



Changes in Phenolic Compounds due to Processing Black Tea Drink

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ABSTRACT

Black tea samples represented three commercial varieties from India, Kenya, and Malawi were selected and chemically evaluated. Chemical characteristics such as moisture, ash, total fibers and caffeine content within the acceptable limits. Moreover, phenol compounds and flavonoids content in these samples and their drinks were determined. The results indicated that phenol compounds varied according to the origin as well as preparation methods of black tea drinks. The amounts of some phenol compounds in black drinks augmented by extended the period and temperature of processing. The highest total phenol compounds content was found in the treatment 2 (T_2). Two grams of dust black tea was added to 100ml distilled hot water and then boiling continued for 2 minutes. In all varieties of black tea drinks, and ranged 4062.63 to 5904.87mg/100g for Malawian and Kenyan black tea drinks. Total flavonoids content among black tea samples ranged 73.40 to 553.14mg/100g) before treatments. However, the highest flavonoids content of black tea drinks was found in the treatment 2 (T_2) in all varieties of black tea drinks, and ranged from 612.41 to 1312.72mg/100g) for Malawian and Indian black tea drinks.

The study was conducted in the treatment 2 (T_2) given the highest phenols and flavonoids content of three varieties black tea drinks.

Keywords: Phenol compounds, flavonoids, caffeine, salicylic acid, apiginin.

INTRODUCTION

Tea currently is the hot topic in both nutritional and therapeutic research worldwide depending on the chemical constituents present and the most preferred drink in Egypt. It is an affordable beverage such as soft drinks. The quality of tea is a function of several compounds, as presence and percentage. These components are phenolic compounds,

during processing reactions are done to form the compounds of pleasant taste and aroma, although separately each has unpleasant taste (*Owuor et al.*, 1987).

Tea is prepared in different ways specially in rural areas and low socioeconomic communities. It is prepared by boiling in water for a period of time. This technique allows the majority of phenolic compounds to be present in the drink. Total polyphenolics content in tea includes catechins, epicatechins, etc.

Polyphenols present in tea are the good source of antioxidants in plants (*Cabera*, 2005), and have recently received much attention due to their pharmaceutical functions, such as antioxidative, antitumor and anticarcinogenic activities (*Saknaka et al.*, 1989; *Matsuzaki et al.*, 1985; *Conney et al.*, 1992; *Chung K. et al.*, 1998). Tea contains large amounts of flavonoids and phenolic acids which make up to 30% of fresh green tea leaves dry weight and only 10% dry weight of black tea (*Wang et al.*, 2000). Among the three main varieties of commercial tea, Indian, Kenyan and Malawian, the scope of present research is planned to throw light on the effect of different conditions of treatments on phenolic and flavonoid compounds compared with their drinks.

MATERIALS AND METHODS

Collection of samples :

Tea samples of three countries India, Kenya and Malawi were collected randomly from General Organization for Exports and Imports Control in Port-Said and investigated (dust black tea: the most imported tea to Egypt).

Preparation of drinks beverages :

Tea drinks were prepared using three traditional methods for every variety of dust black tea (three varieties) as following :

- a- Two grams of dust black tea was added to 100 ml cooled distilled water, then heated to boil for 2 minutes, treatment 1 (T₁).
- b- Two grams of dust black tea was added to 100 ml distilled hot water and then boiling continued for 2 minutes (T₂).
- c- Two grams of dust black teas was added to 100 ml boiled distilled water (90-95°C) and stand for infusion for 2 minutes (kushary tea) (T₃).

At the end of preparation time, tea extracts were allowed to cool at room temperature, then subjected for analysis.

Methods :

Moisture, total ash, water soluble ash, water insoluble ash, alkalinity of soluble ash, total fibers (Free amino nitrogen, FAN), free amino acids and total acidity (as acetic acid) were measured according to the A.O.A.C (2000).

Total phenolic compounds were determined using Folin- denis reagent as described by *Swain and Hillis* (1959) the concentrations of flavonoids in methanol extract were measured spectrophotometrically at 440 nm according to *Zhisen* (1999).

The pH values of all dust black tea were adjusted by pH meter (Backman pH meter with glass electrode at 25°C as described in A.O.A.C (2000).

Phenolic and flavonoid compounds were determined by HPLC according to the method of *Mattila et al.*, (2000).

RESULTS AND DISCUSSION

Chemical composition of black tea varieties :

The chemical composition of black tea varies depending on their origin and the type of processing in Table (1). Variation in moisture content of black tea samples (2.48- 2.58%) could be related to the level of fermentation, condition of drying and backing, storage period and the technology of processing (*Yamanishi et al.*, 1992). All samples of tea have low content of moisture, which makes them not highly susceptible to microorganisms attack. This might be advantageous in terms of the shelf life of tea samples.

Black tea from Kenya contained the lowest ash content (5.32%) and the highest content of total ash was found in Malawian black tea (6.07%). It was within the range given by *Yamanaish et al.* (1992) and *Salama* (2000).

The average of the total ash content of black tea was around (5.70%) of the dry matter. On the other hand, the water soluble ash in samples of tea was ranged between 3.21 to 3.49% for Malawian and Indian black tea. However, the results from the water insoluble ash content showed that these samples were found to be within the documented range. The levels were 2.83, 2.01- 2.86% for Indian, Kenyan and Malawian respectively. pH value of Indian black tea was found to be slightly acidic than the other black tea samples.

The results indicate that Indian black tea contained the higher levels of total fiber (14.74%) than those of both varieties of black tea samples, which ranged from 10.88 to 11.08%.

Table (1) indicated that total free amino nitrogen (FAN) was present only at low levels. The FAN of Kenyan black tea was higher 0.66% than that of Indian and Malawian black tea which ranged from 0.002 to 0.01%. These results are in agreement with those given by *Nemata* (1996). On the other hand, *Belitz and Grosch* (1987) found that FAN of fermented tea was 7%.

Total polyphenols make up 12.16% and 18.05% of the dry matter content of black and green tea, respectively. The data indicated that Indian black tea contains higher amounts of polyphenolic compounds

(18.05%) than those in black samples. Whereas Kenyan black tea had the lowest amount, which differ depending on manufacturing procedure. The results indicated also little variation in the content of polyphenolic compounds in black tea samples.

Caffeine constitutes (2.75-3.024%) of the dry matter of black tea. It plays an important part in determining the tea taste and briskness of the beverage. The highest amount of caffeine in samples analyzed was found in Indian tea sample while the lowest was found in Kenyan tea samples. The obtained data was in agreement with the results of *Belitz and Grosh* (1987) and *Salama* (2000). The displayed results confirmed that caffeine content depends on the age of tea leaves and process involved in the production, (*Barone and Roberts*, 1996, and *Athayde, M.L. et al.*, 2000)

Table (1) : Chemical composition of black tea varieties (On dry matter basis)

Constituents (%)	Resources of black tea		
	Indian	Kenyan	Malawian
Moisture	2.58	2.48	2.52
Total ash	5.72	5.32	6.07
Water soluble ash	3.49	3.31	3.21
Water insoluble ash	2.83	2.01	2.86
Alkalinity of soluble ash	30.53	52.21	32.92
Total fibers	14.74	10.88	11.08
Free amino nitrogen (FAN)	8.01	0.66	0.002
Total polyphenols	18.05	12.16	12.64
Flavonoids	1.18	1.20	1.07
Caffeine	3.02	2.75	2.79
pH value	4.45	4.50	4.47
Total acidity	1.79	1.99	2.10

Identification of phenolic compounds of black tea samples using HPLC before treatments :

High performance liquid chromatography (HPLC) was used for identification of phenolic compounds in three varieties of black tea samples. The results in table (2) indicated that black tea samples contained fifteen phenolic compounds. The data cleared that the total phenols which measured in the black tea and among the samples analyzed the range was narrow all within and between countries. Indian black tea contained low levels of total phenols (3765.07 mg/100g) while Kenyan could be considered a rich or high source of phenolic compounds (4366.64 mg/100g)

Caffeine was the most predominant phenolic acid with all the samples of tea varieties and were also identifies as antioxidative compounds, representing about 1396.51, 2020.51 and 1895.31mg/100g

in Indian, Kenyan and Malawian black tea samples respectively. Followed by salicylic acid were 399.30, 678.35 and 537.11 mg/g). Indian black tea contained also the highest amounts of ellagic acid, benzoic, coumarin and hydroxytyrosol 370.77, 389.99, 37.57 and 714.94 mg/100g respectively, than those of Kenyan and Malawian black tea.

On the other hand, Kenyan black tea contained the highest amounts of ferulic acid, protocatechoic acid and catechol which represented about 182.07, 67.58 and 144.70 mg/100g. While Malawian black tea contained the highest amounts of vanillic acid, gallic acid, cinnamic, catechin, chlorogenic acid and caffeic acid were 13.01, 201.66, 19.63, 77.46, 515.00 and 70.93 mg/100g respectively.

Table (2) : Fraction of phenolic compounds by HPLC for the samples of black tea (mg/100g).

Phenol compounds	Indian	Kenyan	Malawian
Vanillic acid	10.15	3.49	13.01
Caffeine	1396.51	2020.51	1895.30
Ferulic acid	38.06	182.07	74.37
Salicylic acid	399.30	678.35	537.11
Gallic acid	193.27	113.71	201.66
Cinnamic acid	12.23	Nd	19.63
Ellagic acid	370.77	212.918	263.39
Benzoic acid	389.99	288.96	Nd
Catechin	33.58	16.11	77.46
Protocatechoic	29.05	67.58	28.63
Chlorogenic acid	101.02	26.21	515.00
Caffeic acid	15.82	29.76	70.93
Coumarin	37.57	34.40	37.50
Catechol	52.76	144.70	89.42
Hydroxytyrosol	714.94	547.0	363.55
Total	3765.07	4366.64	4187.00

Generally, the results in Table (2) are in agreement with those reported by (*Balentine, 1997, Hera et al., 1995d and Mukhtar et al. 2000*). They reported that the phenolic acid in tea leaves consist of caffeic acid, benzoic acid, vanillic acid, ferulic acid and catechin. Most of these compounds are widely distributed in nature and have been shown to possess anti-oxidative properties (*Van Gadow et al., 1997*).

Table (3): Fraction of phenolic compounds by HPLC for the samples of black tea drinks (mg/100g)

Phenolic compounds	Indian tea			Kenyan tea			Malawian tea		
	T ₁	T ₂	T ₃	T ₁	T ₂	T ₃	T ₁	T ₂	T ₃
Vanillic	5.54	Nd	0.39	0.12	1.38	0.017	Nd	1.03	Nd
Caffeine	598.27	641.40	489.98	755.20	992.46	259.55	716.41	815.23	522.14
Ferulic	216.16	Nd	Nd	297.68	347.25	Nd	250.41	80.84	78.53
Salicylic	63.99	863.68	498.35	122.24	1167.70	377.28	898.62	795.51	Nd
Gallic	312.23	261.89	255.34	357.43	438.80	146.20	351.99	518.83	187.42
Ellagic	226.96	253.77	Nd	Nd	413.33	149.48	155.46	333.14	216.39
Pyrogallol acid	266.45	323.62	140.16	635.42	396.30	182.32	421.12	678.88	323.25
Benzoic acid	Nd	642.52	233.01	644.49	389.73	Nd	Nd	Nd	339.24
Syringic acid	5.46	0.90	0.93	5.99	2.02	2.47	6.24	13.17	4.74
Catechin	319.40	396.99	310.40	502.13	496.62	277.73	379.33	481.77	231.37
Protocatechoic acid	770.56	759.45	488.12	1073.98	309.05	413.64	626.115	1026.77	524.35
Chlorogenic acid	238.32	495.02	307.47	244.83	562.72	284.60	378.16	500.98	186.10
Caffeic acid	Nd	109.04	77.55	Nd	Nd	Nd	57.15	82.84	Nd
Coumarin	45.93	8.48	5.59	6.22	12.13	2.91	4.55	4.95	Nd
Epicatechin	330.10	339.49	356.44	407.69	295.18.	121.46	Nd	571.26	Nd
P-OH-Benzoic acid	325.73	Nd	155.00	501.60	446.15	Nd	531.26	Nd	178.38
Catechol	151.44	Nd	Nd	298.94	Nd	59.60	383.26	Nd	104.40
Total	3769.43	5717.68	3094.77	5452.96	4062.63	2206.25 7	5107.43	5904.87	2899.27

T₁ : Treatment 1 T₂ : Treatment 2 T₃ : treatment 3 Nd : Not detected

Changes in phenolic compounds of processing black tea drinks :

The results given in Table (3) and figure (1) indicate that in Indian black tea sample after heat processing. The amounts of caffeine were decreased in three treatments comparing their amounts before treatments, whereas caffeine in treatment 2 (T₂) was 641.40 mg/100g more than treatment 1 (T₁) 588.27 mg/100g and treatment 3 (T₃) 489.98 mg.100g. It could be seen that salicylic acid was increased in both (T₂) and (T₃), in (T₂) 863.69 mg/100mg more than (T₃) 498.35 mg/100mg. Followed by benzoic acid which increased in (T₂) 642.52 mg/100mg only, while decreased in other treatments. Similarly, decreases were found in the case of ellagic acid (226.76 and 253.77mg/100mg) in (T₁) and (T₂). We can notice that the amounts of ferulic, catechin, protocatechoic, chologenic and catechol were increased than those in pre-treatments. The increase of phenolic compounds was believed to result from the isomerization of higher temperature treatment (*Seto et al.*, 1997 and *Lee et al.*, 2008). The decreases in total phenolic content were mainly due to the fact that the phenolic compounds in black tea drinks were oxidized or polymerized during heating and processing.

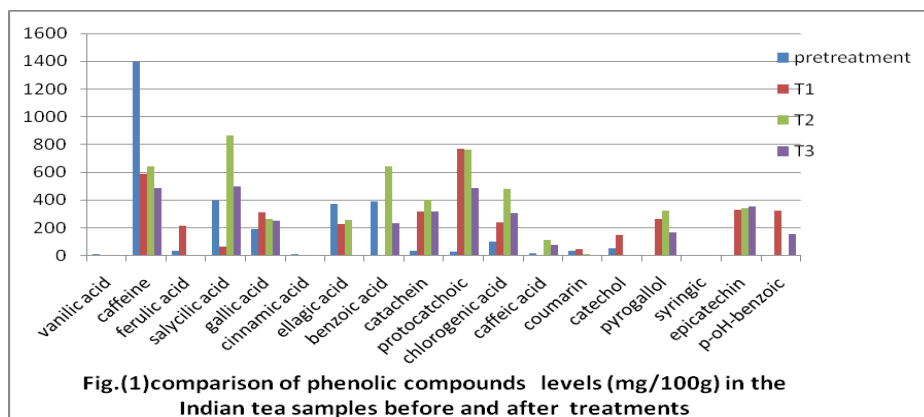


Figure (2) shows the phenolic compounds in Kenyan black tea drinks. After heat processing the amounts of caffeine and salicylic acid were decreased while salicylic acid was increased in (T₂). During processing protocatechoic, catechin, benzoic acid and chlorogenic acid were much higher amounts comparing their original amounts.

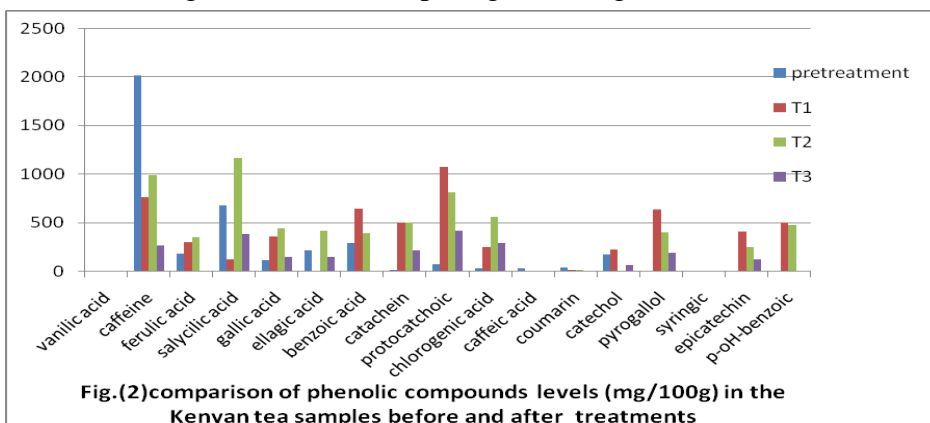


Figure (3) show that decreases of caffeine and chlorogenic acid in all treatments of Malawian black tea drinks, whereas ellagic acid increased in (T₂) and decreased in (T₁) and (T₃). All ferulic acid, salicylic acid and protocatechoic acid were increased in all treatments, while gallic acid was increased in (T₁) and (T₂) but was decreased in (T₃).

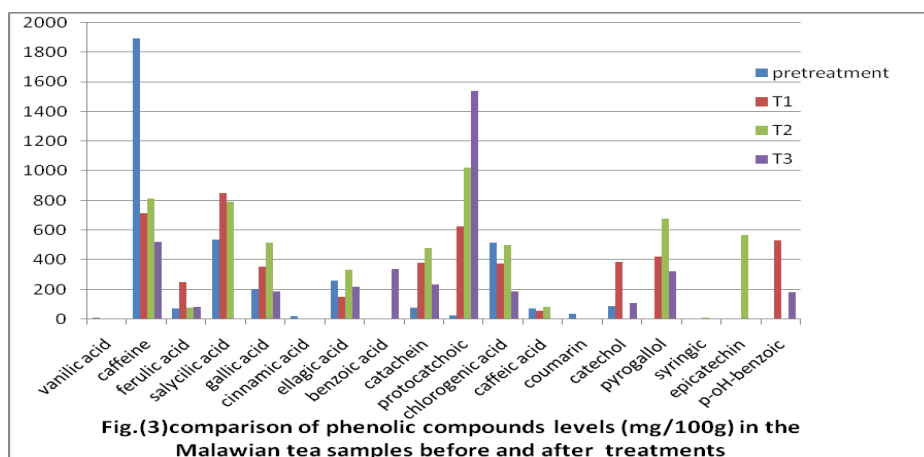


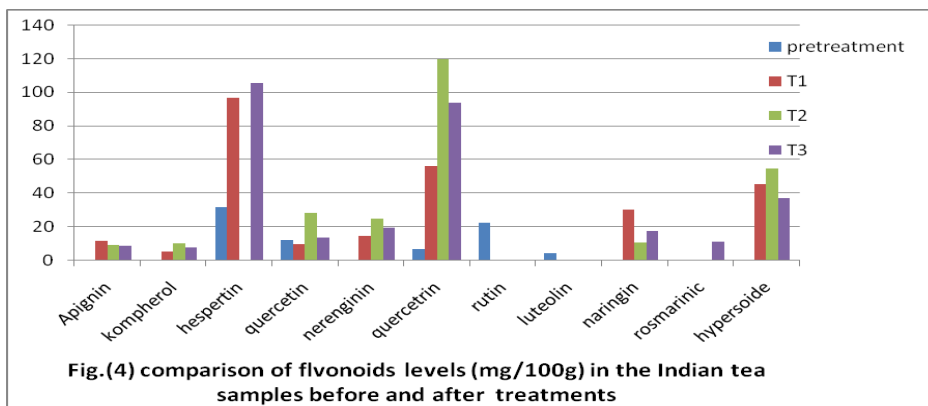
Fig.(3)comparison of phenolic compounds levels (mg/100g) in the Malawian tea samples before and after treatments

Looking into the processing methods of black tea drinks, treatment 2 (T_2) with a much higher phenolic compounds. During (T_2) more phenol compounds could stay in the drink. Therefore, phenolic compounds were judged to be the key elements that determine both colour and taste qualities of tea beverage during processing.

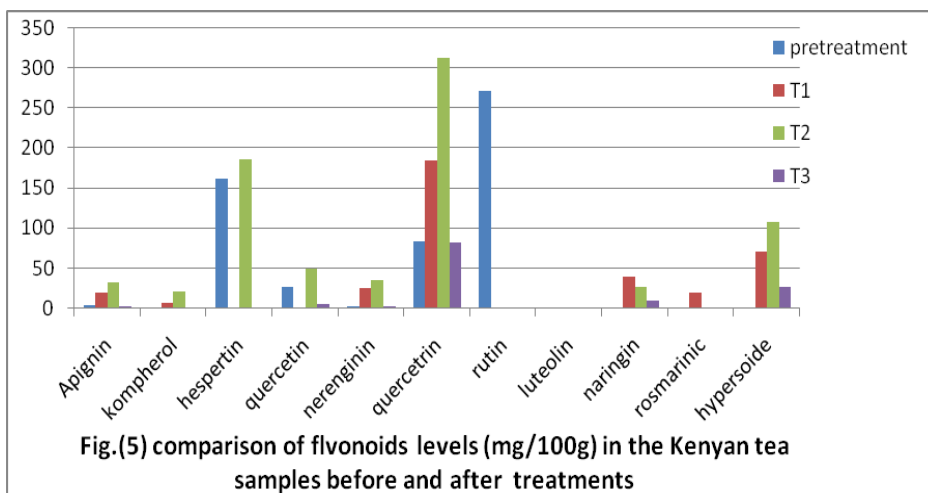
Fraction of flavonoid compounds by HPLC of black tea drinks :

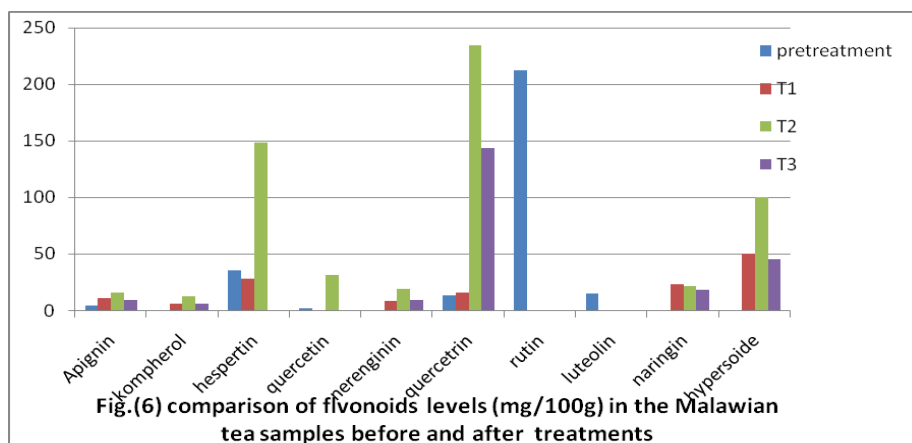
A major source of flavonoids in the Egyptian diet is tea. The most frequently consumed tea are black tea. Flavonoids are claimed to be responsible for most of the positive health effects of tea (Shankar et al., 2007 and Fraser et al., 2007). Eight flavonoids were detected in dust black tea samples. They were identified by comparing their retention times with those of the authentic standards on the HPLC chromatograms. Table (4) shows the quantity of phenolic compounds by mg per 100g. As seen in the results of Kenyan black tea recorded the highest amount of total flavonoids (553.14mg/100g).

After heat processing Table (5) shows the highest quantity of flavonoid compounds were found in all (T_2) of all varieties (1312.72, 984.44 and 612.41mg/100g) Indian, Kenyan and Malawian black tea drinks respectively Fig (4).



The amounts of kampherol and marenginin were increased in all treatments of all varieties, while apiginin increased in all treatment of all varieties except (T₃) in Kenyan black tea drinks was (3.01 mg/100g), in this connection quercetrin was increased in Indian black tea drinks except (T₁) while increased in (T₂) 0.27 mg/100g) only in Kenyan black tea drinks and also in (T₂) (30.98 mg/100g) in Malawian black tea drinks. On the other hand, rutin was not detected in all treatments Fig. (5 and 6).





The results are in agreement with those reported by *Hertog et al.* (1993), who reported that black tea contains substantial concentrations of the flavonoids quercetin, kampherol and myricetin. Flavonoids have been shown to protect against the oxidation of LDL *in vitro*, to inhibit platelet aggregation, to reduce inflammatory process and to improve vascular function (*Duffy et al.*, 2001 and *Leenen et al.*, 2000).

Table (4) Fraction of flavonoid compounds by HPLC for the samples of black tea (mg/100g).

Flavonoid compounds	Resources of black tea		
	Indian	Kenyan	Malawian
Apignin	0.60	4.54	4.41
Kampherol	Nd	Nd	Nd
Hesperten	31.47	162.12	35.57
Quercetin	11.99	26.98	1.5
Narenginin	0.47	2.31	Nd
Quercetrin	6.99	83.70	13.11
Rutin	22.46	271.63	211.89
Luteolin	4.46	1.83	14.77
Total	73.40	553.14	231.13

Nd : Not detected

Table (5): Identification of flavonoid compounds for the samples of black tea drinks (mg/100g)

lavonoid	Indian black tea	Kenyan black tea	Malawian black tea
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compounds	T ₁	T ₂	T ₃	T ₁	T ₂	T ₃	T ₁	T ₂	T ₃
Apiginin	11.67	9.37	8.69	19.80	33.19	3.01	10.58	15.53	8.65
Kampherol	5.19	10.10	7.56	6.53	21.53	Nd	6.14	12.08	5.80
Hesperitin	96.79	Nd	105.48	Nd	186.39	Nd	28.04	148.44	Nd
Quercetin	9.64	23.32	13.43	Nd	50.27	6.12	Nd	30.98	Nd
Narenginin	14.52	25.11	19.55	25.35	36.04	2.36	7.89	18.90	9.11
Quercetrin	56.24	129.36	93.87	185.18	312.85	82.18	15.76	234.58	143.85
Rutin	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Naringin	30.04	10.56	17.72	40.14	27.71	10.15	22.97	21.56	17.99
Rosmarinic	Nd	Nd	11.45	20.05	Nd	Nd	Nd	Nd	Nd
Hisperdin	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Hypersoid	45.21	54.46	36.83	71.79	108.54	27.72	49.76	99.78	45.10
Total	269.3	1312.72	314.63	388.65	984.44	131.54	157.81	612.41	230.5

T₁ : Treatment 1 T₂ : Treatment 2 T₃ : treatment 3 Nd : Not detected

In general, high quality tea is related to their content polyphenol compounds, when black tea drink is boiled in heated, many complex chemical reactions occur and substances of tea began to be degradation. The polyphenol compounds break done into both volatile and non-volatile compounds while are soluble in the water and these compounds contribute to both desirable and undesirable sensory characteristics of the boiled black tea.

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التغيرات التي تحدث للمركبات الفينولية نتيجة إعداد الشاي الأسود

السيد محمود الصعيدي، منال عباس الجندي
معهد بحوث تكنولوجيا الأغذية- مركز البحوث الزراعية- جيزة- مصر

الملخص العربي

استهدف البحث دراسة التغيرات التي تحدث للمركبات الفينولية نتيجة لعمليات إعداد الشاي الأسود من مصادر مختلفة وارادة إلى مصر من الهند وكينيا ومالايو، وشملت الدراسة التقديرات الكيميائية (الرطوبة والرماد والرماد الذائب والغير ذائب في الماء وقلوية الرماد الذائب والألياف والكافيين) واختلفت النتائج فيما بينها وكانت جميعها في حدود المستويات المقبولة. كما أوضحت النتائج محتوى المركبات الفينولية والفلافونويدية، قبل وبعد عمليات الإعداد، وكذلك تفريد والتعرف على تلك المركبات ومدى التغير الذي طرأ عليها، حيث اختلفت تلك المركبات باختلاف المصادر وطرق الإعداد وبعضها ازداد بارتفاع درجة الحرارة وزمن التعرض أثناء عمليات الإعداد، ووجد أن أكثر محتوى للمركبات الفينولية في كل الأصناف في المعاملة الثانية، وتراوح بين ٥٤٥٢.٩٦ إلى ٥٩٠٤.٨٧ ملليجرام/١٠٠ جرام لمشروب الشاي الأسود الكيني والمالايو.

كما تراوح محتوى مركبات الفلافونويد بين ٧٨.٤٠ إلى ٥٥٣.١٤ ملليجرام/١٠٠ جرام قبل عمليات الإعداد، وسجل أعلى محتوى لمركبات الفلافونويد في المعاملة الثانية لكل أصناف الشاي وتراوح بين ٦١٢.٤١ إلى ١٣١٢.٧٢ ملليجرام/١٠٠ جرام لمشروب الشاي الأسود الهندي والمالايو. وأوضحت الدراسة أن المعاملة الثانية أدت لأعلى محتوى من المركبات الفينولية والفلافونويد لمشروب الشاي في الأصناف الثلاثة.