theaflavins, thearubigins, color, brightness, thickness and strength of the black tea.

**Therefore, the General objective of this experiment was**

- To standardize the withering time for the different clones at Wush Wush in view of improving the quality of black tea

**Specific Objective**

- To determine the influence of withering duration on black tea quality

- To determine the effect of selected tea clones on black tea quality

# 2. LITERATURE REVIEW

## 2.1 Tea Withering

Withering is the first processing step in the factory and is a process in which freshly plucked leaf is conditioned physically, as well as, chemically for subsequent processing stages. Indeed, withering is one of the most important tea production process steps and can be said to constitute the foundation for achieving quality in tea manufacture (Bohea, 1991). Based on achieving the desired level of withering, one can make better quality teas and, on neglecting, can invite serious problems in subsequent steps of manufacture (Blog, 2006).

Withering is an important step in the manufacture of black tea from the young soft shoots of *Camellia sinensis* (L) O. Kuntze and the most important step in quality improvement of black tea (Takeo,1984; Mohammed ,1984) and also quality of tea is determined by the withering time (Willson and Clifford, 1992). Plucked tea that arrives at the factory contains moisture between 70 and 80% depending on atmospheric conditions, usually 77 to 80% under very wet condition (Blog, *2006)*. Withering is a process of reducing the moisture level of tea leaf from 77 to 80% by weight down to 65 to 68%. Ideally, the leaf is physically withered to 68 to70% moisture content over a period of 18 hours. By this time it should be ready for processing (Bohea, 1991). Withering time is determined by the factory equipment, weather conditions, loading depth of a leaf, clone type and temperature of withering (Owuor *et al*., 1990; Owuor and Orchard, 1990).

Withering of tea shoots is slow and time consuming yet an essential step in black tea manufacture. It has two components namely desiccation of shoots (physical wither) and slow but rate limiting biochemical changes (chemical wither) both of which are essential for quality of tea. Both are independent, but due to long storage time (10-16 h) needed to achieve chemical wither, black tea processing has remained a batch process (Ravindranath, 2008).

The withering stage has two purposes. It allows certain chemical changes to occur, and a reduction in the moisture content from 75 to 80% by weight, down to 55 to 70%, depending on the subsequent process to be employed. Throughout the process, the temperature of the leaf should be maintained at around ambient levels; otherwise faster moisture removal would be offset by adverse chemical changes. Withering time is usually in the range of 16 to 20 hours. During withering, a certain amount of the solid matter, as well as moisture, is lost from the leaf. This is due to the loss of carbon dioxide by respiration, and can account for 3 to 4% of the dry weight content of the incoming leaf (Mahanta and Baruah, 2006). Natural withering of plucked tea shoots for 16 to 18 hours was considered adequate for achieving both physical and chemical wither, though natural wither for 12 hours qualified the requisite chemical wither for cut tear curl (CTC) teas. Natural wither beyond 20 hours appeared detrimental to tea quality (Blog, *2006)*.

During withering polyphenol oxidase activity was depressed. Very hard wither affected PPO activity and the formation of TF, but the formation of TR continued; producing teas with body and without adequate briskness and brightness unwithered CTC teas produced higher TF and lower TR resulting in bright, brisk and thin liquor (Muthumani and Kumar, 2006). Natural withering produces better quality teas than leaf withered at 37.8°C in a chamber for 13 hours. The TF, TR and brightness in tea were observed higher in 18 hrs wither than those of teas of less wither (TRA, 2008). However, withering practices vary with climate, producing region, the type of manufacturing process and demands by the markets. Mechanized withering may improve quality by reduced handling of the leaf (Tomlins and Mashingaidze, 1997).

#### 2.1.1. Physical withering

Physical withering is moisture loss of fresh tea leaf and related physical changes (Mohammed *et al*., 2006). Physical withering reduces the moisture content in the leaf and correct withering is essential for quality, although it has always been a difficult task to determine the end-point of withering. The same reduction in moisture percentage and increase of flaccidity of leaf to the desired level can be achieved in a shorter period, a longer period is necessary for chemical wither. Therefore, physical wither is regulated at a slower rate, so as to reach the desired physical withering in the same interval as required for the chemical wither. The objectives are achieved by passing air through the leaves (TRA, 2008).

Physical withering refers to loss in moisture and change in cell membrane permeability which is result in the leaf becoming flaccid. It is achieved by passing air through the leaf at ambient by applying heat (Tomins *et al*., 2010). Immediately after plucking the fresh leaf starts to lose water as vapor. In the factory the maximum initial drying rate has been reported to be 0.075kg of water per kg of green leaf at ambient temperature (Wickremasinghe, 1975). As the degree of wither progresses, the permeability of the cell membranes in tea shoot increase and the leaf become more flaccid (Bohea, 1991).

During withering some solid matter (3 to 4 per cent of the dry weight) in the leaf lost through respiration in addition to the moisture initial present in the leaf. The rate of loss of moisture during physical withering is related to leaf surface moisture, how often the leaf is turned humidity of the air altitude air flow and whether heat is applied (Tomlins *et al*., 2010). The rate of loss of moisture and temperature of the leaf during withering is related to surface moisture, humidity of air, altitude, dry bulb and wet bulb temperature air flow plucking density and whether heat is applied during withering (Hampton, 1998). In compacted leaf a temperature rise of $3^0$C per hour can occur, but the effect on quality is not known although regular turning of leaf was recommended (TRA, 2008).

### 2.1.2. Chemical withering

The term is perhaps misleading as it does not involve the addition of chemical to the leaf. It refers to natural biochemical changes that occur inside the leaf that may or may not influence the quality of the made tea. These biochemical reactions are considered necessary for formation of the tea aroma which is more important for flavor teas. A chemical wither is achieved by blowing air either intermittently or continuously at low flow rate through the leaf to keep it cool without losing moisture for between 4 and 18 hours (Tomins *et al*., 2010). Desirable biochemical changes from plucking to initiation of processing (manufacturing), normally last 14 to 20 hrs (Anon, 2008). Blowing air through the tea is vital since temperature rise of $3^{O}C$ per hour are considered common if air is not passed through. A very important role of this type of withering is in storing the leaf to assist in the factory management of leaf. Hence, chemical withering could also be referred to as storage wither (Tomlins *et al.*, 2010). It is noted by many experienced tea makers that change take place to the liquor properties of leaf during the withering period. For instance, color increase and greenness reduced. A well withered tea tends to produce round liquor (Bohea, 1991).

Chemical wither starts immediately after plucking. It is independent of the rate of loss of moisture and is a function of time and temperature of withering although the desired moisture level may be reached in a few hours, the catabolic changes, which had been initiated at the time of plucking, will take time (Obanda *et al.*, 2001). The chemical composition of the leaf will thus be unsuitable for manufacture after the leaf has been desiccated for a few hours. It is, therefore, necessary to continue to supply sufficient air and wait for breakdown of large organic molecules to simpler structures. The following chemical changes occur during withering:

- Release of carbon dioxide and water due to break down of larger molecules.
- Changes in enzyme activity.
- Partial break down of proteins to amino acids which act as precursors for aroma.
- Increase in caffeine, sugar and organic acids content
- Production of Volatile Flavor Components (VFC): Some of these compounds contribute to the grassy odor and others are responsible for the flowery aroma.
- Reduction in chlorophyll, lipid and fatty acids, catechines and carbohydrate content.

The above chemical changes are all intrinsic of the biochemical structure of the leaf, but the range and the extent of the reactions depend on the clone, cultural practices and physical parameters like temperature, humidity, time etc. This process normally takes about 12 to 16 hours and cannot be hastened (TRA, 2008).

#### 2.1.2.1 Changes in the biochemical constituents of the green leaf during chemical withering

A large number of chemical changes occur during withering. At the time of plucking many biochemical and physiological processes occur in the green leaf. During manufacturing some of these processes will be altered and new processes commenced (Obanda *et al.*, 2001). The known biochemical changes involved are the breakdown of proteins in to amino acids, a decrease in lipid and fatty acid carotenoids and chlorophyll, an increase in caffeine content and change in sugar, organic acid, enzyme activity and flavor volatile component (Tomlins *et al*., 2010).It is independent of the rate of loss of moisture and is a function of time and temperature. Although the desired moisture level may be reached in a few hours, the catabolic changes, which had been initiated at the time of plucking, will take time. The chemical composition of the leaf will thus be unsuitable for manufacture after the leaf has been desiccated for a few hours. It is, therefore, necessary to continue to supply sufficient air and wait for breakdown of large organic molecules to simpler structures (TRA, 2008).

**Catechins and enzyme activity**

In freshly plucked leaf the catechines account for about 30 percent (dry weight) of the green leaf and their content decrease during withering (Owuor *et al*., 1987). Catechins form theaflavin (TF) and thearubigin (TR) mostly in the presence of polyphenol oxides (PPO) and peroxides (PO) enzymes although recent work indicates that their formation can also occur without enzyme. The TFs and TRs are responsible for the brightness, briskness, color and strength of black tea (Obanda *et al*., 2001).

The activity of the PPO enzyme has been reported to decrease during withering as a result of moisture loss but can be restored when the withered leaf was rehydrated although the degree of moisture loss has not been clearly defined. The activity of enzyme is also reduced at temperature above $35^{O}C$ (Tomlins *et al.*, 2010).

**Protein and amino acids**

Protein accounts for 15 % (dry weight) of the green leaf. During withering leave are reduced by approximately 1.2% and the change accompany an increase in amino acids which are thought to be involved in flavor formation. Some amino acids give rise to poor flavors while other can improve it (Tomlins and Mashingaidze, 1997). The influence of withering on the amino acid composition varies with the duration ambient temperature and moisture .Withers of short duration have little effect on amino acids composition whereas, long wither allow for built up of amino acids. When moisture loss was prevented amino acids formation was reduced. The formation of amino acid was reduced at temperature below $18^{0}C$ and accreted as the temperature increases to $40^{0}C$ formation stopped after 6h because of the death of tissue (Tomlins *et al.*, 2010). During chemical withering proteins content decreases and free amino acid content increases. High temperature cause proteins break down to increase (Obanda *et al*., 2001).

**Carbohydrates and simple sugars**

The free sugars found in tea shoot are glucose, fructose, sucrose, raffinose and stachyose. Maltose in Assam variety and rhamnose in china variety appeared special. Pectic substances contain galactose, arabinose, galacturonic acid, rhamnose and ribose. Free sugars are responsible for the synthesis of catechins in tea shoot, formation of heterocyclic flavor compounds during processing of black tea and contributing towards water-soluble solids in tea liquor. Cellulose, hemi-cellulose, pectin’s and lignins are responsible for the formation of crude fibre content in black tea (Anon, 2001).

Carbohydrates account for about 34% (dry weight) of the green leaf .simple sugars formed from the breakdown of Carbohydrates increase during withering and may be incorporated in to amino acids to form volatile flavor compounds (Tomlins *et al.,* 2010). During withering carbohydrate and soluble gums reduce, the surge content increase typically surge content of 0.84% in green leaf and 1.23% in withered leaf However, the surge quantity varies in different clones (Obanda *et al.*, 2001).

**Caffeine**

Caffeine is an important quality parameter for green and black tea. Caffeine contributes to the astringency along with the catechines and their derivatives in green tea and is responsible for briskness and creaming in the black tea infusion. Cream is a thick turbid layer that develops on the surface when a good cup is cooled. Cream is a complex of theaflavin, thearubigin and caffeine and is formed by weak hydrogen bonds (Owuor *et al*., 1987). The complex formed modifies positively the taste characteristics of both caffeine and theaflavin. The tender younger leaves of tea bushes contain higher caffeine contents than older leaves. Caffeine content decreases from about 4.7% in the bud to about 2.9 % in the third leaf. Leaf stalks accumulate low levels of caffeine. Fine plucking standards ensure high caffeine content in brewed tea. There exists a positive and significant correlation established between caffeine levels in the green two and a bud shoot, and tasters' preferences of black teas (Gulati, 2011).

Black tea with low level of caffeine lacks the ability to cream. The creaming properties of made tea are adversely affected by insufficient wither. Although, caffeine is thought to increase during both chemical and physical wither most of the increase occurs during the letter (Tomlins *et al.*, 2010).

The increase this important is probably due to the increase in amino acid Caffeine is a purine derivative, which is 1, 3, 7-tri- methyl xanthine. Caffeine content in black tea is around 3 – 4% of dry weight. It has stimulating property and removes mental fatigue. The contribution of caffeine to the infusion is the briskness and creamy property resulting from the complex formed by caffeine with polyphones. Briskness is a taste and sensation while creaming is the turbidity that develops from a good cup of tea when cooled (Anon, 2001).

**Lipid and fatty acids**

Lipid content increased with shoot maturity. It varied from 30 mg/g in bud to almost 72 mg/g in the fourth leaf. The $C_6$ aldehydes (hexenal responsible for fresh aroma) are obtained from $C_{18}$ unsaturated fatty acids by lipid oxidase in leaf chloroplast. The lipids during withering are converted to fatty acids. These fatty acids are acted upon by lipoxygenase enzyme to give aromatic $C_6$, $C_7$ and $C_8$aldehydes. These aldehydes impart fresh green aroma to tea. These compounds are indicators of freshness of tea (Gulati, 2011).

Lipids are important in the development of aroma compound in the black tea. The leave of saturated fatty acids (FA) decreases during withering but their influence on black tea flavor is not known .more is known about the role of unsaturated FA during withering and their influence on black tea quality .during withering lipid degradation occurs and the unsaturated FA decreases to undergo region and enantio selective oxidative cleavage to form aroma compound. Aroma compound formed from unsaturated FA breakdown are thought to have negative effect on flavor the strong grassy odor from the withering troughs is a result of aroma compound produced from FA. Volatile compounds formed as are results of the breakdown of lipid and fatty acid 3%odry weight of the green leaf during withering are thought to have a detrimental effect on flavor in particular, the strong grassy odor on the withering troughs is a result of the volatile compound produced from fatty acids (Tomlins *et al.*, 2010).

The neutral, glycol and phospholipids contents and their fatty acid composition varied in Assam, China and Cambodial varieties and also during different stages of black tea manufacture. Total lipid contents (%) and total fatty acids (1g/g) at different stages i.e. green leaf, withered leaf, rolled leaf, fermented leaf and black teas are about 6.5, 5.7, 4.5, 4.3 and 2.8 and 9.8, 8.4, 6.6, 4.8 and 3.7 respectively and major fatty acids available in tea are linolenic, linoleic, oleic and palmitic (Anon, 2001)

**Carotenoids**

The degradation of carotenoids during withering and fermentation leads to important flavor in high quality tea compound. Carotenoids are significantly reduced during withering where as B-carotene and lutein only decline during fermentation. Carotenoids are yellow pigment in the green leaf which assists with photosynthesis. During withering and fermentation they degraded to form volatile flavor compound important in the aroma of tea. Their decline in concentration is more significant during the physical rather than the chemical wither (Tomlins *et al.*, 2010).

Carotenoids and chlorophylls have been identified as important for quality in Kenyan teas and have been suggested as the basis of predicting quality .Carotenoids are significantly reduced during withering which is during physical withering (Tomlins and Mashingaidze,1997).

**Chlorophyll**

Chlorophyll is green pigment essential in plant for photosynthesis .Teas with high chlorophyll may result in infirerior liquor that have a grassy odor .Their concentration decreases during withering but their presence is however important as they break during drying to contribute to the black color (Tomlins et al., 2010).

It is though that the conversion of chlorophylls to pheophytins during fermentation and firing contributes to black tea appearance .During withering level of chlorophylls decline by 15% forming chlorophyllides.It has been noted that green leaf high in chlorophyll produce tea with a grassy odor, but the cause of the grassy odor has not been identified (Tomlins and Mashingaidze,1997).

### 2.1.2.2 Influence of withering on the biochemistry of the fermentation process

**Theaflavin (TF)**

Theaflavin, (TF) the golden yellow pigment, constitute around 0.5%- 2% of dry wt., depending on the type of manufacture of black tea. The attractive color of the tea infusion is due to theaflavins and it emerges as an important quality index of black tea. TF are responsible for the development of the bright color and brisk taste of the liquor. These compounds are mostly formed during the maceration stages in black tea manufacture. These groups of compounds contribute about 30% of the total color and are the result of rolling and fermentation. Theaflavins are unique having a seven-member benzotropolone ring (Bohea, 1991). Theaflavins also provides briskness, freshness and aliveness to the infusion, which is highly valued in taster's parlance. The different types of Theaflavins are Theaflavin, Isotheaflavin, Neotheaflavin, Theaflavin-3-gallate, Theaflavin-3'-gallate, Theaflavin-3, 3'-digallate, Epitheaflavic acid, Epitheaflavic acid-3-gallate, theaflavic acid, Epitheaflavic acid-3'-gallate. Recently, some new theaflavins has been isolated namely, neotheaflavin-3-monogallate, isotheaflavin-3'-monogallate and theacitrin A (TRA, 2008). TFs in tea are responsible for the briskness and brightness color. Unwithered leaf is associate with tea that are bright, brisk but thin and lower in correspondingly the teas are higher in TFs .Wither produce teas with less brightness but improved body the TFs are lower and the TRs higher(Tomlins *et al.*, 2010).

**Thearubigins**

Thearubigins (TR), the orange-brown compounds constituting about 6 to 18% of dry weight are another important group of pigments formed during the processing of black tea. They are heterogeneous complexes and are responsible for taste, total color and body of the liquor. They contribute around 35% of the total color and also play a significant role in brown appearance of made tea. Thearubigins are difficult to separate from the other tea components (TRA, 2008). Roberts (1962) defined thearubigins as acidic, brown phenolic pigments. Later, evidence from chemical degradation led to the conclusion that thearubigins are polymeric proanthocyanidins. The thearubigin anions are more deeply coloured than the undissociate form and the color due to thearubigin is determined by the relative proportion of anions and undissociate forms. Color of thearubigins is therefore dependent on pH of water and acidic pH reduces the intensity of color. Tea brewed in hard water is darker in color. Thearubigins are separated as coloured complexes. Five coloured complexes have been separated in Sri Lanka in 1967 of which the two deeply coloured fractions were found to contain corilagin, theanine and protein. Three of the less coloured complexes also contain corilagin, theanine, theogallin, chlorogenic acid etc. Three thearubigin fractions have been separated at Tocklai (Obanda *et al.*, 2001).

The low molecular weight fraction is reddish brown in color and is astringent. It consists of amino acids, caffeine, fatty acids, theogallin and traces of chlorogenic acid and chlorophyll. This fraction has important contribution to quality. The other two fractions were light brown and ashy coloured and were complexes of amino acids, chlorogenic acids, p-coumarylquinic acids. Biogenetic pathways to thearubigins cannot be established till the structures have been fully elucidated, but it has been demonstrated that thearubigins form directly from catechin as well as by oxidative degradation of theaflavins (TRA, 2008).

**Volatile flavor compounds**

Flavor of tea can be divided into two components. One is an inherent flavor due to the presence of some compounds in the leaves which do not undergo changes and the other is acquired flavor due to the transformation of different compounds during processing (Sanderson, 1964). More than 700 compounds have been identified so far, as contributing to flavor either positively or negatively. The main sources of these compounds are carotenoids/terpenoids, lipids and amino acids. This group compounds impart undesirable grassy odor. Carotenoids/terpenoids are the main sources of floral flavor of black tea which are geraniol, linalool and its oxides (Anon, 2001).

Withering is thought to improve the aroma of tea .The aroma of black tea is complex being composed of over 600 volatile compound, none of which on their own have the characteristic aroma of tea. During chemical withering of plain teas in Kenya, the concentration of volatile in the made tea decrease but the flavor improve because those compound that impart an inferior grassy odor to black tea declaim faster than those that impart a sweet flowery aroma (Tomlins *et al.*, 2010).

### 2.2 Factor That Affect Withering of Tea Leaves

#### 2.2.1 Duration of withering

The period of wither is ascertained by taking both physical and chemical wither; physical wither can be achieved in 3 to 4 hours but chemical wither requires 12 to 16 hours. In case the leaf is under withered, the following problems are envisaged:

- Rolling of unwithered leaf leads to breaking up into small flakes, which would not respond to the subsequent processing steps and produce unacceptable teas.
- If the leaf were under withered valuable water-soluble solids would be lost during the leaf conditioning process.
- Under withered leaf when rolled turns into a wet water soaked mass; the sogginess restricts supply of oxygen and hinders uniformity in the subsequent oxidation reaction (fermentation).
- Maceration of under withered leaf also leads to formation of lumps during fermentation.
- At temperature more than 25°C with under withered leaf, chances of bacterial contamination increase.
- Proper physical wither reduces load on the dryers (Ramaswamy and Ravichandran, 1995).

And all so unwithered fresh leaf CTC produces a higher proportion of TF and lower TR, resulting in bright, brisk and thin liquors. Hardness of wither was accompanied by a further depression in PPO activity, and formation of TF also declined with a concomitant loss in brightness and briskness. The TR content increased up to a certain degree of withers and thereby improved the ‘body’ of the liquor. Very hard wither, accompanied by a large reduction in moisture content restricts PPO activity (Mohammed and Roy, 1982). It appears that in the fresh leaf, high moisture levels together with dissolved oxygen accelerate the enzymic oxidation and produce large amounts of TF. Senescence of the leaf seems to facilitate the production of TR (Mohammed *et al.*, 2006).

Long wither is believed to initiate the floral taste and aroma biological processes in the leaf. During the long withering, the leaves are preferably tumbled at least once, preferably in a simple roll-cage. This has the effect of causing controlled damage to the perimeter of the leaf and is essential to obtaining good floral taste and aroma. If the damage is more extensive, as would be brought about by freezing the leaves, then the floral taste and aroma are lost (Anon, 2011).

### 2.2.2. Temperature of withering

High leaf temperatures during the withering process of black tea manufacture decrease the theaflavin, brightness, flavor index and sensory evaluation scores of black tea. Black teas manufactured with withering temperature above 30°C have high thearubigins and total color levels but lack briskness (Sud and Baru, 2000). Highest percentage of TF was observed when leaves were withered at $25^0$C and as the temperature increased, there had been a steady decline in the quality (Tomlins *et al*., 2010).

### 2.2.3. Clonal Difference

There was a wide variation between enzyme activities of different clones, as well as variation due to seasonal changes and shoot maturity. Field practices such as plucking rounds and pruning had a great impact on enzyme activities (Hulya and Yilmaz, 2009). The enzyme activities positively correlated with tasters' scores. The extent of change in enzyme activities at different stages of manufacture differed widely. The loss of activity during withering could be restored by rehydration. Residual activity was observed in made tea (Ramaswamy, 1989).

High altitude grown teas are more aromatic than low altitude grown black teas, implying that the low altitude grown teas are plain in character. Thus producers at high altitudes should aim at producing aromatic black teas, although yields will be lower than same genotypes at lower altitudes. Producers growing teas at low altitudes should focus on high output and ensure optimal conditions for production of plain black teas (Owuor and Orchard, 1989). There are seasonal black tea quality and yield variations. Cold seasons lead to slow growth resulting in low yields, but high black tea quality. Provided soil moisture and temperatures are adequate, warm temperatures lead to fast growth, leading in turn to high yields, but low black tea quality (Tomins *et al.*, 2010).

### 2.2.4 Relative humidity during withering

The quality of rainy season teas suffered owing to high chlorophyll content and low degree of withering. Hot air circulation through the withering troughs to assist evaporation of leaf moisture increased the brightness and total color of rainy season teas (Temple *et al.*, 2001). High atmospheric demand during the dry season assisted loss of green leaf moisture, but withering and brightness exhibited significant negative correlations with high relative humidity and rainfall (Sud and Baru, 2000).

Withering did not adversely affect the quality of the end product. Thus, it was concluded that it is possible to store fresh tea leaves at controlled temperature and RH conditions to preserve them until the time of processing and at the same period it is possible to achieve withering. This can give the opportunity to small capacity tea processing factories to store their raw tea for about one month and continue processing without loss of product quality because of poor preprocess handling conditions (Hulya and Yilmaz, 2009).

### 2.2.5. Depth of load of tea on the trough

The spread of leaves actually act as a valve and create the necessary system resistance for the airflow helping to even out the static pressure in the plenum chamber (Botheju, 2009). Leaf is generally spread uniformly on the trough at the rate of 23-25 kg $m^{-2}$ for CTC or 13-16 kg $m^{-2}$ for orthodox tea manufacture in the plains and 8-10 kg $m^{-2}$ for orthodox tea manufactured in the hills (Sud and Baru, 2000).

Thin layer withering  can ensure that each particle is exposed to as near identical conditions as possible, and thick layer can long time for withering and affect the chemical composition of the tea in additions to these it consume time continuous monitoring of bed weight gives moisture monitoring in real time (Temple *et al.*, 2001).

### 2.2.6 Effect of physical damage of leaf on withering and made tea quality

Apart from damage of the quality attributes in green leaf due to heat generation, leaf can be damaged through rough handling, causing bruise and tear in the leaves (Willson and Clifford, 1992). Cell damage is the ultimate objective in the manufacture of black tea. However, if the damage is initiated before withering and is indiscriminate, the leaf may undergo uneven oxidation process adversely affecting the desirable chemical constituents in the end product. Also, damaged leaf withers at a faster rate, as the structure of cuticular waxy layer of the leaf is disturbed. This ultimately influences the water holding capacity of the leaf and will lead to uneven wither (Mohammed *et al.*, 2006). The green leaf can turn brown/red as a result of physical damage arising from bruising of leaf and heat stress.

The red color is observed to be prominent when the leaf temperature exceeds 35 $^0$C .The increase of red leaf percentage has got detrimental effect on quality. The cause of red leaf formation is mainly due to oxidation of polyphones. Reddening of leaf may occur more rapidly when the leaf temperature exceeds 40$^0$C (Hampton, 1998). With the increase in percentage of red leaf formation there is significant increase in undesirable TRs. It will reduce brightness and briskness of liquor along with reduction in essential volatile flavor constituents (VFC) of made tea. Careless handling and transportation resulting in bruising and tearing, therefore, can lead to reduction in quality (TRA, 2008).

## 2.3. Processing Reasons for the Physical Withering

We need to remove moisture from the leaf so that it is easier to handle during processing and can affect the following stage

### 2.3.1. Maceration

Fresh green leaf arriving at the factory typically has moisture content above 75% on a wet basis and can be as high as 83%. Maceration machines operate most effectively within a fairly narrow green-leaf moisture range (68-72%) on wet basis and hence some moisture must usually be lost during withering (Hampton, 1998). For LTP (lawrie tea processor) maceration, a target moisture content on a wet basis of 71 to 72%is desirable.CTC (Cut-Tear-Curl) maceration can operate

Over a wide range of moisture in the green leaf may clog both the rotor vane and CTC rollers. The orthodox method requires leaf that had harder wither to 60 to 66% moisture content and can be as low as 50% for the manufacture of darjeling. This is to ensure that the leaves are sufficient flaccid for the rollers to produce the desire twist in the leaf (Tomlins *et al*., 2010).

Manufactured CTC teas, unwithered fresh leaf CTC produces a higher proportion of TF and lower TR, resulting in bright, brisk and thin liquors. Whereas thearubgine content increased up to a certain degree fresh leaf are produce lower proportion of theaflavin and higher proportion of thearubgine and resulting in lower brightness and briskness and there by improved the 'body' of the liquor (Mohammed *et al.,* 2006).

### 2.3.2. Fermentation

Physical wither is necessary for producing an even fermentation. Fig.1 below Show the ideal temperature is $26.5^0$ to $29.5^0$c Excessive moisture more than $32^0$C in the dhool reduces aeration and temperature control resulting in uneven ferment. Excessive withering may concentrate the catechins to leave that inhabit polyphenol oxides (PPO) activity and reduce the expansion of cell content onto the surface of the macerated leaf hence a significant proportion of the fermentation may occur within the leaf particle under limiting oxygen condition (TRA, 2008). Moisture loss during withering does not appear to alter the optimum fermentation. Too hard physical wither may inhibit the enzyme (Tomlins *et al.*, 2010).

During fermentation many complex chemical changes take place, the main effect of which was to give a palatable character to the liquor. Over and under withering practices can result in poor quality during fermentation (Borah 2005). During the withering of tea leaf (*Camellia sinensis*) in black tea manufacture affected the fermentation condensation of tea flavanols in forming theaflavin (TF) and thearubigins (TR), which are associated with brightness, briskness and 'body' of tea liquors. In contrast to conventionally manufactured CTC teas, it appears that in the fresh leaf, high moisture levels together with dissolved oxygen accelerate the enzymic oxidation and produce large amounts of TF. Senescence of the leaf seems to facilitate the production of TR (Mohammed *et al.*, 2006).

### 2.3.3. Drying

Too high a moisture content (unwithered) tea can affect the drying as the wet dhool will tend to form ball and stick to the bed plate of fluidized bed driers causing uneven draying and off-flavors. Some people reported their result as moisture contents and other as percentage wither. These are not the same moisture content refers to the quantity of moisture in the leaf on a wet basis where as percentage wither refers to weight loss in relation to the fresh weight (Tomlins *et al.,* 2010).For the tea that are over withered are higher rate of moisture removal and hared crust are formed at the surface. When the rate of moisture removal is above optimum and core moisture is not removed properly tea gets case hardened .If the tea are un withered tea gets under fired then the enzyme not deactivated and post fermentation will continue (Sud and Baru, 2000).

Wush Wush Tea Manufacturing Process Flow Chart Green Leaf (Bud +Two)

Withering (18hr)
68-70%    65-68%
↓
Cut, Tear and Curl Through Rotor vane
↓
Fermentation (108min)
$26.5^0$ to $29.5^0$c
↓
Drying
Air Inlet 105-140 $^0$c    Outlet 42-$55^0$c
↓
Quality Control Visual Inspection and Cup Test
↓
Storing and Grading
↓
Storage
↓
Blending /Packing

Figure 1 .Wush Wush black tea processing flow chart
Source :- (Borah, 2005)

## 2.4. Quality Analysis Method of Tea

Quality in tea is a relative term, which depends on customers' preference. There are three methods of quality monitoring so far in tea industries. The first one is subjective assessment method, cup taster judged color, briskness, brightness, strength and thickness of liquor. These are important ‘parameters’ that are might be considered in different tea processing stages in order to determine the quality of tea. The second assessment method is biochemical parameters in which the total amount theaflavin, thearubigin, brightness and infusion color are measured and the third method is E-nose (computer assessment method) (Borah, 2005). In some tea factories chemical tests are conducted to find out the correct withering of orthodox and CTC tea, spectrophotometer method, is also used for the made tea for quality judgment (Borah and Bhuyan, 2003). Color and flavor are the two key parameters for the subjective determination of the overall product quality of many foods processing industries (Borah, 2005). However, the subjective assessment is most frequently adopted in tea industries (Borah, 2001). Human sensory panel’s judgment by using organoleptic methods (visual inspection, sniffing)  are good in justification of Color of objects in particular regions But due to different factors such as human variability, insensitivity due to prolonged exposure, mental state, age difference etc. Human organoleptic methods of tasting show great inconsistency so, this must supported by biochemical analysis. Biochemical analysis helps the human sensory panel to evaluate the quality of made tea (Borah, 2005).

# 3. MATERIALS AND METHODS

## 3.1. Study Area

The study was undertaken at Wushwush Tea Plantation, located in the Southern Nations, Nationalities and Peoples Regional State (SNNRP) of Ethiopia, in Kafa Zone Gimbo Woreda Wush Wush Kebele. Wushwush Tea Plantation farm is located at 7º16´N and 36º11E. The altitude of the area is 1900 m above sea level (Fig. 2). The Mean annual temperature at Wushwush is18.5 $^{0}$C ranging from a mean annual minimum of 11.25 $^{0}$C to a mean annual maximum of 25.5$^{0}$C .The mean annual rainfall is 1,820 mm/year. The area under tea production is 1249 ha (Mekuria, 2005).

Moreover the chemical analyses for theaflavine, thearubgine, and brightness and infusion color were conducted at Jimma University post harvest laboratory. The site is located at 7$^{0}$, 33' N latitude and 36$^{0}$, 57' E longitude at an altitude of 1710 m.a.s.l. The mean maximum and minimum temperature are 26.8$^{0}$C and 11.4$^{0}$C, respectively and the mean maximum and minimum relative humidity are 91.4% and 39.92% respectively (BPEDORS, 2000). The area receives an annual rainfall of 1500-1800mm (Melaku, 2008).

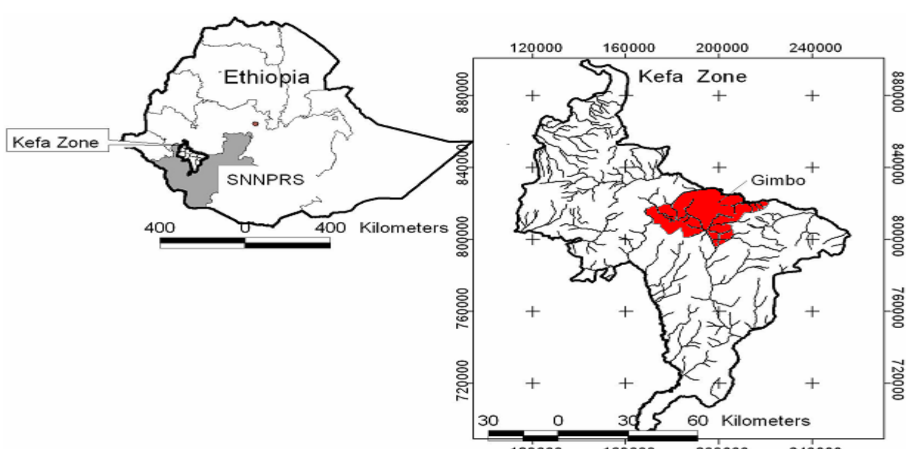


Figure 2. Map of Southern Nations, Nationalities and Peoples Regional State
Source: (Mekuria, 2005)

## 3.2. Experimental Materials

The plantation grows five clones of tea: BB-35, 6/8, 11/4, 11/56, and 12/38. These five different tea clones were receiving 150kg N $ha^{-1}$ $year^{-1}$ as NPK-25:5:5 compound fertilizers at the end of rainy season, which is three months prior to experiments. The tea gardens in field which are having relatively similar growing condition and range of age were identified and used for the study.

### 3.3. Experimental Design

The experiment was laid out in Randomized Complete Block Design (RCBD) with 5x5 factorial arrangement totally 25 treatment combination (Table.1) and replications three times. Randomization was held separately and independently for each of the replications as described by Gomez and Gomez (2009).

**The model**

Yijk = $\mu$+ T1i+T2j+ (T1T2) ij+€k (ij)

Yijk =is dependent variable observed in replication k of treatment ij

μ =is the overall mean effect.

T1i=is the effect of level i of different variety of clones

T2j =is the effect of level j of factor withering duration different variety of clones

(T1+T2)ij=is the effect of using level i of factor different variety of clones with level j of Factors withering time

€k(ij)=Is the error effect

Table 1. Clones and duration of withering combination

| Clone | Duration of withering (hours) | | | | | Total made tea (kg ) |
|---|---|---|---|---|---|---|
| | 16 | 17 | 18 | 19 | 20 | |
| $C_1$(11/4) | $C_1$16 hr | $C_1$17 hr | $C_1$18 hr | $C_1$19 hr | $C_1$20 hr | 200 |
| $C_2$(BB/35) | $C_2$ 16hr | $C_2$ 17hr | $C_2$18hr | $C_2$ 19hr | $C_2$ 20hr | 200 |
| $C_3$(11/56) | $C_3$ 16hr | $C_3$ 17hr r | $C_3$ 18hr | $C_3$ 19hr | $C_3$ 20hr | 200 |
| $C_4$(6/8) | $C_4$ 16hr | $C_4$ 17hr | $C_4$ 18hr | $C_4$ 19hr | $C_4$ 20hr | 200 |
| $C_5$(12\38) | $C_5$ 16hr | $C_5$ 17hr | $C_5$ 18hr | $C_5$ 19hr | $C_5$ 20hr | 200 |

## 3.4. Plucking and Processing

A 1000 Kg of tender fresh leaves (bud + two green tea leaves) were plucked from each tea clone, and transported to the factory after which the leaves were spread out on wire trays for withering. Leaves were withered separately for a period of withering time (16, 17, 18, 19, and 20) and rolled and macerated by CTC machine. The macerated leaves were spread out in fermentation tank for 90 min for oxidation. During fermentation time, room temperature and humidity were maintained at $22^0$C and 95%, respectively. The fermented tea leaves were dried according to the procedure of Wushwush fluid bed drier standard at 105°C for 25 min to stop chemical reaction. The dried product was then winnowed to remove the fiber and dust. Finally, 500g of dry made tea sample was packed from each experimental unit for further chemical and sensory analysis.

### 3.5. Chemical Analysis

### 3.5.1. The chemical analysis for Theaflavin, Thearubigin, Infusion Color and Brightness

Preparation of infusion: 9 g of made tea was added in to 375 ml of boiling water in a thermos flask. After ten minutes with occasional shaking, filtered and left the infusion to cool

**Reagents:** Disodium hydrogenphosaphate (1% w/v in $H_2O$), Oxalic acid (10% w/v in $H_2O$), Ethyl acetate, methanol.

Step.1.Ten ml of infusion added to 10 ml of 1% disodium hydrogen phosphate and 10ml Ethyl acetate in a separating funnel was taken. By slowly moving the Separating funnel in a to-from motion for 10 minutes the two phases were separated and Sodium sulfate was added to separate the emulsion. Fig 3 showed that from this mix the organic layer (A) was then taken.

Step. 2. For the first set of readings, 4 ml of A added to 4ml ethyl acetate in a 25 ml volumetric flask was taken. The volume was made to full volume with methanol. Blank contains 4 ml ethyl acetate and the volume was made to full with methanol Step E1

Step .3 For the second set of experiment 1 ml of infusion was taken added to 1ml of Oxalic acid 8 ml water in a 25 ml volumetric flask. Methanol was added to bring the volume to full. Blank was made from 1ml of Oxalic acid (10%) added 9 ml water in a 25 ml volumetric flask in which case methanol was added to bring the volume to full (E2).

Step.4 For the third set of experiments 1 ml of infusion was added to 9 ml water in volumetric flask and made up the volume to 25 ml with methanol. The blank had 10 ml of water in a 25 ml of volumetric flask in which methanol was added to bring the volume to full (E3).

Finally, the absorption of all sets of experiment units was read at 380 and 460 nm using spectrophotometer . The system is auto zeroed using blank and the reading was made against blank in each case (Hafezi, *et al*., 2006).

Theaflavins percent= 2.25 x 2 E1 (absorbance of E1 read at 380 nm)

Thearubigins percent= 7.06 (4E2 - 2E1) (absorbance of E1 and E2 read at 380 nm)

Infusion color= 4E3x 6.25 (absorbance of E3 read at 460 nm)

Brightness= (2E1/4E3) x 100 (absorbance of E1 and E3 read at 460 nm).

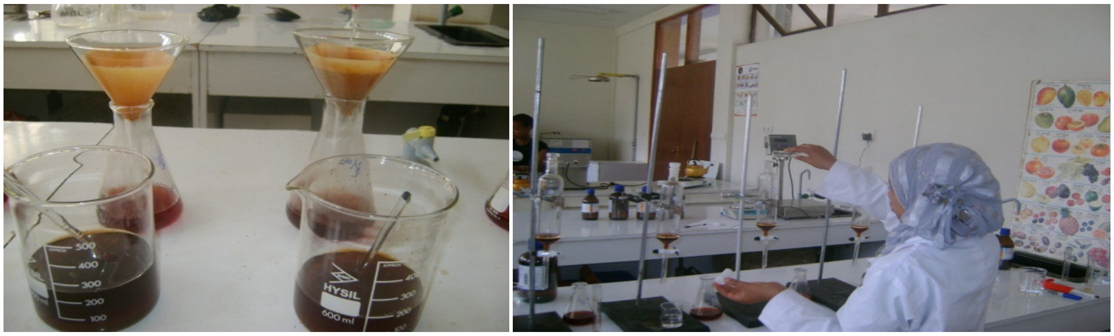

Figure 3 Chemical analyses for theaflavin, thearubigin, infusion color and brightness

**3.6. Organoleptic Evaluations**

Organoleptic evaluation can indicate the degree of acceptance of the product by the consumer (Muthumani and Kumar, 2006). Black tea tasting that is carried out during  processing is said inline tasting, and the experts that test are  called “tea tasters,” Tea cup testing done by two professional tasters and scores  were based on briskness, brightness, color, strength of liquid and overall quality, each on a scale of 1 to 5 for Taster (Owuner and Obanda, 2003). The five parameters are the best and simplest descriptors used to describe the sensory characters. According to Wushwush inline tasting method, tea tasting takes place immediately after drying. Tasters give scores for five different withering times per variety on a five scale for brightness, briskness, strength, color and thickness of liquor (Where 5= excellent, 4=very good, 3=good, 2=satisfactory and 1=poor). The standard procedure for the preparation of brew for cup tasting was as follows. Three grams of made tea leaves was added to 200 ml of boiled water and infused for 5min. The infusion was poured out for evaluation (Yan, 2007).

The cup was covered and let to sit for about 5-6 min. When the temperature was dropped, the brew was tested and its brew liquid was differentiated in to its textural classes:  briskness, strength, thickness, color and brightness. The quality is said to be worst if brew is greenish in color, raw, lack brightness, briskness and develop dull blurred red color. The brew is said to be best marketable quality if it develops bright golden red color with strong aroma. In addition to this the cup testers give high attention to the point where the briskness directly fits with strength. During cup tasting Tea Evaluation Room requires natural homogenous and adequate lighting. The change of light intensity has significant effect to the evaluation color.

### 3.7. Data Analysis

Data were first checked for all assumptions and subjected to the analysis of Variance (ANOVA) by using SAS version 9.2. Software and significant means separated using least significant difference (LSD) test.

# 4 RESULTS AND DISCUSSION

Withering is the first processing step in the factory and one of the most important tea processing steps. In short, it constitutes the foundation for achieving quality in tea manufacture. Based on achieving the desired level of withering, one can make better quality teas and, on neglect, can invite serious problems in subsequent steps of manufacture. As a matter of fact withering makes or destroys the tea quality. The overall result from this research attempts to give an answer to the poor quality tea. Moreover, not only responding to the research questions but also majority of the parameters attained results that can have paramount significance in to filling the gaps that exist in tea processing. Detailed explanations on the results are discussed below.

## 4.1. Effects of Clones and Duration of Withering on Chemical Composition of Black Tea

### 4.1.1. Theaflavin

The results of this experiment showed that the interaction effect of different withering durations, and clone had showed very highly significant *($P<0.001$)* differences on the theaflavin content of black tea (Table 2 and Appendix Table 1). Based on the treatments applied, the highest theaflavin content was recorded in clone 6\8 at 17h whereas the lowest theaflavine was registered in clone 11\56 at 20h. In addition, all clones attained their maximum point of theaflavine content at the withering duration of 16-18hrs.

In this finding clone 6\8 was identified as clone with higher amount of theaflavin among the clones that are produced in Wush Wush farm. Each clone produced the maximum amount of theaflavin at 16hrs and 17hrs of withering time. Moreover, the longer the withering time, the less theaflavin content was mainly due to high production of carotenoids during the first two withering treatment. These carotenoids degrade to form volatile flavor compound responsible for tea aroma. Similar finding reported by Tomlins and Mashingaidze (1997) also indicated that the shorter withering time, about 16hrs resulted in good flavor and quality of tea. The amounts of theaflavin contents and rates of development of theaflavin are highly dependent on variety (clone) and duration of withering as well as their interaction effects. The results reported elsewhere using different clones also showed that the longer the wither time; the lower was the TF levels (Owuor and Obanda, 2006).

The correlation coefficients (Appendix Table 5) showed that, theaflavin content was positively and significantly correlated with brightness(r=0.30333**), briskness (r=0.34182**) and significantly correlated to thickness (0.25823*).

Table 2 Effects of clones and withering duration for theaflavine content of made black tea

| Clone | Duration of withering (hours) | | | | |
|---|---|---|---|---|---|
| | 16 | 17 | 18 | 19 | 20 |
| 11\4 | $3.073^{ab}$ | $3.033^{abc}$ | $2.933^{abcd}$ | $2.700^{abcdef}$ | $2.733^{abcdef}$ |
| BB\35 | $2.633^{abcdefg}$ | $2.793^{abcdef}$ | $2.517^{bcdefg}$ | $2.303^{defgh}$ | $2.300^{efghd}$ |
| 11\56 | $2.170^{fgh}$ | $2.203^{efgh}$ | $2.307^{defgh}$ | $1.976^{gh}$ | $1.800^{h}$ |
| 6\8 | $2.563^{bcdefg}$ | $3.283^{a}$ | $2.867^{abcde}$ | $2.800^{abcdef}$ | $2.633^{abcdefg}$ |
| 12\38 | $2.567^{cdefg}$ | $2.3967^{cdefgh}$ | $2.313^{defgh}$ | $2.300^{efghd}$ | $2.190^{efgh}$ |
| LSD (5%) | 0.6729 | | | | |
| CV (%) | 14.90 | | | | |

*Different letters in the table indicate the difference between two means is statistically significant (P<0.001) for each of the treatment combinations*

### 4.1.2. Thearubgine

The analysis of variance indicated that there were highly significant *(P<0.01)* differences among the clones and withering durations with respect to thearubgine content (Table 3 and Appendix Table 1). Thearubgine content, in all clones, was increased as the withering period increased from 16 hr to 19hrs. This indicates that the longer the withering time, the higher is the Thearubgine content. Table 4 below depicts that there is a fluctuating nature of thearubgine content in each clones having minimal and maximum point. The maximum point of thearubgine content was observed in clone 12\38 at 19hrs while the minimum was in clone 6\8 at 16hrs. For all clones there was no significant differences between durations of 18 to 20hrs during which good thearubgine contents were observed. From this result a withering duration of 18h is considered ideal time for all clones to get good theaflavine. The result of the present study is in line with the work Tomlins and Mashingaidze (1997), who reported that wither tea leaves for longer hours resulted in maximum thearubgine but tea of poor quality and flavor. As illustrated in Table 4 below, tea that is withered for shorter time is lower in thearubgine content while long withered teas produce highest thearubgine content. The present finding is in line with the findings of Tomlins *et al*. (2010), who reported that, unwithered leaf is associated with teas that are lower in thearubgine.

The correlation coefficients (Appendix Table 5) showed that, thearubgine content was positively and highly significantly correlated with color (r=0.31810**), and significantly correlated to infusion color (0.25066*).

Table 3 Effect of clones and withering time for thearubgine content of made black tea

| Clone | Duration of withering (hours) | | | | |
|---|---|---|---|---|---|
| | 16 | 17 | 18 | 19 | 20 |
| 11\4 | $12.980^{f}$ | $16.520^{abcde}$ | $17.527^{abc}$ | $17.847^{abc}$ | $17.363^{abcd}$ |
| BB\35 | $14.763^{bcdef}$ | $15.407^{abcdef}$ | $15.780^{abcde}$ | $16.703^{abcde}$ | $15.237^{abcdef}$ |
| 11\56 | $14.063^{cdef}$ | $14.733^{bcdef}$ | $15.403^{abcdef}$ | $17.490^{abcd}$ | $16.783^{abcde}$ |
| 6\8 | $11.173^{f}$ | $13.267^{def}$ | $16.590^{abcde}$ | $18.860^{ab}$ | $13.923^{cdef}$ |
| 12\38 | $15.163^{abcdef}$ | $16.233^{abcde}$ | $17.770^{abc}$ | $19.240^{a}$ | $19.037^{a}$ |
| LSD (5%) | 4.2584 | | | | |
| CV (%) | 16.99 | | | | |

*Different letters in the table indicate the difference between two means is statistically significant (P<0.001) for each of the treatment combinations*

### 4.1.3. Brightness

The result with respect to brightness showed that the interaction between clones and withering durations was very highly significant (*P<0.001*). In addition, the separate effect of both withering duration and clone types showed very highly significant differences with respect to brightness (Table 4). From the result, the brightness varied depending on the type of clones. This indicates that all tea clones have their own chemical compositions. Maximum point of brightness ( 26.8) was observed in clone 11\4 withered for 16hrs of withering time while the lowest was in clone 11\56 withered for 20hrs.Clone 12\38 attained its maximum point of brightness with withering time of 16hrs after which tended to decrease slowly. On the other hand, clone 6\8 and BB\35 reached their maximum point of brightness at 17h after which it started to decrease. On the other hand clone 11\56 reached its pick point at 18h and afterwards slowly decreased. This indicates that the tea clones vary in their responses with respect to brightness to the different withering duration. Similar research by Tomlins and Mashingaidze (1997) confirmed that unwithered leaf is associated with teas that are bright while withered teas produced less brightness.

The correlation coefficients (Appendix Table 5) showed that, brightness was positively and significantly correlated with theaflavin (r=0.30333**), and positively correlated to briskness(r=0.01457) thickness (r=0.15538) and leaf appearance (r=0.08546).

Table 4 Effect of clone and withering duration on brightness

| Clone | Duration of withering (hours) | | | | |
|---|---|---|---|---|---|
| | 16 | 17 | 18 | 19 | 20 |
| 11\4 | 26.85$^{a}$ | 25.167 $^{ab}$ | 20.28 $^{cde}$ | 20.23$^{cde}$ | 17.233$^{ef}$ |
| BB\35 | 20.557 $^{cde}$ | 22.37$^{bcd}$ | 19.833cdef | 19.66$^{cdef}$ | 17.76 $^{ef}$ |
| 11\56 | 20.36$^{cde}$ | 22.73$^{abc}$ | 22.93$^{abc}$ | 18.93$^{cdef}$ | 15.33$^{f}$ |
| 6\8 | 19.30 $^{cdef}$ | 20.07$^{cde}$ | 19.57 $^{cdef}$ | 18.23$^{def}$ | 17.63 $^{ef}$ |
| 12\38 | 23.27 $^{abc}$ | 23.200$^{abc}$ | 22.933$^{abc}$ | 20.80$^{bcde}$ | 20.500$^{cde}$ |
| LSD (5%) | 4.4559 | | | | |
| CV (%) | 13.19 | | | | |

*Different letters in the table indicate the difference between two means is statistically significant (P<0.001) for each of the treatment combinations*

### 4.1.4. Infusion color

The result pertaining to infusion color showed that the interactions between clones and withering durations were significantly different (*P <0.05)* while the separate effect of duration of withering and clones showed highly significant different (*P*<0.001) with respect to this parameter. The maximum point of infusion color was observed for clone BB\35 with 19hrs of withering whereas the minimum point was with 16hrs withering of clone 6\8. As per this result, each clone is characterized by its own withering duration requirement in attaining good color. For instance, clone 11\4 and 12\38 showed good color with 20hrs and 18hrs withering, respectively. While in clone 6\8, 11\56 and BB\35 good infusion color was observed with withering duration of 19hrs. For all clones under the present study, good color was observed with withering duration ranging between 18 and 20hrs, therefore, a withering time of 19hrs can be considered as the ideal time for production of good infusion color tea without compromising the other quality parameters.

The correlation coefficients (Appendix Table 5) showed that, Infusion color was positively and significantly correlated with thearubgine (r=0.25066*) and positively correlated with brightness (r=0.21339) and strength(r= 0.12133).

Table.5 Effect of clone and withering duration on infusion color of black tea brew

| Clone | Duration of withering (hours) | | | | |
|---|---|---|---|---|---|
| | 16 | 17 | 18 | 19 | 20 |
| 11\4 | 9.8567abcd | 9.833abcd | 10.000 abcd | 10.140abcd | 10.3433 abc |
| BB\35 | 9.1267cd | 10.067 abcd | 10.033abcd | 11.1500 a | 10.450 abc |
| 11\56 | 9.800abcd | 9.703abcd | 10.023 abcd | 10.500 abc | 10.433 abc |
| 6\8 | 8.853d | 9.933 cdab | 10.033abcd | 10.767 a | 10.283abcd |
| 12\38 | 9.133cd | 9.560 bcd | 10.543 abc | 10.170 abcd | 9.867abcd |
| LSD (5%) | 1.4543 | | | | |
| CV (%) | 10.36 | | | | |

*Different letters in the table indicate the difference between two means is statistically significant (P<0.001) for each of the treatment combinations*

## 4.2. Effects of Clones and Duration of Withering On Sensory Quality of Black Tea

### 4.2.1. Briskness

With regard to briskness, results of the present study explain that there was highly significant *(P<0.01)* interaction effect of withering time and clone (Table -6 and Appendix table- 2). The maximum point of briskness was obtained during the first three treatments. The best quality was obtained in clone 11\4 and 11\56 with 16hrs and 18hrs, respectively and the lowest observed with 20hrs withering of clone 6\8. Results reported elsewhere using different clones showed that the longer the withering duration, the lower was the briskness (Mohammed *et al.,* 2006). The biochemical reaction like carotinoid, protein and catechins that contribute for the formation of the tea aroma were produced during shorter period of withering and they are responsible for briskness of tea (Tomins *et al*., 2010).

The correlation coefficients (Appendix Table 5) showed that, briskness was positively and significantly correlated with theaflavine (r=0.34182**), and positively correlated to brightness (r=0.01457) and thickness (r=0.14326).

Table 6 Effect of clone and withering duration on Briskness of tea brew

| Clone | Duration of withering (hours) | | | | |
|---|---|---|---|---|---|
| | 16 | 17 | 18 | 19 | 20 |
| 11\4 | $2.67^{a}$ | $2.33^{ab}$ | $2.00^{ab}$ | $2.00^{ab}$ | $1.67^{b}$ |
| BB\35 | $2.33^{ab}$ | $2.33^{ab}$ | $2.33^{ab}$ | $2.33^{ab}$ | $2.00^{ab}$ |
| 11\56 | $2.00^{ab}$ | $2.33^{ab}$ | $2.67^{a}$ | $2.33^{ab}$ | $2.00^{ab}$ |
| 6\8 | $2.33^{ab}$ | $2.33^{ab}$ | $1.67^{b}$ | $2.00^{ab}$ | $1.67^{b}$ |
| 12\38 | $2.33^{ab}$ | $2.33^{ab}$ | $2.00^{ab}$ | $2.00^{ab}$ | $2.00^{ab}$ |
| LSD (5%) | 0.6759 | | | | |
| CV (%) | 18.79 | | | | |

*Different letters in the table indicate the difference between two means is statistically significant (P<0.001) for each of the treatment combinations*

### 4.2.2. Thickness

The result from thickness indicated that withering duration and clones have showed very highly significant differences *at (P<0.001).* As indicated in table-7 and appendix Table- 2, the thickness of the tea brew was not constant within each clone having their own minimal and maximal points for thickness. For instant clone 11\4, 6\8 and 11\56 were found to be good with respect to thickness at 16-18hrs of withering duration. On the other hand, clone 11\4 and 12\38 showed a very good thickness values at 16h and BB\35 and 6\8 at 17hrs. This indicates each clone has its own time to give good thickness

The correlation coefficients (Appendix Table 5) showed that, thickness was positively and significantly correlated with theaflavin (r=0.25823*) and positively correlated to brightness (r=0.15538) and briskness (r=0.14326).

Table 7 Effects of clone and withering duration on thickness of tea brew

| Clone | Duration of withering (hours) | | | | |
|---|---|---|---|---|---|
| | 16 | 17 | 18 | 19 | 20 |
| 11\4 | $2.67^{a}$ | $2.33^{ab}$ | $2.00^{abc}$ | $2.33^{ab}$ | $2.33^{ab}$ |
| BB\35 | $2.33^{ab}$ | $2.33^{ab}$ | $2.33^{ab}$ | $1.67^{bc}$ | $2.00^{abc}$ |
| 11\56 | $2.00^{abc}$ | $2.33^{ab}$ | $2.67^{a}$ | $2.33^{ab}$ | $2.33^{ab}$ |
| 6\8 | $2.00^{abc}$ | $2.67^{a}$ | $2.33^{ab}$ | $2.00^{abc}$ | $1.33^{c}$ |
| 12\38 | $2.33^{ab}$ | $2.00^{abc}$ | $2.00^{abc}$ | $2.00^{abc}$ | $1.67^{bc}$ |
| LSD (5%) | 0.8223 | | | | |
| CV (%) | 18.82 | | | | |

*Different letters in the table indicate the difference between two means is statistically significant (P<0.001) for each of the treatment combinations*

### 4.2.3. Strength

The result of this experiment showed that the interaction between clones and duration of withering was very highly significant *(P<0.001)*. As can be seen from Figure- 4, all clones responded differently to the withering period with respect to the strength of the brew. For example, the maximum point of strength was observed at 18h in clone 6\8 while the minimum were recorded in clone11\56 at a withering period of 16hrs and clone 6\8 at 16h and 20hrs. Therefore, there is no constant hour to get good strength which is each clone have their own time to get good strength. The maximum value for strength was observed when withering duration took 18 to20hrs. Similar finding was also observed by Tomlins *et al*. (2010), who reported that, unwithered leaf is associated with teas that are lower in strength.

The correlation coefficients (Appendix Table 5) showed that, strength was positively correlated with thearubigin (r=0.12501), color (r=0.12133) and infusion color (r=0.14326).

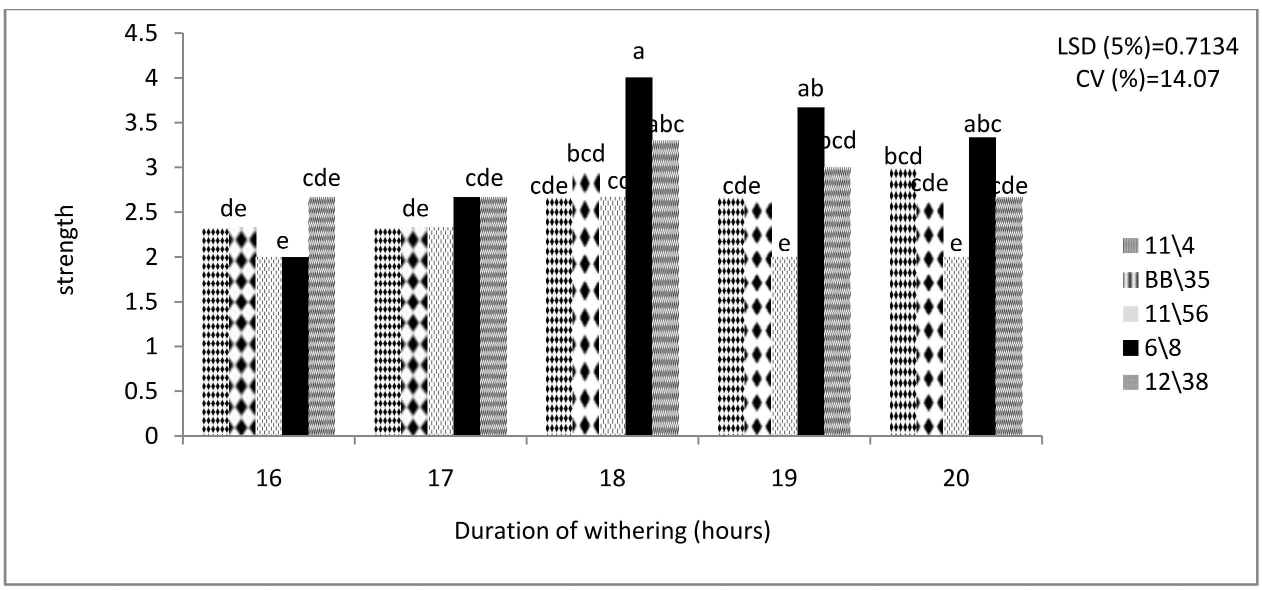


Figure 4 Effect of clone and withering duration on strength of black tea brew

*Different letters in the table indicate the difference between two means is statistically significant (P<0.001) for each of the treatment combinations.*

### 4.2.4. Color

Results obtained from this experiment showed that there were very highly significant differences *(P<0.001)* in color due to the interaction effects of clones and withering durations. The result (Figure 5 and Appendix Table 3) illustrated that longer withering periods are necessary to produce good colored tea as short withering periods produces grayish colored and poor quality tea. From this result, in all clones a withering period of 18 to 19hrs were the best for developing good colored tea. Withering periods greater than 19hrs though also produced good colored teas , however, resulted in the loss of their flavor and quality. Research results of Tomlins and Mashingaidze (1997) confirmed that the longer withering period (usually above 19hrs) result in teas of good color but of poor quality and flavor.

The correlation coefficients (Appendix Table 5) showed that, color was positively and significantly strongly correlated with thearubgine (r=0.3181**) and positively correlated to leaf appearance (r=0.21796)

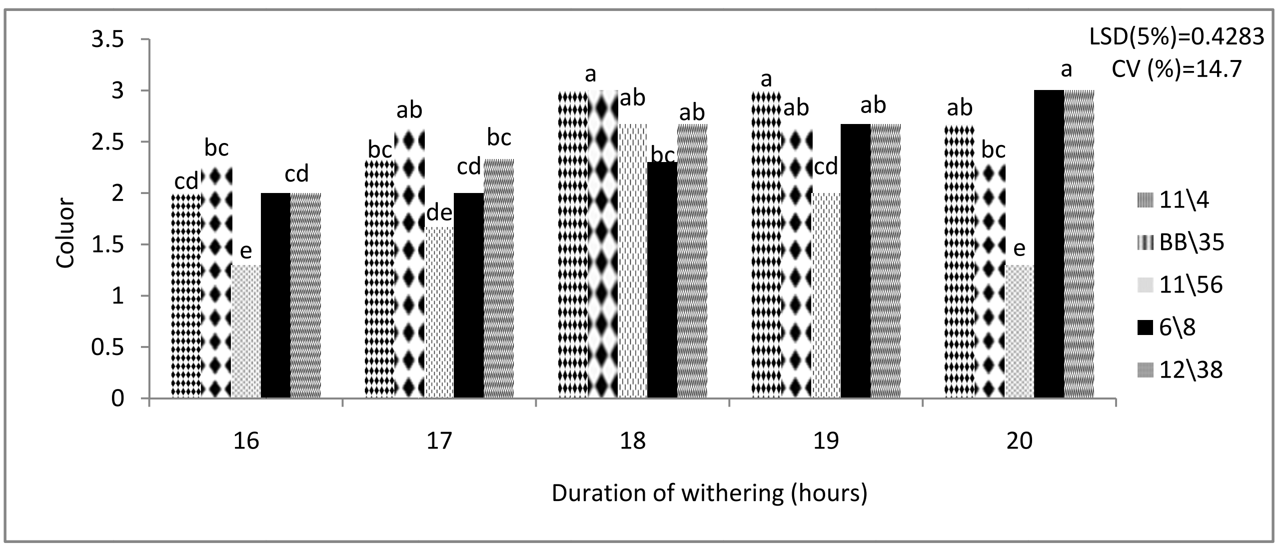


Figure 5. Effect of clone and withering duration on color of black tea brew

*Different letters in the table indicate the difference between two means is statistically significant (P<0.001) for each of the treatment combinations*

### 4.2.5. Leaf appearance

The result with respect to Leaf appearance showed that the interaction between clones and withering durations was very highly significant (*P<0.001*). Good leaf appearance was observed with 16hrs of withering duration in clone 11\4 whereas poor quality teas were observed in clone 11\4 with 20h. The results in Table 9 depict that there was no uniform duration of withering to attain good leaf appearance for all the clones as they have their own optimum duration to develop good leaf appearance. Clone 11\4 and 12\38 develop good leaf appearances with 16hrs of withering period whereas clones BB\35and 6\8 at 17hrs and clone 11\56 required 18hrs.

The correlation coefficients (Appendix Table 5) showed that, leaf appearance was positively correlated with thearubigin (r=0.02892) brightness (r=0.08546) and color (r=0.21796)

Table 8 Effects of clones and withering time on the appearance of made black tea leaf

| Clone | Duration of withering (hours) | | | | |
|---|---|---|---|---|---|
| | 16 | 17 | 18 | 19 | 20 |
| 11\4 | $3.667^{a}$ | $3.33^{a\ b}$ | $3.333^{ab}$ | $2.333^{cd}$ | $2.000^{d}$ |
| BB\35 | $3.00^{abc}$ | $3.33^{ab}$ | $3.000^{abc}$ | $3.000^{abc}$ | $2.667^{bcd}$ |
| 11\56 | $2.667^{bcd}$ | $3.000^{abc}$ | $3.333^{ab}$ | $3.000^{abc}$ | $2.667^{bcd}$ |
| 6\8 | $2.333^{cd}$ | $2.667^{bcd}$ | $2.667^{bcd}$ | $2.667^{bcd}$ | $2.667^{bcd}$ |
| 12\38 | $3.333^{ab}$ | $2.667^{bcd}$ | $2.667^{bcd}$ | $2.333^{cd}$ | $2.667^{bcd}$ |
| LSD (5%) | 0.8047 | | | | |
| CV (%) | 18.04 | | | | |

*Different letters in the table indicate the difference between two means is statistically significant (P<0.001) for each of the treatment combinations*

### 4.2.7. Overall quality

Results obtained from this experiment showed that there were significant differences *($P<0.05$)* in Overall quality due to the interaction effects of clones and withering durations (Table. 9). By considering overall quality, clones 11\4 and 12\38 needed withering duration not more than 16hrs. Clones BB\35 and 6\8 could be withered for duration of 17 hrs while clone 11\56 withered for 18hrs. Withering more than this duration leads to loss in the overall quality made black tea.

**Table 9.** Summary of the effect of clones and withering duration on the overall quality of made black tea

| Clone | Duration of withering (hours) | | | | |
|---|---|---|---|---|---|
| | 16 | 17 | 18 | 19 | 20 |
| 11\4 | 3.33$^{a}$ | 3.00$^{ab}$ | 2.67$^{abc}$ | 2.33$^{bc}$ | 2.33$^{bc}$ |
| BB\35 | 3.00$^{ab}$ | 3.33$^{a}$ | 2.33$^{bc}$ | 2.33$^{bc}$ | 2.00$^{c}$ |
| 11\56 | 2.67$^{abc}$ | 2.67$^{abc}$ | 3.00$^{ab}$ | 2.33$^{bc}$ | 2.33$^{bc}$ |
| 6\8 | 2.67$^{abc}$ | 3.00$^{ab}$ | 2.67$^{abc}$ | 2.33$^{bc}$ | 2.00$^{c}$ |
| 12\38 | 3.33$^{a}$ | 2.00$^{c}$ | 2.67$^{abc}$ | 2.67$^{abc}$ | 2.33$^{bc}$ |
| LSD (5%) | 0.7014 | | | | |
| CV (%) | 15.93 | | | | |

*Different letters in the table indicate the difference between two means is statistically significant ($P<0.05$) for each of the treatment combinations*

# 5. SUMMARY AND CONCLUSION

Withering is the first processing step in the factory and is a process in which freshly plucked leaf is conditioned physically, as well as, chemically for subsequent processing stages. Indeed, withering is one of the most important tea production process steps and can be said to constitute the foundation for achieving quality in tea manufacture. At Wush Wush, during processing in the factory leaves of all five clones are treated with similar withering time and the company overcomes the quality problems by its experienced cup taster. However, the existing subjective assessment of tea is not enough to produce competitive product in the international market. In view of this, biochemical parameters have been used to detect the amount of theaflavins and thearubigin formation at different withering duration. Hoping that output of the experiment would definitely contribute to optimize withering duration in the tea industry to produce quality tea, the present study was conducted to standardize the withering duration for the different clones at Wush wush in view of improving the quality of black tea. Accordingly, five tea clones (11\4, BB\35, 6\8, 11\56 and 12\38) and five levels of withering duration (16, 17, 18, 19 and 20hour) were used. The experiment was lied out in 5x5 factorial arrangements in (RCBD) design with three replications.

The result of the study showed that interaction of clone and duration of withering had significantly influenced the theaflavin (TF), thearubgine (TR), brightness (BR), thickness (TH), briskness (BS), strength (ST), leaf apperiance (LA) and color (CL*)* and the highest (3.2%) TF was obtained with 17h duration of clone 6\8 and lowest (1.8) with 20h for clone 11\56 on the other hand the highest (19.2%) TR at was recorded with 19h withering of clone 12\38 and lowest(11.1%) with 16h withering of clone 6\8 .The maximum value for BR (26.8) was observed with 16h withering of clone 11\4 and the minimum (15.3) was noted from clone 11\56 which was withered for 20h. On the other hand, TH was maximum (2.67) when withering duration was between 16 and18h and lowest (1.3) at 20hr. The highest BRS (2.67) developed from 16hrs and 18hrs withering of clone 11\4 and 11\56 while the lowest (1.67) was from 20h withering in clone 6\8. The strength of the tea was at its maximum (4) when the withering duration was adjusted to 18h in clone 6\8 and lowest (2) in clone 6\8 and 11\56 withered for 16 and 20h. the highest LA(3.67)when withering duration is between 16 to 17hrs Moreover, the highest value for CL (2.67) was registered when teas leaves were withered for 20h in clone 6\8 and 12\38 and lowest (1.3) in clone 11\56 withered for 16h and 20hrs. It was generally observed that almost all clones gave good quality when the withering duration was fixed between 16 and 18 hours.

Both chemical and sensory analysis results showed that the optimum withering duration for clone 11\4 and 12\38 are16h and clones BB\35 and 6\8 showed good quality with 17 hour and clone11\56 with 18h withering. Clone 11\56 id somewhat slow withered than that of the 11\4 and 12\38. Clone11\4 and 12\38 needed shorter period of time than the11\56 to attained good quality. Therefore, by considering these optimum withering hours for the different clones we can save time, money, manpower and most importantly improve the quality of black tea.

Even though this processing step is the first and the most important in maintaining the quality it is provided with limited attention in the Ethiopian tea industry. By considering the advantages of reducing the losses of labor, time, quality and also increases the demand difficulties in tea processing, these recommended withering durations are beneficial to the tea processing industries especially for Wush Wush agro ecology.

The present research standardized the withering duration for each clone at Wush Wush tea state condition. However, the following gaps need to be considered as future line of work:

- Duration of withering during summer when they use fuel instead of natural air
- Effect of withering duration and drying temperature
- The temperature of the air used for withering need to be studied for its impact on the withering duration of different clones.
- Depth of leaf on the withering trough could also affect the withering duration therefore needs to be optimized for different clones and hence deserve attention of researchers

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# Appendix

**Appendix Table 1** Analyses of variance for Chemical analysis

| Source of Variation | DF | Mean Squares | | | |
|---|---|---|---|---|---|
| | | TF (%), | TR% | BR | IFC |
| Block | 2 | 0.04 | 6.65 | 1.626 | 0.268 |
| Duration of withering | 4 | 4.44** | 62.94* | 323.65*** | 17.78*** |
| Clone | 4 | 0.92*** | 98.85*** | 102.1*** | 6.98*** |
| Duration of withering*clone | 16 | 1.13*** | 16.23** | 39.76*** | 1.61* |
| Error | 48 | 0.15 | 7.39 | 7.41 | 0.74 |

NS, *, ** and *** = Non-significant, significant, highly significant and very highly significant differences at 0.5%,0.1% and 1% levels of probability level respectively

TF=Theaflavin, TR=Thearubigin, Brightness=BR, IFC=Infusion color,

Appendix Table 2 Analyses of variance for sensory analysis

| Source of Variation | DF | Mean Squares | | |
|---|---|---|---|---|
| | | TH | BRS | ST |
| Block | 2 | 0.28 | 0.09 | 0.12 |
| Duration of withering | 4 | 1.06*** | 0.45* | $0.3^{ns}$ |
| Clone | 4 | 4.10*** | 0.5* | 1.2*** |
| Duration of withering*clone | 16 | 0.92*** | 0.7** | 1.83*** |
| Error | 48 | 0.25 | 0.16 | 0.13 |

NS, *, ** and *** = Non-significant, significant, highly significant and very highly significant differences at 0.5%,0.1% and 1%  levels of probability level respectively

TH=thickness, BRS=briskness, ST=strength

Appendix Table 3 Analyses of variance for physical analysis

| Source of Variation | DF | Mean Squares | |
|---|---|---|---|
| | | LA | CL |
| Block | 2 | 0.36 | 0.01 |
| Duration of withering | 4 | 3.25*** | 2.41*** |
| Clone | 4 | 3.2*** | 1.78*** |
| Duration of withering*clone | 16 | 0.67*** | 1.06*** |
| Error | 50 | 0.26 | 0.12 |

NS, *, ** and *** = Non-significant, significant, highly significant and very highly significant differences at 0.5%,0.1% and 1% levels of probability level respectively

L A=leaf appearance, CL=color

Appendix Table 4 Glossary of Tea Terms

| Glossary of Terms | Definition |
|---|---|
| Color | A measure of the depth of the tea's physical color. Based on season/growth/grade factors. |
| Briskness | A lively, astringent quality, characteristic of all fine teas, not flat.. |
| Brightness | A lively, clean style that refreshes the palate. |
| Strength | Liquor possesses strong of body and flavor. |
| Infusion color | A measure of the color depth of the teas liquid extracts (liquor). |
| Thickness | A term describing tea that has good body or strength |

Appendix Table 5. Simple correlation on quality attributes of black tea

| | TF | TR | IFC | BR | LA | BRS | TH | CL | ST | Mc |
|---|---|---|---|---|---|---|---|---|---|---|
| TF | 1 | -0.24* | 0.06 | 0.30** | -0.12 | 0.34** | 0.25* | -0.23* | -0.19 | 0.01 |
| TR | | 1 | 0.256* | -0.26* | 0.03 | -0.33** | -0.07 | 0.31** | 0.12 | -0.09 |
| IFC | | | 1 | 0.21 | -0.23* | -0.09 | -0.07 | -0.02 | 0.12 | -0.08 |
| BR | | | | 1 | 0.08 | 0.01 | 0.15 | -0.16 | -0.09 | -0.07 |
| LA | | | | | 1 | -0.20 | -0.23* | 0.21 | -0.11 | -0.02 |
| BRS | | | | | | 1 | 0.14 | -0.28* | -0.31** | -0.21 |
| TH | | | | | | | 1 | -0.04 | -0.37** | -0.03 |
| CL | | | | | | | | 1 | 0.06 | -0.03 |
| ST | | | | | | | | | 1 | 0.16 |
| Mc | | | | | | | | | | 1 |

*, ** = Correlation is significant at the 0.05 and 0. 01 level respectively.

TF=Theaflavin, TR=Thearubigin, IFC=Infusion color, BR=Brightness, L A=Leaf appearance, BRS=Briskness, TH=Thickness, CL=Color, ST=Strength, MC=Moisture content

Appendix figure 1 Pictures clones planted in Wush Wush

Appendix Figure. 2 Laboratory figures


**Fig.3 chemical analysis**