



## Phytochemical screening of different black tea brands

A. Imran, M. S. Butt, M. Yasin, M. Imran, R. Batool and A. Naz

*National Institute of Food Science and Technology, University of Agriculture, Faisalabad.*

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### Abstract

*Tea is largest consuming drink in the world. Many health claims is attributed towards the tea due to its distinguished phytochemical array. Role of tea is well established as a nutraceutical and many studies elucidate its pharmacological worth. This project was conducting to explore the chemical composition of the Pakistani tea brands. Result regarding the moisture, fat, protein, fiber and ash fluctuate from 6.09 to 7.08, 7.67 to 4.02, 3.13 to 1.82, 1.36 to 2.51 and 4.95 to 5.11% respectively. Mineral contents for sodium ranges from 2.3 to 3(mg/100g) as compared to ca that ranges from 13.9 to 17.2 (mg/100g). Appreciable level of other minerals was also detected in the study. In concluding Pakistani teas performed satisfactory in context of international standers and provide additional health attribute that may be proved important to mitigate different ailments bestowed by this computer driven world.*

**Key words:** black tea , minerals, theaflavins, phytochemical profile

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### Introduction

In recent years, increasing attention has been paid to the role of diet in human health. Nutraceuticals are widely accepted as an adjunct to conventional therapies for enhancing general well being of body that induces resistance against diseases. It demands that the value of food is recognized for its health benefits beyond basic nutrition. The value of such "alternative" therapy is now being revitalized by many researchers that support the use of traditional remedies to cure diseases (Klein et al., 2000; Ramaa et al., 2006).

Dietary phytochemicals are considered as an effective tool to cure body disorder. They play important roles as therapeutic agents in prevention of many diseases, possesses antimutagenic effects and indeed modulating and stimulating the immune system resulting in normal functioning of whole body (Rates, 2001; Raskin et al., 2002). Several epidemiological studies have indicated that a high intake of natural products is associated with reduced risk

of a number of chronic diseases like atherosclerosis and cancer (Hashimoto et al., 2002; Gundgaard et al., 2003; Gosslau and Chen, 2004). Such beneficial effects are attributed to the compounds i.e. flavanoids, anthocynids and carotenoids. Flavonoids are secondary plant metabolites and contribute to the first defense line against oxidative stress, because they quench singlet oxygen radicals (Krinsky, 2001; Shi et al., 2001). Food rich in these healthful compounds hold a prominent position in food chain generally flavonoids are abundant in fruit, vegetables, tea and coffee.

Tea (*Camellia sinensis*) is cultivated in more than thirty countries around the world and belongs to family Theaceae. It is one of the most commonly consumed caffeinated beverages in the world. It is an evergreen plant that grows mainly in tropical and sub-tropical climates. However, it is commercially cultivated from the equator too as far north as Cornwall on the UK mainland (Baer, 2003).

The consumption of tea is ancient habit and was initiated about five thousand years ago. Now it attains a special position in economy of countries, china holds first position in term of its production while India and Srilanka are also among the leading producers (Abe et al., 2005).

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\* Corresponding author:  
Ali Imran  
E-mail: aliimran.ft@gmail.com  
Cell no. 03336575583

The basic steps for manufacturing for all types of tea are similar except difference in their degree of fermentation during tea processing. Green tea is non-fermented product, while black tea is a fully fermented product. Step of fermentation brings specific characteristics e.g taste, color and flavor to the final product (Hara, 2001; Katiyar et al., 2007).

Black tea phytochemistry is blessed with certain compounds and also accompanied by some components that become thereat when used in an excessive amount. Major components are catechins, theaflavins, thearubigins, caffeine, flavonols, phenolic acids, amino acids, methyl xanthines, carbohydrates, proteins, minerals and volatile (Luczaj and Skrzydlewska, 2005).

Black tea polyphenols are further subdivided into two classes: flavanols and flavonols. The flavanols comprise of catechins, theaflavin and thearubigins. The major catechins in fresh tea leaves are epigallocatechin gallate, epi-gallocatechin, epicatechin gallate, and epicatechin. Catechins are colorless, water-soluble compounds that impart bitterness and astringency to tea infusion (Balentine et al., 1997). Black tea flavonols are quercetin, kaempferol and myricetin. They make up 2–3% of the water-soluble extract in tea. Flavonols are pre-dominantly present as glycosides rather than as their nonglycosylated forms (aglycones). Quercetins, kaempferol, and myricetin together proved effective against cancer and have the ability to inhibit the growth of malignant cell (Wang and Helliwell, 2000).

Theaflavins and thearubigins oxidation help to develop typical color and flavor of black tea. Classically the pigments of black tea have been divided into orange-colored theaflavins (TFs) and brownish thearubigins (TRs) (Chopra and Thurnham, 1999) however theaflavins comprises a group of four constituents theaflavin, theaflavin 3-gallate, theaflavin, 30-gallate, and theaflavin 3, 30-digallate. This group formation occurs due to reaction between quinones derived from a simple catechin and a gallo catechin. They contributed towards tea brisk, astringent taste and bright golden color to the infusion (Cheng et al., 1997). TRs are a heterogeneous group of phenolic pigments molecular mass in the range 700–40,000 Da. They are water soluble, acidic, and are rust-brown in color that gives the richness in taste of tea (Yuan, 1983).

Levels of theaflavin and thearubigins are the necessary tools to assess the tea quality along with other parameters like caffeine and theabronins. Theaflavin and thearubigins has positive impact on tea quality and important for trade while caffeine and theabronins considered a obsolete in tea so tea quality indicate by the highest level of theaflavin and thearubigins and lower level of caffeine and theabronins (Taguri, et al., 2004). Solvents selected for the extraction of tea active molecules based upon their ability to preserve all the desired compounds. According to the nature of the tea different solvents methanol, acetonitrile and ethanol

considered good but the importance of extraction time and temperature cannot be denied (Houghton and Raman 1998). Present research was conducted to explore the chemical profile of different Pakistani teas to set their role in modern life

## Material and Methods

### Procurement of Samples

Different commercial brands of black tea i.e. paper packed, loose and tea bags were procured from the local market (Table 1). Reagents and standards were purchased from Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan) and Merck (Merck KGaA, Darmstadt, Germany). The collected tea samples were stored at ambient temperature for further study.

### Proximate analysis

The tea samples were analyzed for moisture, ash, crude protein, crude fat and crude fiber according to their respective methods as described in AACC (2000).

### Moisture contents

The moisture content was estimated according to official method 44-01 of AACC(2000) by drying the sample in a hot air oven at  $105 \pm 5^\circ \text{C}$  till the weight of the sample became constant. The moisture content was calculated as:

$$\text{Dry matter (\%)} = \frac{\text{Wt. of dried sample (g)}}{\text{Wt. of fresh sample (g)}} \times 100$$

$$\text{Moisture (\%)} = 100 - \text{Dry matter}$$

### Total ash

Total ash was estimated by direct incineration of sample taken in a tarred crucible according to AACC (2000) method 08-01. The crucible was heated on the oxidizing flame till it gave no fumes and then ignited in a muffle furnace at  $550^\circ \text{C}$  till grayish white residue were obtained. Finally, ash was calculated as.

$$\text{Ash (\%)} = \frac{\text{Wt. of ash (g)}}{\text{Wt. of sample (g)}} \times 100$$

### Crude protein

The percentage of nitrogen in the sample was determined by using the method given by AACC(2000) Kjeldahl's method 46-13. The sample was first digested with concentrated  $\text{H}_2\text{SO}_4$  in the presence of digestion tablets for 2-3 hours or until the digested material attained light greenish or transparent color. This material was diluted (250 mL using distilled water) and distillation was done by taking 10 mL of diluted material and 10 mL of 40% NaOH solution in the distillation apparatus. The ammonia thus liberated was collected in 2% boric acid solution containing methyl red as an indicator. Finally the distillate was titrated against 0.1 N  $\text{H}_2\text{SO}_4$  till golden brown end point. The crude protein percentage was calculated by multiplying nitrogen (N) with a factor given below:

$$N (\%) = \frac{\text{Vol. of } 0.1 \text{ N H}_2\text{SO}_4 \times \text{Vol. of dilution} \times 0.0014}{\text{Wt. of sample (g)} \times \text{Vol. of dill taken (mL)}} \times 100$$

$$\text{Crude Protein} = N (\%) \times 6.25$$

#### **Crude fat**

The crude fat content was determined by taking 3 g sample using n- Hexane as a solvent in Soxhlet apparatus according AACC(2000) official method 30-10.

$$\text{Crude fat (\%)} = \frac{\text{Wt. of fat (g)}}{\text{Wt. of sample (g)}} \times 100$$

#### **Crude fiber**

The crude fiber was estimated by digesting defatted free sample and digesting first with 1.25% H<sub>2</sub>SO<sub>4</sub>, followed by 1.25% NaOH solutions. The residue was weighed and ignited in a muffle furnace at 550° C till white residue left. Fiber percentage was calculated according to the AACC(2000) method 32-10. The crude fiber was calculated as

$$\text{Crude fiber (\%)} = \frac{\text{Wt. loss on ignition (g)}}{\text{Wt. of sample (g)}} \times 100$$

#### **Mineral analysis**

For mineral determination, wet digestion of the all samples was carried out according to the method of AACC (2000) 0.5 g of the sample was taken in a conical flask. The sample was digested with 10 ml HNO<sub>3</sub> at a temperature of 60-70° C for 20 minutes and then digested with 5 ml HClO<sub>4</sub> at a temperature of 60-70° C for 20 minutes and subsequently increasing the temperature to 195° C till the volume of the content was reduced to 1-2 ml. The digested sample was transferred to 100 ml volumetric flask and volume was made up to the mark using distilled water and then filtered. After filtration, the digested samples were stored for different mineral determination according to their respective methods. The minerals Fe and Mn, were determined using Atomic Absorption Spectrophotometer (Varian AA240) while Na, Ca and K were estimated through Flame Photometer (Sherwood 410).

#### **Statistical Analysis**

Data obtained for each parameter was subjected to statistical analysis by applying completely randomized design (CRD) using Statistical Package (Statixtic V-8.1). Significant ranges were further compared using least significance test (LSD).

### **Results and Discussion**

Plants are bestowed with varying beneficial properties in order to combat certain disorders. The health promoting properties are attributed to the presence of specific phytochemistry. Tea holds rich phytochemistry and its potential needs to be further explored. Theaflavin thearubingins and theabronins along with caffeine

percentage are very important for concluding its properties; however its importance to assess the nutritional and functional quality before recommended its use. So present research plan was a step forward in this direction and various brands of tea were explored for their nutritional quality and their quality criteria also exploring their hypoglycemic potential.

#### **Proximate analysis**

Composition of raw materials enable researcher to generate an idea about possible application of the product under study. The purpose of the present study was to evaluate chemical attributes of Commercial selected tea brands prior to extraction. Means values for moisture of tea brands have been presented (tables 1) indicating that the moisture content varies from 6.9(CB1) to 7.08% (LP). The highest moisture content was found 7.08% in (LP) followed by 7.02% in (CB3) and lowest detected in CB1 as 6.9%. Similarly mean values for fat content of different tea brands in table 1 showed that fat content were highest as 7.67% in CB1, which was similar to the CB2 while the lowest value 4.2% found in CB3. Statistical analysis showed that CB1 and CB2 have similar mean while CB3 is similar to LP and TB1 while mean of TB2 is different from all others. Data concerning about protein means represented (table 1) showed the protein constituent among different tea brands maximum value found 3.13% in TB2 while lowest found 1.82% in CB2. Statistical analysis cleared that CB1 has similar mean like CB3 and TB1 while CB2, LP and TB2 was different from all others. Means regarding the fiber (table 2) disclosed its variation from 1.36 to 2.51% From them highest found 2.51 % in the LP while lowest found 1.36% in the TB2 which revealed that less fermentation in the loose and more in the CB2. It is revealed from (Table 2) that ash content in different tea samples ranged from 5.2 to 4.17% while, in tea bags it ranged from 4.95% to 5.11%. Highest ash content 5.11% was found in CB3 followed by 5.11% in TB2 while lowest claimed 4.18% by CB1. Statistically LP and TB1 were similar to each other and different from the rest.

Water content determination is the most frequent analysis performed in food products and it is quite significant in many aspects (Isengard, 2001). Nearly every food product contains water and this parameter affects many others, both of physical and chemical nature. Evaluation of most chemical parameters is based on dry mass and therefore water content must be measured. Also, water content affects microorganism growth and enzymatic activity, affecting the stability and shelf life of foodstuffs. The moisture contents are very important as these contents limit the storage of the material. Higher amount represent that food commodity has lower shelf life (Mendonca et al., 2007).

Results indicating that packaging holds a prominent position for conforming tea quality. The moisture percentage almost same within same kind of packaging only LP tea have more moisture which indicating entrance of moisture from external sources so importance of

packaging should given priority. The importance of moisture was reported by many researchers for measurement of its self-life and quality .it has been reported an inverse relation ship between moisture continents and tea quality parameters as moisture increase they decrease. Moisture also strongly influences the theaflavin and thearubingins ratio which are the important tool for tea freshness and quality (Mumin et al ., 2006).

Saleem et al .,( 2002) evaluate the different brands of Pakistani teas and they conformed the result for moisture and according to them moisture were varies between 6.5 to 7.5% among the brands depend upon the packaging as well as method for preservation of the samples. These finding having agreed with the early finding of Kloss (2002) they found the fat content between different varieties and conclusion based upon the fact that the significant difference is due to variety and climatic difference moreover the region also influence the results.

Hara et al .,(1995) determined the fat contents (%) during different manufacturing stages they found the fat 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. This significant difference may be related with the difference in sampling techniques of the companies as well as the processing, moreover, the picking stage of leave also consider important in that regard.

#### **Mineral analysis**

Minerals are inorganic compounds and their structure is usually nothing more than a molecule, or molecules, of an element. The functions of minerals do not include participation in the yielding of energy. But they do play vital roles in several physiological functions, including critical involvement in nervous system functioning, in cellular reactions, in water balance in the body, and in structural systems, such as the skeletal system. The determination of mineral nutrients in tea is of great interest, considering the great consumption of this product for millions of people world-wide (Santos and Oliveira, 2001).

Data concerning means for minerals of various tea brands was presented in table 3 revealed that maximum sodium contents as 3.0(mg/100g) were given by TB1 followed by LP as (2.9mg/100g) while least sodium contents were recorded in CB2 as (2.3mg/100g). The sodium contents of different tea samples were in the range of 2.3to-3.0 (mg/100g). The present results are identical to the findings reported by the other scientists such as Santos and Oliveira, (2001) they reported that the sodium content of different tea and herbal tea samples proved non significant between the same origin and clone. Means for K (table 3) exposed that maximum potassium contents as 17.2(mg/100g) were given by CB1 followed by CB3 as (16.4 mg/100g) while the least potassium contents were recorded in CB2 (13.9mg/100g). This was statistical at par to CB3. According to the data CB2, TB1 and TB2 exhibited non-significant relation with one another and displayed significant relation with other tea

samples. The potassium contents of different teas samples were in the range of 13.9-17.2(mg/100g) which was supported by the findings of Grembecka et al .(2007) that gives the concentration of potassium in the range of 11.75-15.82 (mg/100g) in tea sample. The means of various brands of tea is presented in (table 3) revealed that maximum calcium contents as (18.8mg/100g) were given by CB1 followed by TB1 as (16.8mg/100g). While the least calcium contents were observed in CB3 as (4.6mg/100g). According to the data The calcium contents of different tea samples were in the range of 4.6-18.8(mg/100g) which was supported by the findings of Grembecka et al ., (2007) that exhibited the concentration of calcium in the range of 51.3-162 (mg/100g) in caffeinated coffee, and tea this significance was due to their different origin and difference of fertilizer application. Gallaher et al., (2006) analyzed the ten type of commercially available teas for their calcium contents in U.S.A. and determined the health effects of them. Their founding suggest that the mineral contents (mg/100g) of the herbal infusions studied included commercial blends of peppermint, Echinacea, red clover, Siberian ginseng, dandelion, red raspberry were found to be Ca was in the range of 10 to 20 (mg/100g).

Means concerning Fe (table 4) revealed that TB1 showed the highest iron content as (73.2mg/100gm) followed by CB2 as (64.9 mg/100gm) and least iron contents were recorded in TB2 as (48.4 mg/100gm) whilst, means of Mn presented in table 4 exhibited that TB2 showed the highest manganese content as 96.6% followed by LP as 72.5 (mg/100gm) and least Mn contents were recorded in CB1 sample as 40.8 (mg/100gm). Difference between mineral contents was due to their agronomic practices their botanical and genetic factors as well as region influencing on their contents. Difference among the iron content may be due to the difference in the agronomic application, fertilizer utilization and due to product specific genetic variation. Influence of climate and region also hold significant position on that regard. The iron contents of different tea samples were in the range of 73.2 to 64.9 (mg/100g) which was supported by the findings of Santos and Oliveira, (2001). Martin et al.,(1999) determined the concentration of Fe in the range of 42.5to52.5 (mg/100g) in caffeinated coffee, and teas samples .Gullhar (2004) investigated the mineral composition of Chinese green tea for, Fe, contents and value revealed that the iron content was in the range of 50 to 80 (mg/100g) all the scientist were in the opinion that the mineral contents varies due to region and climate .

Sang et al., (2002) compared the different type of black teas from different origins for there mineral contents and his founding revealed that manganese for different black tea samples ranges from 60 to 110(mg/100gm). This variation is the result of region and climate influence on the mineral contentions.

Table: 1 Means for moisture, protein and fat contents of different tea brands

Tea Samples	Moisture	Fat	Protein
CB1	6.9±0.02a	7.67±0.02a	2.58±0.26bc
CB2	6.7±0.04b	7.10±0.01b	1.82±0.02e
CB3	7.02±0.04a	4.23±0.01d	2.46±0.01c
LP	7.08±0.05a	7.08±0.10bd	2.06±0.04d
TB1	6.85±0.01a	5.65±0.04c	2.73±0.09b
TB2	6.74±0.03c	7.16±0.01b	3.13±0.01a

Values are mean±SD

- CB1 commercial brand :1
- CB2 commercial brand :2
- CB3 commercial brand :3
- LP Loose tea
- TB1 Tea bag :1
- TB2 Tea bag

Table: 2 Means for fiber and ash contents of different tea brands (values are mean± sd)

Tea SAMPLES	Fiber %	Ash %
CB1	2.51±0.01 b	4.18±0.01e
CB2	1.97±0.02 d	4.24±0.01d
CB3	4.18±0.03a	5.02±0.08c
LP	2.47±0.02c	4.97±0.01b
TB1	1.36±0.01e	4.95±0.05b
TB2	1.38±0.04e	5.11±0.01a

Table: 3 Means for Sodium, Potassium and Calcium contents of different tea brands

TEA SAMPLES	Na (mg/100g)	K (mg/100g)	Ca (mg/100g)
CB1	2.4±0.2a	17.2±0.05a	18.8±0.51a
CB2	2.3±1.0a	13.9±0.54c	7.7±5.30b
CB3	2.4±0.2a	16.4±0.96ab	4.6±0.1c
LP	2.9±1.0a	16.4±0.96ab	15.5±1.07a
TB1	3.0±0.9a	15.8±1.48bc	15.5±1.07a
TB2	2.3±0.2a	14.9±1.23abc	14.9±1.23abc

values are mean± sd

Table: 4 Means for iron and Manganese contents of different tea brands

Tea samples	Fe( mg/100g)	Mn ( mg/100g)
CB1	52.03±8.01d	40.8±1.10b
CB2	64.9±0.72b	55.7±1.0d
CB3	61.2±1.82c	54.8±8.59d
LP	48.4±1.36e	72.5±3.40b
TB1	73.2±4.38a	61.6±1.6c
TB2	48.4±1.62e	96.6±3.0e

values are mean± sd

## Conclusion

Black tea phytochemicals that contributed towards its cup quality in Pakistan are in close proximity with international market. Difference between the key chemical parameters might be due to the processing technology, fermentation and storage period. The result of recent investigation indicates that tea is a hidden tool for enhancing the human resistance against various ailments. Furthermore, there is a dire need for exploration of its potential that reputed its role as a functional drink that can be applied as a nutraceutical intervention.

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