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Effect Of Withering Temperature and Time on Biochemical Properties of Vegetatively Propagated and Seedling Tea

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Abstract

Black tea quality depends on chemical composition of tea leaves and physical and chemical processes involved in its manufacturing. Different literature reports indicated that the main constituents of tea leaves belong to the polyphenol group and also various chemical constituents including methylxanthines, amino acids, chlorophyll, carotenoids, lipids, carbohydrates, vitamins, and more than 600 volatile compounds. This study investigated the effect of withering conditions (time and temperature) on thearubigins (TRs) and theaflavins (TFs) content in seedling and vegetatively propagated tea leaves. Tea leaves were withered at different times (14, 16, 18, and 20hours) at constant temperature (25°C) and different temperatures (23, 25, 27, and 29°C) at constant duration (18hours), prior to HPLC determination of TFs and TRs concentration. Parameters measured included, TFs, TRs, taste, liquor appearance and aroma. Withering times of 20hours for seedling tea and 16hours for vegetatively propagated tea at 25°C promoted the optimum development of theaflavins (3.77+0.06mg/g) and thearubigins (3.93+0.05mg/g) thus, excellent taste, liquor appearance, and aroma of tea. There was a significant difference ($p<0.05$) in theaflavins and thearubigins concentration between seedling and vegetatively propagated teas.

Keywords: Withering; Thearu begins; Theaflavins; Seedling tea; Vegetatively propagated tea

Introduction

Tea is made from the tender leaves of the tea plant Camellia Sinensis (belonging to the Theaceae family) which is native to East and South-East Asia (Roberts, 2004) [1]. It is one of the most popular and widely consumed beverages in the world because of its refreshing taste, attractive aroma and health benefits (Hajra et al., 2015) [2]. Different literature reports show that the main constituents of tea leaves belong to the polyphenol group, which accounts for 25 to 35% on a dry weight basis and also contains various chemical constituents. These chemical constituents include methylxanthines, amino acids, chlorophyll, carotenoids, lipids, carbohydrates, vitamins, and more than 600 volatile compounds. Some health benefits of tea include lowering blood glucose levels, eliminating fats, reducing fatigue, promoting good blood circulation and a steady heart rate, enhancing high bone mineral density and improving cognitive functions Lee et al., 2002; Khan & Mukhtar, 2007; da Silva Pinto, 2013 [3]. Tea processing consists of five distinct stages, which are withering, leaf maceration, fermentation, drying, and sorting. The withering stage is the most important because of the reduction of moisture levels in tea leaves from about 75-80% to 68-70% (wet basis) for 18 hours and its influence on some biochemical changes that occur as a result of the changes in the leaf structure [1]. These changes are in the levels or contents of tea phenolic compounds (catechins, theanine, theaflavins (TFs), thearubigins (TRs), proteins, lipids, sugars, enzyme activity, and organic acids Hilton et al. [4]. Tea phenolic compounds are responsible for the creaming effect of tea, proteins and theanine for the flavour, TRs for the body of the tea (liquor colour and strength), and TFs for tea brightness and briskness or astringency of black tea Obanda et al. [5].

A number of factors or conditions affect the biochemical changes, and these include withering duration, temperature, air flow rate and relative humidity Horie and Kohata, 2004. For example, high withering temperatures above 30 °C result in unfavourable enzymatic reactions, which can lead to the production of unfavourable amounts of TFs and TRs causing a decrease in brightness, flavour indexes, and sensory evaluation scores of black teas. Temperatures between 25 and 28°C induce favourable biochemical changes for enhancement in flavour and brightness of tea liquors. Long withering durations (17-20hours) can produce tea with good biochemical properties and sensory scores, whereas short durations (6-8hours) produce tea of low-quality Owuor & Orchard ; Jabeen et al. [6,7] .

Besides processing conditions, tea quality also depends on the cultivar of the tea, type of shoot (1+b, 2+b or 3+b leaf) harvested, growing environment, plucking standard, plucking interval and plucking season, method of processing, size of ground tea leaves and infusion preparation. Despite the numerous studies on the effects of withering time-temperature conditions on the quality attributes of tea, limited studies have been conducted to compare the effect of time- temperature conditions on biochemical attributes in vegetatively propagated (plant propagated vegetatively from high-yielding plant material) and seedling tea (plants raised from seed). Therefore, this study aimed at getting more insight into the effects of withering temperature and duration on TFs and TRs in vegetatively propagated and seedling tea. Insights from this study could enable optimization of the withering process for vegetatively propagated and seedling tea and to help identify improvement opportunities.

Methodology

Tea sampling and treatment

For each tea type (vegetatively propagated or seedling tea), 2000kg of 2+b(3+bL) green leaf was hand (machine) harvested, weighed using a weighbridge scale and equally added to 4 trough beds with two heat fans. Four withering durations, 14, 16, 18, and 20 hours at 25 °C, were used to determine the effect of withering duration and four temperature treatments of 23, 25, 27, and 29 °C for 18 hours were applied to determine effect of withering temperature on the TRs and TFs content in vegetatively propagated and seedling tea. In both treatments, relative humidity, and air flow rate were kept constant at 73%, and 6.3 m^3/min/kg, respectively. After leaf maceration, 800g to 1kg of the dhool samples were then selected randomly for the analyses of TFs and TRs.

Extraction and analysis of polyphenols, theaflavins (TFs) and thearubigins (TRs)

Fifteen (15) gram dhool samples from the withered seedling and vegetatively propagated tea were weighed and 120 ml of boiled distilled water was added, and the mixture was left to infuse for 5minutes. The samples were then filtered using a tea strainer and the filtrate was stored in a reagent bottle covered with aluminium foil before it was injected into the HPLC (Agilent 50 LC model) equipped with a UV-VIS detector. The mobile phase consisted of 100% acetonitrile solution (A) and distilled water (B). Gradient elution was programmed as follows: 90%A and 10%B, raised to 20%A (7-9 min) and 90%A (9-15min). Detection peaks were scanned at a wavelength of 280 nm. A calibration curve was prepared by serial dilutions of the standard stock solution. The procedure was repeated for all the 3 dhool samples from vegetatively propagated and seedling leaves and the fresh tea leaves (before the withering process). Three replicates were used for each treatment.

Statistical analysis

The data from the Significance test (ANOVA) for the effect analysis experiments was analysed using one-way ANOVA (analysis of variance) SPSS statistical package version 23.

Results and Discussion

Effect of withering duration on thearubigin and theaflavin concentration in seedling and vegetatively propagated tea leaves

The TFs in vegetative propagated and seedling tea did not differ significantly between the withering duration of 18 hours. Seedling tea produced the highest concentration of TFs (3.06mg/g+0.05) and TRs (3.90mg/g+0.06) at 20°C followed by 18 hours 3.04mg/g+0.04 TFs and 3.81mg/g+0.09 TRs, then 16 hours with 2.97mg/g+0.07 TFs and 3.74mg/g+0.05 TRs and 14hours with 2.86mg/g+0.04 TFs and 3.66mg/g+0.04 TRs.

For vegetatively propagated tea, the highest concentration of TFs (3.24mg/g+0.05) and TRs (3.93mg/g+0.05) were obtained at a withering duration of 16 hours. This was followed by 14 hours with 3.15mg/g+0.05 TFs and 3.89+0.03mg/g TRs then 18 hours with 3.06mg/g+0.05 TFs and 3.86mg/g+0.06 TRs. Duration of 20 hours produced the least concentration values of TFs (2.93mg/g+0.05) and TRs (3.76 mg/g+0.006). The results indicate a significant difference ($p<0.05$) in the concentration of TFs and TRs in seedlings and vegetatively propagated (Table 1) and withering duration had a significant effect ($p<0.05$) on TF and TR concentrations for both seedling and vegetatively propagated teas (Table 1). Vegetatively propagated tea leaves had higher concentrations of both the TFs and TRs than the seedling tea.

The TRs content for seedling tea changed significantly during the withering process such that withered leaves at the duration of 20 hours had the highest concentrations of TRs whilst those at 14 hours had the least TR concentrations. These changes could be attributed to the oxidation of catechins in the tea leaves (synthesised by the free sugars found in tea e.g., fructose, glucose, raffinose, stachynose) in the presence of the naturally occurring polyphenol oxidase and peroxidase enzymes forming TFs and TRs to a larger extent that are reddish brown in colour, water soluble and highly responsible for the body and colour of tea. This is in agreement with Ullah et al. (1984)'s findings that there is an increase in TR content during withering resulting in a stronger taste, tea liquor appearance/colour and body.

Borah et al., [8] reported that seedling tea withered for a shorter duration is lower in TR content due to inadequate catechin degradation to TR and long withered teas produce the

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highest TR content due to adequate TR formation from catechins. This could be the explanation for the low TR concentration at a duration of 14 hours and high TR concentration at 20 hours in the study. Short withering durations result in inferior black tea quality for seedling tea since TR formation will not be maximized. According to Owour [9], vegetatively propagated tealeaves and withering duration interaction have a significant influence on TF, TR, brightness, infusion colour, thickness, strength, briskness, leaf and liquor appearance and almost all vegetatively propagated (clones) produce good quality between 16-18 hours. Duration of 16 hours had the highest TF and TR concentrations (Table 1) which is in line with Owour (2003)'s study. As withering duration increased from 16 to 20 hours for vegetatively propagated leaves, TR content decreased (Table 1). This is similar to the findings by Tomlins and Mashingaidze [10] who stated that TR content decrease with an increase in withering duration but different from the seedling trend where TR content increases with an increase in duration.

The highest TF content (3.24+0.05mg/g) was recorded at 16 hours duration whereas the lowest (2.93+0.05mg/g) at 20 hours for vegetatively propagated leaves (Table 1). These results are in agreement with those by Sanderson [11], who reported that the longer the withering time, the less TF content; mainly due to high production of carotenoids which degrade to form volatile flavour compounds that are responsible for tea aroma. Similarly, studies by Tomlins and Mashingaidze [10] indicated that the shorter withering time for vegetatively propagated tea (12-17hours) resulted to tea of good flavour and quality by efficient poly phenol oxidase enzyme activity which converts catechins to TF and TR. Tomlins and Mashingaidze confirmed that unwithered tea leaves are associated with teas that are bright while withered teas produce less brightness. Brightness is directly related to TF content or concentration in tea which explains the very low initial concentrations of TFs from the unwithered or fresh tea leaves from this study.

The results indicate a significant difference in the concentration of TFs and TRs in seedlings and vegetatively propagated leaves. Vegetatively propagated teas have higher concentration of TFs and TRs. This could be attributed to a greater proportion of polyphenols in vegetatively propagated teas as compared to seedling teas, implying that the catechin amount is higher hence sufficient or adequate degradation of catechins to TRs and TFs [12].

Effect of withering temperature on thearubigins and theaflavins content in seedling and vegetatively propagated tea leaves

The results in Table 2 show that withering temperature had a significant effect ($p<0.05$) on TF and TR formation. The TFs (0.15mg/g+0.04) and TRs (0.16mg/g+0.04) concentrations before withering (fresh leaf) for seedling tea were not significantly different for TFs (0.16mg/g+0.04) and TRs 0.16mg/g+0.03 for vegetatively propagated leaves). Table 2 shows that withering temperature of 23°C recorded the highest TRs (3.08mg/g+0.05) and TF (3.66mg/g+0.06) concentrations, both seedlings tea and vegetatively propagated tea (3.77+0.06mg/g; 3.26mg/g+0.08 respectively). Tea treated at 29°C had the least concentrations of TRs (2.73mg/g+0.10) and TFs (3.46mg/g+0.08). The same trend of results was noted for vegetatively propagated teas as well, though they had higher concentrations than seedling tea (Table 2).

Table 1: Theaflavin (mg/g) and Thearubigin of black tea in relation to withering duration.

Duration (hours)	Seedlings		Vegetatively propagated	
	TFs	TRs	TFs	TRs
0	0.15 ± 0.02^{a}	0.26 ± 0.06^{a}	0.16 ± 0.06^{a}	0.26 ± 0.04^{a}
14	2.86 ± 0.04^{b}	3.66 ± 0.04^{b}	3.15 ± 0.05^{cd}	3.89 ± 0.03^{bc}
16	2.97 ± 0.07^{bc}	3.74 ± 0.05^{bc}	3.24 ± 0.05^{d}	3.93 ± 0.05^{c}
18	3.04 ± 0.04^{c}	3.81 ± 0.09^{bc}	3.06 ± 0.05^{bc}	3.86 ± 0.06^{bc}
20	3.06 ± 0.05^{c}	3.90 ± 0.06^{c}	2.93 ± 0.05^{b}	3.76 ± 0.01^{b}

Table 2: TFs (mg/g) and TRs of black tea in relation to withering temperature (°C).

Temperature (°C)	Seedlings		Clones	
	TFs	TRs	TFs	TRs
Fresh leaf	0.15 ± 0.04^{a}	0.15 ± 0.05^{a}	0.16 ± 0.04^{a}	0.16 ± 0.03^{a}
23	3.66 ± 0.06^{c}	3.08 ± 0.05^{d}	3.77 ± 0.06^{c}	3.26 ± 0.08^{c}
25	3.57 ± 0.07^{bc}	3.06 ± 0.05^{cd}	3.64 ± 0.06^{bc}	3.18 ± 0.04^{c}
27	3.48 ± 0.06^{bc}	2.87 ± 0.08^{bc}	3.56 ± 0.08^{b}	2.95 ± 0.05^{b}
29	3.46 ± 0.08^{b}	2.73 ± 0.10^{b}	3.48 ± 0.05^{b}	2.92 ± 0.06^{b}

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An increase in withering temperature resulted in a decrease in TF and TR concentrations. Withering tea leaves at 23°C temperature produced teas with the highest TF concentrations. A study carried out by Sanderson [11] also reported that at lower temperatures, overall biochemical reactions are kinetically and thermodynamically controlled for optimal formation of quality components in black tea.

The results indicate that TR concentrations were significantly lower than TFs in the withering temperature treatment and lower temperatures (23 and 25°C) produced higher TR concentrations whilst higher temperatures (27 and 29°C) had lower TR concentrations (Table 2). These findings are in accordance with Howard [13] who confirms that black tea manufactured with withering temperature above 25°C has low TR content, total colour levels, lacks briskness and has a very low TF content as well. The results in Table 2 shows that tea leaves subjected to high temperatures produced teas with the least TF and TR contents because enzyme activity at these high temperatures is reduced such that the rate of catechin degradation to TFs and TRs is apparently reduced (Table 2). This then gives the need to control temperatures below 27°C during withering; explaining why teas at lower temperatures had good quality attributes in the study. Comparison of the effect of withering temperature on seedling and vegetatively propagated tea leaves

The results indicate that there was a significant difference in the concentration of TFs and TRs in seedling and vegetatively propagated tea. Vegetatively propagated teas have higher concentrations of TFs and TRs. This could be attributed to their constitution of a greater proportion of polyphenols than seedlings; implying that their catechin content is higher hence sufficient or adequate degradation of catechins to TRs and TFs which enhances tea brightness, briskness, body and quality [12-17].

Conclusion

The amount of TF and TR content in tea is dependent on the type of tea leaf, withering duration as well as their interaction effects. The study showed that withering times of 20 hours for seedling tea, 16 hours for vegetatively propagated tea, and a withering temperature of 23°C are the most desirable since they promoted the optimum development of significant quality attributes of tea, that is high TF and TR concentrations. Furthermore, the results showed that withering seedling tea leaves are the most desirable since they promoted the optimum development of significant quality attributes of tea with good biochemical properties through high TF and TR content. The results of this study can help operators in the tea industry to improve the quality of their tea.

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