RESEARCH ARTICLE

Seasonal Variations in Catechins and Caffeine Profiles among Tea Cultivars Grown in Kenya

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Abstract

Consumption of beverage tea rich in flavonoids is associated with a wide range of health benefits. However, the industry in Kenya has over-relied on black tea whose sustainability is not guaranteed due to glut in the market resulting in low income to farmers. Product characteristics and prices are determined based on the accumulation of the phenolic compounds that are profoundly influenced by environmental and cultivar differences. Profiles of quality-related components were assessed in six new and existing cultivars over three distinct annual seasons. Weather data indicated the wet-cold (April–August) season received the largest amount of precipitation and highest relative humidity, while the hot-dry season (January to March) was the driest with a lower relative humidity. Total catechin (TC) contents were significantly ($P \leq 0.05$) different among the cultivars, seasons, and interactions season x site and cultivar x season x site. Overall, the purple-leafed cultivars had lower TC content compared to the green-leafed teas, whereas teas obtained during the WW season (September–December) had higher levels (14.3%) that are suitable for processing of high-quality tea products. Esterified catechins, EGCG and ECG, formed the major component among individual catechins studied. EGCG was significantly higher during both the CW and WW seasons. Cultivars of 'China type' accumulated higher +C content as compared to the 'Assam type'. EC was highest during the WW season in Timbilil, while in Kangaita, the CW season had slightly higher content. Generally, the Cambod type cv. TRFK 301/1 had the highest caffeine content, while China type cv. TRFK 73/1 had the lowest content. Unlike catechin, caffeine content was highest during the HD season. This information is crucial for interventions on product diversification, value addition and novel marketing strategies in tea.

Keywords: Flavonoids, Germplasm, Weather.

International Journal of Tea Science (2019); DOI: 10.20425/ijts1418

Introduction

In recent years, world tea [*Camellia sinensis* (L.) O. Kuntze] production and consumption has steadily risen to over 5 million ton/year.[1] Increase popularity is attributed to the taste, aroma and enormous health benefits associated with the beverage.[2] Cultivated tea are classified into three main taxa namely *C. sinensis* var. sinensis (China type), *C. sinensis* var. assamica (Assam type) and *C. sinensis* var. Assamica ssp Lasiocalyx (Cambod type) that are differentiated by their morphological, biochemical and molecular affinities.[3-6] Phenolic compounds contribute significantly to the quality (appearance, taste and flavor) of food products as well as to the health properties.[7,8] In tea, the quality of the beverage is determined by the accumulation of flavan-3-ols (catechins), comprising 70–80% of the tea polyphenols found in the young leaves of the plant.[9] These include (–)-epigallocatechin gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin gallate (ECG), (–)-gallocatechin (GC), (–)-epicatechin (EC), and (+)-catechin (C).[10,11] Consumption of tea beverage rich in catechins is associated with a wide range of health benefits.[12]

Tea is an evergreen tree species that is regulary pruned to a bush of about 0.9 to 1.25 m to ease plucking of apical bud and the associated two leaves used for commercial production of the beverage [13]. The crop is a major source of foreign earnings to most producing countries [1,14]. Beverage tea can be classified based on the processing procedure with green tea being unfermented, oolong tea is semi-fermented, black tea is fermented and pu-erh tea is post fermented [15]. For instance, Kenya earned over USD 1.36 billion from about 443,000 metric tons of processed tea in 2015, representing 4% gross domestic product (GDP) [1]. Kenya is the leading producer of black tea which is largely sold in bulk with little value addition and product diversification. This has caused a glut in the market with the price of proceeded tea stagnating or decreasing. In the recent past however, naturally occurring purple pigmented teas have been developed. Cultivar 'Zijuan' [16] and cv. 'Ziyan' [17] in China, cv. 'Sunrouge' in Japan,[18] and cv. 'TRFK 306' in Kenya[12] are among the commercialized germplasms. Unlike the green-leafed cultivar (Figure 1a) that is rich in catechins (Figure 1a), the purple-leafed cultivar (Figure 1b) is rich in anthocyanins known for enhanced health properties.[12] Anthocyanins are a group of water soluble pigments found in many plant species.[17] The phenylpropanoid pathway, and more specifically the flavonoid pathway, that is responsible for the accumulation of the flavonoid compounds such as the flavonols, the condensed tannins, flavan 3-ols (catechins) and anthocyanins is well characterized in tea.[19,20]

Since the introduction of tea in Kenya in the early 1900s [14], cultivation has widely spread to the east and west of the Great Rift Valley [21]. These areas receive well-distributed annual rainfall of above 1400 mm and mean air temperature of 13-30°C [22]. Accumulation of quality related compounds in plants is profoundly influenced by changes in environmental parameters and cultivars

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How to cite this article: Cheptot, L., Maritim, T., Korir, R., Kipsura, E., Kamunya, S., Matasyoh, L., Muoki, R. Seasonal Variations in Catechins and Caffeine Profiles among Tea Cultivars Grown in Kenya. International Journal of Tea Science 2019; 14(1):56-61

Source of support: Nil

Conflict of interest: None

Figures 1A and B: Illustration of (A) green- and (B) purple-leafed tea cultivars

which ultimately affects product characteristics and quality [9,23-25]. Annual weather patterns in the regions are generally grouped into three distinct seasons: the main dry season from mid-December to end of March; cool wet season from April to August; and a warm wet or warm dry season from September to mid-December [26]. To overcome the market glut, the industry needs appropriate interventions that will contribute to expansion of market outlets. Such interventions can be driven by product diversification, value addition and novel marketing strategies. Continuous studies on the effects of seasonal weather changes on new and existing cultivars are crucial if we are to improve tea quality and maximize on product diversification.

Materials and Methods

Study Site and Cultivars

Two experimental fields growing in different agro-ecological tea-growing zones at Tea Research Institute: Timbilil centre, Kericho county west of the Rift Valley (altitude 2178m asl, 0°22´S, 35°21´ E) and Kangaita centre, Kirinyaga County east of the Rift Valley (altitude 2020m asl, 0°26´S, 37°15´E) were used in the study. Six mature (over 15 years old) tea cultivars representing both green- and purple-leafed tea cultivars planted at 1.52m x 0.762m spacing were selected for the study in 2014 (Table 1). The trial was planted in a completely randomized block design with three replications and maintained as per the recommended agronomic practices. Weather stations situated within the two sites were used to collect environmental parameters during the study period.

Biochemical analysis

Catechins were analyzed using high-performance liquid chromatography (HPLC) method as described by Kerio *et al.*[12] The reference standards (Gallic acid (GA), epigallocatechin (EGC), (+) catechin, epicatechin (EC), epigallocatechin gallate (EGCG), caffeine and epicatechingallate (ECG) were obtained from Sigma–Aldrich (St Louis, MI, USA). Individual catechins were identified by comparing the retention times of unknown peaks with those identified from the peaks obtained from the mixed catechin standards (Graph 1). Catechin quantification was done using a caffeine calibration curve together with the consensus relative response factors (RRFs), with respect to caffeine, calculated on a dry matter basis. The total catechin content of the teas as a percentage mass on a sample dry matter basis was determined by the summation of individual catechins as follows:

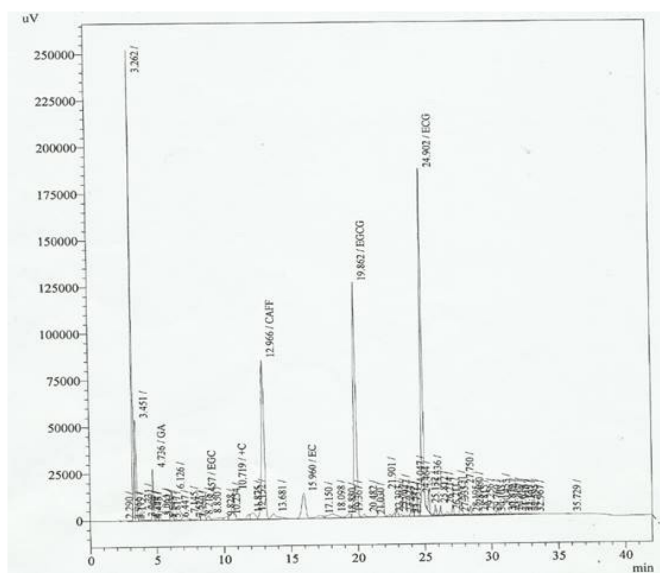
% Total catechins = (%EGC +%C + %EC + %EGCG + %ECG).

Table 1: List of selected green- and purple-leafed tea cultivars used in the study

S/No.	*Cultivar*	*Leaf color*	*Variety*
1.	TRFK 73/1	Purple	China
2.	TRFK 306/4	Purple	Assam
3.	GW Ejulu-L	Green	China
4.	TRFK 31/8	Green	Assam
5.	TRFK 301/1	Green	Cambod
6.	TRFK K-Purple	Purple	China

Statistical analysis

Data were analyzed using GENSTAT software Version 15 (VSN International Ltd., UK) and subjected to a factorial analysis of variance (ANOVA) to test for the effects of cultivar, season, site and their interaction on phenolic content. Means were separated by Duncan Multiple Range Test (DMRT).



Graph 1: HPLC chromatogram of catechin fractions on the green-leafed cv. GW Ejulu-L

Results and discussion

Weather parameter

In general, the wet-cold season received the largest amount of precipitation and had the highest relative humidity, while the hot-dry season (January to March) was the driest season with a lower relative humidity (Table 2). Cumulative precipitation in the hot-dry season, was higher in Timbilil (221 mm) as compared to Kangaita (150 mm) site, while maximum air temperature was above 25°C in Timbilil as compared to cooler temperatures in Kangaita (Table 2). Lower minimum temperature (9.0°C) was experienced in Timbilil as compared to Kangaita (10°C) leading to higher diurnal variation and lower relative humidity in Timbilil (Table 2). Similarly, precipitation was higher in the cold-wet season at Timbilil (1218 mm) as compared to Kangaita (923.0 mm) over the cold-wet months, beginning April to August (Table 2). Both sites experienced increased precipitation and temperatures, while relative humidity decreased in the warm-wet season, from September to December.

Seasonal changes in total catechin

Total catechin (TC) contents which constitutes 10–25% of the total dry matter of the leaf was significantly ($P \leq 0.05$) different among the cultivars, seasons, and interactions season x site and cultivar x season x site (Table 3). However, no significant difference was observed between the two sites. Generally, TC was significantly higher in cv. GW Ejulu-L (13.8%), a China type of cultivar known for premium black tea quality (Table 3).[12,27] Further, it is noteworthy that green-leafed teas comprising a Cambod type cv. TRFK 301/1 and Assam type cv. TRFK 31/8, and a purple-leafed China type cv. TRFK 73/1 had comparable TC content to the best cultivar. Tea cultivars with high catechin content are potential raw material for commercial extraction of functional components. The variations in catechin content among cultivars with seasons can be attributed to differences in respective pathway regulation.[3] Studies have shown that phenylalanine ammonialyase (PAL), flavonoid 3, 5-hydroxylase (F3-5H) and dihydroflavonol 4-reductase (DFR) are positively correlated with catechin concentrations in tea shoots harvested during spring and autumn, whereas flavanone 3-hydroxylase and anthocyanidin synthase are downregulated in autumn.[28] Overall, the purple-leafed cultivars had lower TC content compared to the green-leafed teas, confirming previous reports.[12] Teas obtained during the WW season (September-December) had higher TC levels (14.3%) giving great potential for processing of high-quality tea products as well as extraction of natural catechins for industrial application. The HD season, characterized by drought and frost stress periods, had the lowest TC content. It is clearly evident that the accumulation of catechins in the tender shoots of the plant is significantly affected when the plant is under stress.[29]

Seasonal variation in esterified catechins

Individual catechins were identified by comparing the retention times of sample peaks with those obtained from mixed catechin standards. Esterified catechins, EGCG and ECG are derived from non-esterified catechins during flavan-3-ols biosynthesis, a process catalyzed by flavan-3-ol gallate synthase.[28] They formed the major component among individual catechins studied. The catechins have unique bioavailability, bioactivity and physiological pharmacokinetics attributed to their chemical structures.[30] EGCG was significantly higher during both the CW and WW seasons (Table 3). Studies have reported significant increase in EGCG during the warm season attributing it to stronger sunlight and increased sunshine hours during the warmer months.[31] Cultivar TRFK 73/1 (5.7%) had the highest EGCG content as compared to cv. TRFK K-purple (3.2%) that had the least, both purple-leafed. Notably, green-leafed cultivars TRFK 31/8 and GW Ejulu-L had comparable yields to TRFK 73/1. The esterified catechin ECG was highest in GW Ejulu-L (3.9%) (Table 3). However, no season, site or interactions were significant.

Seasonal variation in non-esterified catechins

The catechins comprising +C, EGC and EC are synthesized through stepwise reactions catalyzed by leucoanthocyanidin 4-reductase (LAR), anthocyanidin synthase (ANS), and anthocyanidin reductase (ANR) in the flavonoid pathway.[32] Simple catechin (+C) content varied significantly ($P \leq 0.05$) between cultivar, across seasons and interaction between cultivar and season. Cultivar TRFK K-purple had the highest +C content though comparable to cv. GW Ejulu-L (Table 3). The findings suggest that varieties of 'China type' accumulated higher +C content as compared to the 'Assam type'. This is contrary to previous reports where 'Assam type' was reported to have higher levels compared to Chinary tea as the later contain more quercetin and Kaemferol-3-glucoside compounds.[33,34] Both EC and EGC have 3', 4'-dihydroxyl groups in the B-ring with EC having H^+ compared to OH^- in EGC at position 5' in the B-ring.[29] The configuration of the hydroxyl group in the B-ring is associated with potency of free radical scavenging. EC was significantly accumulated during the WW season in Timbilil, while in Kangaita, the CW season showed slightly higher content (Table 3). Generally, the Cambod type cv. TRFK 301/1 showed significantly higher levels. Based on the seasonal mean, EGC content was significantly high ($P \leq 0.05$) in the commercial Assam type cv. TRFK 31/8 though comparable to China type cv. TRFK 73/1 with the WW season having highest content (Table 3). Similar results have been observed previously.[11,35,36] Pigmented cv.TRFK K-purple recorded the lowest EGC content across sites.

Seasonal variation in caffeine content

Caffeine, a primary-secondary metabolite responsible for the stimulating effect and bitter taste properties of tea, showed significant variation across cultivars and seasons (Table 3). Based on the overall clonal means, Cambod type cv. TRFK 301/1 had the highest caffeine content, while China type cv. TRFK 73/1 had the lowest content across the two sites. Unlike catechin, caffeine content was high during the HD season (Table 3). Similar results observed in previous studies attributed the high content during the dry period to hydroxylation of *p*-ce toumaric acid to caffeic

Table 2: Seasonal weather parameter recorded at Timbilil and Kangaita sites

	Precipitation (mm)		*Temp max (°C)*		*Temp min (°C)*		*Relative humidity (%)*	
Season	*Timbilil*	*Kangaita*	*Timbilil*	*Kangaita*	*Timbilil*	*Kangaita*	*Timbilil*	*Kangaita*
H-D	221	150	25.8	22.6	9.0	10.0	50.7	70.7
C-W	1218	1130	23.3	19.0	9.2	11.5	70.7	81.9
W-W	793	721	23.5	20.6	9.3	10.8	68.9	76.5

Table 3: Quality components of selected green- and purple-leafed tea cultivars

Cultivar	*Season*	*TC*		*EGCG*		*EGC*		*ECG*		*EC*		*C*		*GA*		*CAFF*	
		Timb	*Kang*	*Timb*	*Kang*	*Timb*	*Kang*	*Timb*	*Kang*	*Timb*	*Kang*	*Timb*	*Kang*	*Timb*	*Kang*	*Timb*	*Kang*
TRFK 73/1	H-D	8.28	10.99	3.42	4.24	2.02	3.89	1.87	1.45	0.97	0.51	0.26	0.55	0.34	0.50	2.05	1.49
	C-W	10.97	13.30	6.08	6.50	1.72	1.33	1.45	2.23	1.53	2.63	0.18	0.63	0.51	0.19	0.72	1.64
	W-W	20.53	14.05	8.84	5.10	6.33	5.15	2.48	1.61	2.12	1.55	0.77	0.65	0.40	0.50	2.32	1.80
TRFK 306/4	H-D	8.61	9.42	3.01	2.25	1.32	2.32	2.46	2.41	1.38	2.08	0.45	0.35	0.51	0.46	1.07	2.23
	C-W	13.34	10.76	5.98	4.33	2.21	2.57	2.89	1.49	1.64	2.11	0.63	0.27	0.36	0.31	2.40	1.71
	W-W	14.40	11.10	4.60	2.92	2.08	2.47	5.33	2.03	1.14	2.25	1.25	1.43	0.76	0.49	1.98	2.08
GW-Ejulu	H-D	13.70	13.69	4.84	4.25	2.18	2.51	4.60	4.39	0.83	1.35	1.25	1.20	0.62	0.34	3.32	2.75
	C-W	12.17	15.00	5.23	7.68	2.07	2.40	2.97	3.15	1.20	1.23	0.71	0.31	0.31	0.49	2.11	1.37
	W-W	13.73	14.37	3.12	4.83	2.96	2.41	4.51	3.80	1.65	1.89	1.51	1.45	0.44	0.28	2.47	2.06
TRFK 31/8	H-D	11.80	10.04	5.75	4.56	3.43	2.49	1.57	1.64	0.84	1.18	0.21	0.18	0.37	0.24	3.47	1.76
	C-W	14.30	9.63	5.57	3.41	3.82	2.79	3.03	1.77	1.13	1.16	0.75	0.49	0.29	0.23	2.28	1.65
	W-W	14.65	12.56	5.83	5.34	5.95	4.91	1.65	1.03	1.00	0.75	0.24	0.52	0.24	0.42	2.53	1.97
TRFK 301/1	H-D	10.46	13.57	2.35	3.52	3.17	3.51	2.89	2.78	1.49	3.12	0.56	0.63	0.30	0.30	4.35	3.05
	C-W	9.72	15.97	2.16	3.89	1.88	2.34	2.77	3.87	2.43	5.43	0.49	0.44	0.17	0.21	1.41	2.40
	W-W	19.29	12.05	4.25	2.95	3.55	1.55	5.11	3.06	5.42	3.91	0.96	0.58	0.25	0.27	2.81	3.22
TRFK K-purple	H-D	6.08	7.64	1.17	1.84	0.97	1.27	2.38	2.56	0.58	1.17	0.98	0.81	0.35	0.26	2.57	1.80
	C-W	12.91	10.57	4.50	3.04	2.01	1.19	3.67	4.35	0.32	0.90	1.34	1.10	0.17	0.35	2.05	2.25
	W-W	13.82	11.29	4.67	3.67	2.06	1.29	4.19	4.09	1.62	1.17	1.28	1.06	0.49	0.43	2.33	2.42
Overall means																	
Cultivars	TRFK 73/1	13.02		5.70		3.41		1.85		1.55		0.51		0.41		1.67	
	TRFK 306/4	11.27		3.85		2.16		2.77		1.77		0.73		0.48		1.91	
	GW-Ejulu	13.78		4.99		2.42		3.90		1.36		1.07		0.41		2.34	
	TRFK 31/8	12.16		5.08		3.90		1.78		1.01		0.40		0.30		2.27	
	TRFK 301/1	13.51		3.19		2.67		3.41		3.63		0.61		0.25		2.87	
	TRFK K-purple	10.38		3.15		1.47		3.54		0.96		1.09		0.34		2.23	
Seasons	H-D	10.35		3.43		2.42		2.42		1.29		0.62		0.38		2.49	
	C-W	12.39		4.86		2.20		2.20		1.81		0.61		0.30		1.83	
	W-W	14.32		4.68		3.39		3.39		2.04		0.97		0.41		2.33	
Site	Timbilil	12.71		4.52		2.76		3.10		1.51		0.77		0.38		2.34	
	Kangaita	12.00		4.13		2.58		2.65		1.91		0.70		0.35		2.09	
LSD (P≤0.05)	Clone (C)	1.81		1.25		0.71		0.89		0.55		0.27		0.14		0.52	
	Season (Ss)	1.28		0.89		0.5		NS		0.39		0.19		0.08		0.37	
	Site (St)	NS		NS		NS		NS		0.32		NS		NS		NS	
	Interaction (CSs)	NS		NS		1.22		NS		0.95		0.47		NS		NS	
	Interaction (CSt)	NS		NS		NS		NS		NS		NS		NS		NS	
	Interaction (SsSt)	1.81		NS		0.71		NS		0.55		NS		NS		NS	
	Interaction (CSsSt)	4.44		NS		NS		NS		1.35		NS		NS		NS	

H-D, Hot and Dry; C-W, Cool and Wet; W-W, Warm and Wet; Timb, Timbilil; Kang, Kangaita; GA, Gallic acid; Caff, Caffeine; C, Catechin; EC, Epicatechin; ECG, Epicatechin gallate; EGC, Epigallocatechin; EGCG, Epigallocatechin Gallate; TC, Total Catechin;*, **,significance level at $p \leq 0.05$ and $p < 0.01$, respectively; NS, No Significance acid in the phenyl-propanoid pathway through the influence of polyphenol oxidase (PPO's) that is induced under water stress condition.[37,38] Since tea plants are exposed to a plethora of abiotic stress during the HD season, the plant responds by accumulating compatible solutes or osmolytes such as proline and caffeine as a defense mechanism.[39]

Conclusion

It is evident that the accumulation of quality related components in different tea germplasms are influenced by seasonal changes in weather. High precipitation accompanied by moderate temperature and relative humidity during the WW season favor the synthesis of catechins in tea. Cultivar and seasonal variations in the accumulation of various compounds would enable selective processing of high quality tea products and extraction of natural compounds for industrial application. Information generated should guide the industry to maximize on product quality for enhanced returns. Molecular studies on the effects of seasons on the biochemical processes for different cultivars are paramount.

Acknowledgment

The authors thank staff of the KALRO-TRI involved for technical support. This paper is published with the permission from the Institute Director.

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