International Journal of Tea Science
Vol 13, No. 1&2 (2017) : 72-79

Clonal Variations in the Response of Hard Physically Withered Leaf to Rehydration Following Long Chemical Wither Durations

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Publication Info

Article history:
Received : 02.08.2016
Revised : 15.04.2017
Accepted : 30.08.2017
DOI:https://doi.org/10.20425/ijts.v13i01-02.9986

Key words:
Plain black tea, chemical wither duration, hard physical withers, rehydration, quality restoration

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ABSTRACT

Long chemical wither durations and hard physical wither reduce the quality of plain black tea, through reduction in total theaflavins production while encouraging thearubigins formation. Such quality reduction may vary with genetic makeup of the tea plant. However, rehydration reactivates activities of oxidative enzymes responsible for producing plain black tea quality parameters. Influence of rehydrating hard physically withered leaf that had undergone long chemical wither durations on two clonal plain black tea quality parameters were assessed. Rehydration restored the formation of plain tea quality parameters from hard physically withered leaf. However, quality deterioration due to long chemical wither duration could not be reversed by rehydration. The patterns of responses of the clones used were similar. Thus in the processing of plain black tea, efforts should be made to maintain chemical wither durations to below 30 hours. However, rehydration reverses reduced plain black tea quality parameters by hard physical withers to equivalent of chemical wither.

INTRODUCTION

Plucking tea (*Camellia sinensis* (L.) initiates biochemical and physiological changes in the leaf (1-4). Collectively, the changes are referred to as chemical wither (5-7), which improve black tea quality (1, 2, 4, 8). During black tea processing, leaf is usually subjected to moisture loss and cell membrane permeability changes, in a process referred to as physical wither (5-7). This process makes leaf flaccid and easy to macerate. This is the most visible withering aspect that tea manufacturers appreciate. Achieving optimal physical and chemical withers presents challenges in black tea processing as withering is slow, difficult to time, requires largest factory space, energy and labour (9). Several approaches have been made to develop technologies of managing withering process. This has partially been necessitated by the fast rise in green leaf production that has made many black tea processors unable to accommodate and process all available leaf. In Kenya, for example, green leaf production has risen faster than expansion of processing capacity. Excess leaf therefore goes to waste during peak production periods (9). The black tea processing stage most adversely affected by fast growth is withering. Technologies enabling factories to accommodate more leaf in the factory without putting up extra buildings including tank withering where only chemical withers either followed by maceration without physical wither (6, 10-13) or followed by physical withers (7, 11, 13-16) have been achieved. These technologies enable factories to handle more leaf with limited processing capacity (9, 17). However, achieving optimal physical wither can be problematic. Often leaf is physically over withered. However, in the processing of plain black teas, provided chemical withers are attained, physical withers reduce quality (7, 11, 14, 16) due to reduction in polyphenol oxidase activity (18-20). Rehydrating hard physically withered leaf has been demonstrated to restore the lost oxidative enzymes activities in the leaf (3, 21). This suggests that lost plain black tea quality due to hard physical withers may be recovered with rehydration prior to maceration.

Timing chemical wither duration is difficult. Chemical wither starts immediately leaf is detached from the plant. In practice, harvested leaf takes long durations in the field before delivery to the factories (12, 22). This problem can be more complicated in the smallholder tea sector. For example, in Kenya, leaf harvesting starts at 6 am. However, such leaf may not be delivered to the factory until late in the evening. Such leaf is mixed with leaf that was plucked continuously during the day. The situation is further complicated by poor state of tea roads, that are particularly difficult to manage during rainy seasons, which usually are also the peak production seasons (22). Plucked leaf can therefore last up to more than 30 hours before arriving at the factory. Although the leaf arriving in the factory may have therefore achieved chemical wither or has undergone past the optimal chemical wither duration (9, 13, 23, 24), for lack of proper ways of timing the process, tea factory personnel start timing withering duration from the time the leaf is spread in withering troughs. Usually, the factories wither for between 12 and 16 hours. For leaf that had stayed in the field, the actual chemical wither duration can therefore be as long as 40 hours. Such long chemical withering duration is usually accompanied by hard physical wither. Whenever, there is delay in leaf collection, farmers aerate the leaf to dissipate heat accumulation arising from respiratory process that may cause leaf cell wall destruction leading to uncontrolled fermentation (25). The resultant plain lack teas from such leaf are usually of lower quality (22). This study evaluated if rehydration may restore plain tea quality parameters of leaf from long and hard physical withered leaf.

Kenyan black teas are classified as plain to medium flavoury in tea trade. Such teas are evaluated based on theaflavins and thearubigins levels (26, 27), which are oxidative products of polyphenols, especially flavan-3-ols (catechins). The oxidation of polyphenols is catalysed by enzymes, especially polyphenol oxidase (28). The levels of flavan-3-ols (29, 30) and polyphenol oxidase (18, 31) vary with cultivars. Clonal variations in response of hard physically withered tea leaves to long wither durations and rehydration were also investigated.

MATERIALS AND METHODS

Leaves and tea processing

Leaves were obtained from clones TRFK 6/8 and TRFK 31/8 planted at the Tea Research Institute (Kenya), Museum of Clonal Tea Plants (altitude 2,180 m above mean sea level, latitude $0^0$22'S, longitude 35° 21'E). The two clones are the most widely cultivated tea plants in Kenya (32) and were receiving recommended agronomic inputs (33). From each cultivar was harvested 9 kgs of green leaf consisting of two leaves and a bud. The total plucking duration was 2 hours. The leaf was divided into three equal portions. One portion was subjected to chemical wither only (5, 11) and the final wither was 75%. The other two portions were subjected to combined slow physical and chemical wither by passing a steam of ambient temperature air to a final wither of 64% (5, 11). One portion was processed at this degree of physical wither and was classified as hard physically withered leaf. The third portion was rehydrated with surface moisture to attain moisture content equivalent to a physical wither of 75%. The portion was classified as rehydrated hard physical withered leaf. From each portion, withering was terminated by macerating one fifth of the leaf after 14, 22, 30, 38 and 44 hours from the time plucking was ended in the field. The leaf was miniature CTC-macerated and fermented for 90 minutes before firing using bench top fluid bed dryer (TeaCraft, UK). The unsorted black teas were subjected to chemical analyses and sensory evaluation. Processing was done on three separate occasions and each occasion was used as a replicate.

Chemical Analysis and Sensory Evaluation

Total theaflavins were determined by the Flavognost method (34) while thearubigins, liquor colour and brightness were determined as described by Roberts and Smith (35). Experienced professional tea tasters, at two tea broking firms in Mombasa, evaluated the black teas. The sensory evaluation was based on brightness, strength, colour, body and infusion, in a scale of 0-20 and 0-10 for each attribute by taster A and B, respectively. The results were subjected to analysis of variance using the MSTATC statistical package as a factorial three trial in a randomised complete block design. Clones were the main treatments, withering technique the sub-treatment and withering duration the sub-sub-treatment.

RESULTS AND DISCUSSION

Changes in plain black tea quality parameters and sensory evaluation due to clones, withering technique and withering durations are presented in Tables 1-3. Theaflavins, thearubigins, brightness and sensory evaluations levels varied (pd"0.05) with clones. Plain black tea quality parameters variations with clones in general (27, 36) and the two clones used in particular (5, 26) had been observed in previous studies. Such variations were also influenced by processing conditions (37). Although both clones are classified as high quality cultivars (5), TRFK 6/8 had superior plain black tea quality characteristics. The difference in the quality parameters of the two clones suggest, their response to processing conditions may also be different.

In both clones long withering duration lowered (pd"0.05) black tea theaflavins, brightness, and sensory evaluations levels but increased thearubigins and total colour. The patterns in the lowering were similar in the two clones. The observations confirm earlier results that long withering durations reduced plain black tea quality (22). However, the results contrast recent findings (23, 24) in which theaflavins levels did not change due to withering durations. Since the polyphenol oxidase (19-21) and other oxidative enzymes (3) decline with long withering durations or hard withers, the levels of theaflavins should also decline with long withers. Brightness of tea liquors is influenced by theaflavins levels and the two parameters were expected to respond in similar pattern as observed in the study. The thearubigins and total colour on the other hand increased (pd"0.05) with long withering durations. Multiple forms of polyphenol oxidase have been claimed to vary with long withering durations (38). Low molecular weight polyphenol oxidase is transformed to high molecular weight polyphenol oxidase due to long withering duration. The low molecular weight polyphenol oxidases are responsible for hydroxylase activities, which increase levels of some volatile compounds, mainly bound C_6 and terpene alcohols, while high molecular weight forms possess catechol oxidase activity (38). Thearubigins and colour of tea infusions are products of oxidised polyphenols, mainly polymeric materials. It is therefore expected that the levels of the two parameters should increase with long withering durations as observed herein.

In processing plain black teas, a minimum of 8 hours chemical wither duration had been determined to be necessary (13). This could be extended to about 20 hours beyond which quality declines under Kenya tea processing conditions (10, 22). In Iran (23) 16 hours of withering was determined to be optimal based on theaflavins formation. The results herein concur with the previous studies as withering beyond 22 hours reduced (pd"0.05) black tea theaflavins, brightness and sensory evaluations but increased thearubigins and total colour, irrespective of clones or withering regime.

The results obtained in the study are useful in the processing of plain black teas. However, in processing flavoury black teas, long withering durations may be desirable. Black tea aroma improve with long withering durations (22). The improvement can be attributed to increase in amino acids levels (39) that transform to volatile flavour compounds (40), increase in glycosidase activity (41) that release bound volatile flavour compounds. These activities increase levels of volatile flavour compounds (2, 42) thereby improving black tea aroma quality.

Increase in the degree of physical wither (moisture loss) causes decline in plain black tea quality (5, 11, 14, 18). Operating conditions in black tea processing including plucking rates, leaf delivery times make it difficult to have defined withering durations. Factory operators overcome this by assuming that withering begins when leaf arrives in the factory. Due to moisture losses on transit, post harvesting and delays in the factory especially in the peak production periods, it is difficult to have uniform withering duration and/or degree. Processed leaf can have large variability in physical withers. Such variability can be overcome in plain black tea by achieving chemical wither only before maceration. Such operation enhances the theaflavins and brightness levels (11, 14, 16). However, achievement of chemical wither only reduces processing speed as rotorvanes cannot effectively handle bulky leaf that is not flaccid (10-12). This reduction in rotorvane speed requires new technological innovations to overcome.

In previous studies, rehydration restored the activities of oxidative enzymes in tea leaves (3, 21). In this study the plain tea quality from leaf that had undergone hard physical withers then rehydrated to equivalent moisture content of chemically withered leaf were compared with quality of leaf from hard physical wither and chemical wither. This tested if surface moisture rehydration prior to maceration restores oxidative enzymes activities lost through hard physical withers and long withering durations. While chemical withers produced black teas with high (pd"0.05) theaflavins, thearubigins, brightness, and total colour leading to high sensory evaluation scores, hard physical withers reduced the parameters (Tables 1-3). The lost quality parameters were restored by rehydration. Although the levels were slightly lower than chemically withered leaf, the differences were not significant. The results demonstrate that irrespective of clones, the quality decline due to hard physical wither can be reversed by adding surface moisture equivalent to that of chemical withered leaf prior to maceration.

Further advantage can be obtained from rehydration. The hard physically withered leaf is flaccid and easy to macerate. Thus, the problems of slow maceration can be overcome. However, such rehydration increases energy consumption as moisture is removed two times, during withering and firing (drying).

Despite the rehydration, quality losses occasioned by long withering durations (10, 16, 23, 24, 43) were not restored by rehydration. This could have been due to changes in the multiple forms of polyphenol oxidase (38) or deactivation of the enzyme (21) that were not reversed. There were no significant interaction effects between clones, withering techniques and withering durations. Thus, changes caused by variations in these parameters occurred in all cultivars in the same patterns.

ACKNOWLEDGEMENT

We thank the staff of the miniature processing unit and biochemistry section of the Tea Research Institute (Kenya) for technical services.

Table 1: Changes in plain black tea theaflavins and thearubigins due to rehydration of hard withered tea leaf and withering duration

Item	Clone	Wither	Withering duration (hours					Mean wither	Mean clone
			14	22	30	38	44		
Theaflavins (μmol/g)	6/8	Chemical	20.94	25.01	23.15	22.23	21.24	22.51	
		Slow hard physical	19.17	21.49	19.78	18.77	16.97	19.24	
		Rehydrate hard physical	22.84	22.81	22.49	23.10	17.49	21.75	
		Mean withering time	20.98	23.11	21.81	21.37	18.57		21.17
		CV (%)			11.76				
		LSD (P=0.05)			3.26			2.91	
	31/8	Chemical	21.62	22.91	21.95	20.28	18.11	20.97	
		Slow hard physical	20.03	20.00	18.33	17.71	16.33	18.48	
		Rehydrate hard physical	22.39	21.01	21.14	18.92	17.29	20.15	
		Mean withering time	21.35	21.31	20.47	18.97	17.24		19.87
		CV (%)			7.92				
		LSD (P=0.05)			2.05			2.47	
	All clones	Chemical	21.28	23.96	22.55	21.26	19.68	21.74	
		Slow hard physical	19.60	20.75	19.06	18.24	16.65	18.86	
		Rehydrate hard physical	22.62	21.91	21.82	21.01	17.39	20.95	
		Withering time	21.17	22.21	21.14	20.17	17.91		
		CV (%)			13.79				
		LSD, (P=0.05)			2.61			1.89	NS
Thearubigins (%)	6/8	Chemical	16.54	17.45	17.64	18.47	19.41	17.90	
		Slow hard physical	15.52	16.10	16.37	17.99	18.10	16.82	
		Rehydrate hard physical	16.22	18.30	17.62	18.39	18.64	17.83	
		Mean withering time	16.09	17.28	17.21	18.28	18.72		17.52
		CV (%)			6.14				
		LSD (P=0.05)			1.41			NS	
	31/8	Chemical	16.95	18.11	18.50	19.38	20.55	18.70	
		Slow hard physical	15.56	17.25	16.91	17.59	18.73	17.21	
		Rehydrate hard physical	17.41	17.22	17.76	19.29	20.70	18.48	
		Mean withering time	16.64	17.53	17.72	18.75	19.99		18.06
		CV (%)			6.06				
		LSD (P=0.05)			1.43			1.23	
	All clones	Chemical	16.24	17.11	17.44	18.69	19.33	17.76	
		Slow hard physical	15.89	17.78	17.27	17.99	18.69	17.52	
		Rehydrate hard physical	16.75	17.25	17.49	18.79	19.71	18.00	
		Withering time	16.29	17.38	17.40	18.49	19.24		
		CV (%)			6.39				
		LSD, (P=0.05)			0.98			NS	1.03

Table 2: Changes in plain black tea total colour and brightness due to rehydration of hard withered tea leaf and withering duration

n	Clone	Wither	Withering duration (hours) 14	22	30	38	44	Mean wither	Mean clone
	6/8	Chemical	4.66	4.75	5.02	5.11	5.30	4.97	
		Slow hard physical	4.36	4.57	4.74	4.99	5.01	4.73	
		Rehydrate hard physical	4.46	5.17	5.24	5.43	5.36	5.13	
		Mean withering time	4.49	4.83	5.00	5.18	5.22		4.95
		CV (%)			10.06				
		LSD (P=0.05)			0.65			NS	
	31/8	Chemical	4.98	5.45	5.26	5.44	5.90	5.41	
		Slow hard physical	4.81	4.83	4.73	4.69	4.91	4.79	
tal colour (%)		Rehydrate hard physical	4.92	4.96	5.65	5.46	5.16	5.23	
		Mean withering time	4.90	5.08	5.21	5.20	5.32		5.11
		CV (%)			9.98				
		LSD (P=0.05)			NS			NS	
	All clones	Chemical	4.82	5.10	5.14	5.28	5.60	5.19	
		Slow hard physical	4.59	4.70	4.74	4.84	4.96	4.76	
		Rehydrate hard physical	4.69	5.07	5.45	5.45	5.26	5.18	
		Withering time	4.70	4.96	5.11	5.19	5.27		
		CV (%)			11.72				
		LSD, (P=0.05)			NS			NS	NS
	6/8	Chemical	34.18	31.56	32.19	28.35	25.56	30.37	
		Slow hard physical	32.09	30.39	27.28	26.49	21.08	27.47	
		Rehydrate hard physical	33.51	30.59	27.90	26.50	23.17	28.33	
		Mean withering time	33.26	30.85	29.12	27.11	23.27		28.72
		CV (%)			10.01				
		LSD (P=0.05)			3.76			1.15	
	31/8	Chemical	29.19	25.79	25.14	21.78	21.12	24.60	
		Slow hard physical	27.55	25.77	23.09	21.15	17.14	22.94	
·ightness (%)		Rehydrate hard physical	29.02	25.90	24.07	22.83	18.56	24.08	
		Mean withering time	28.59	25.82	24.10	21.92	18.94		23.87
		CV (%)			11.71				
		LSD (P=0.05)			3.66			1.03	
	All clones	Chemical	31.69	28.68	28.67	25.07	23.34	27.49	
		Slow hard physical	29.82	28.08	25.19	23.82	19.11	25.20	
		Rehydrate hard physical	31.27	28.25	25.99	24.67	20.87	26.21	
		Withering time	30.92	28.33	26.61	24.52	21.11		
		CV (%)			12.63				
		LSD, (P=0.05)			3.12			1.01	3.01

Table 3: Changes in plain black tea total sensory evaluation due to rehydration of hard withered tea leaf and withering duration

Item	Clone	Wither	Withering duration (Hours)					Mean wither	Mean clone
			14	22	30	38	44		
	6/8	Chemical	86	90	80	71	59	77	
		Slow hard physical	78	67	65	50	41	60	
		Rehydrate hard physical	81	83	70	65	60	72	
		Mean withering time	82	80	72	62	57		70
		CV (%)			17.54				
		LSD (P=0.05)			19			12	
	31/8	Chemical	69	70	63	57	45	61	
		Slow hard physical	40	41	40	22	13	31	
Taster A		Rehydrate hard physical	59	50	45	30	19	41	
		Mean withering time	56	54	49	36	26		44
		CV (%)			19.66				
		LSD (P=0.05)			12			14	
	All clones	Chemical	78	80	72	64	52	69	
		Slow hard physical	59	54	53	36	27	46	
		Rehydrate hard physical	70	67	58	48	40	56	
		Mean withering time	69	67	61	49	40		
		CV (%)			14.14				
		LSD, (P=0.05)			12			11	26
	6/8	Chemical	20	20	19	20	19	20	
		Slow hard physical	19	19	18	18	17	18	
		Rehydrate hard physical	20	20	20	18	17	19	
		Mean withering time	20	20	19	19	18		19
		CV (%)			5.52				
		LSD (P=0.05)			1			1	
	31/8	Chemical	21	21	21	19	19	20	
		Slow hard physical	20	19	18	17	17	18	
Taster B		Rehydrate hard physical	20	20	19	20	18	19	
		Mean withering time	20	20	19	19	18		19
		CV (%)			5.88				
		LSD (P=0.05)			1			2	
	All clones	Chemical	21	21	20	20	19	20	
		Slow hard physical	20	19	18	18	17	18	
		Rehydrate hard physical	20	20	20	19	18	19	
		Mean withering time	20	20	19	19	18		
		CV (%)			6.46				
		LSD, (P=0.05)			1			1	NS

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