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Chemical Analysis and Characteristics of Black Tea Produced in North of Iran

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ABSTRACT: Tea might be considered as the most popular drink in Iran as well as some other parts of the world. Its popularity to some extent depends on its flavor and some other constituents present in its leaves or extract. Black tea samples from various agro climatic zones of northeast of Iran were evaluated for the chemical constituents, polyphenols and metal analysis that determine the quality perception. Black tea epicatechins, namely epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epigallocatechin (EGC) and epicatechin (EC) were also assessed by HPLC. Furthermore, for metal analysis, the level of Mn, Cu, Pb and Ni elements were evaluated in black tea from the five tea growing districts of north Iran. The results confirmed that the total polyphenol concentration in black tea varied from 17.10 g/100g to 12.20 g/100g, whereas the catechins content ranged from 17.20 mg/g to 15.80 mg/g. Furthermore, significant differences were detected in the chemicals content between different black tea samples. In general, the contents of caffeine and water extract in Lahijan sample were found to be the highest. The Mn content was found in the range of 702 and 731 mg/kg and copper ranged between 24.30 and 32.60 mg/kg. The Lead content in the samples were in the range of 0.22 and 0.35 mg/ kg. Nickel concentration in black tea leaves ranged between 2.31 and 3.67 mg/ kg. The results of the evaluation of tea confirmed that the level of elemental contents in all of the tea sample were completely lower than the acceptable limit according to the international standards. It was noted that in different black tea samples, Lahijan produced the best quality tea followed by Tonekabone.

Keywords: *Black Tea, Chemical Composition, Elements Content, North of Iran, Polyphenols.*

Introduction

Tea is one of the traditional and healthy drinks most widely consumed in the world. This beverage is made from the young tender shoots of *Camellia sinensis* (L.) O. Kuntze (Theaceae) and is the most popular for its mildly stimulant and refreshing effects (Harbowy & Balentine, 1997). Tea plant is valued in medicines as the leaves for making cigarettes and fumigating powders for the relief of asthma. Tea infusions have many physiological and biological effects particularly with respect to its potential for preventing and treating cancer, cardiovascular diseases, and low-density lipoprotein oxidation (Zhang *et al.,* 2009).

The chemical composition of tea leaves is complex and is comprised of flavonols, alkaloids, tannin substances, proteins and amino acids, enzymes, aroma forming substances, vitamins, and minerals (Kumar *et al.,* 2005).

Flavanols (Catechins) are the main group of phenolic compounds in fresh leaf and green tea. The most abundant tea catechins are (-)-epigallocatechin 3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin (EC), (-)-epicatechin 3-gallate (ECG), and (+)-catechin (C). Other compounds, such as epiafzelechin and its gallate as well as

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acetylated catechins have also been recognized (Hashimoto *et al.,* 1987). During black tea crushing and full fermentation process, an enzymatic oxidation of tea flavanols, leading to the formation of different coloured polymeric compounds, such as theaflavins (TFs) and thearubigins (TRs) which are mainly responsible for briskness, brightness, colour and strength have been reported (Bhuyan *et al.,* 2009; Robertson, 1992; Senthil Kumar *et al.,* 2011).

Tea has long been considered as a valuable source of antioxidants. The antioxidant activity of black tea is due to its polyphenol contents (Gardner *et al.,* 2007). Theaflavins (TFs) and thearubigins (TRs) have antioxidant activities and also act as antioxidants *in* V*itro* by sequestering metal ions and by scavenging reactive oxygen and nitrogen species (Frei and Higdon, 2003). Theaflavins formation depends upon geographical area of production and genetic variations of cultivar (McDowell *et al.,* 1991). The amount of tea polyphenols is a complementary indicator of the quality of tea (Obanda *et al.,* 1997).

Water extract of *Camellia sinensis*a has been considered as an important international standard for quality control of tea (Hara *et al*., 1995; ISO 3720, 1986; ISO 9768, 1994). The tea water extract is comprised of phenolic compounds, amino acids, sugars, alkaloids and many minor soluble substances, such as minerals and pigments (Harbowy & Balentine, 1997; Wood *et al.,* 1964).

Moreover, tea contains minerals that are essential to human health. Cao *et al*. (1998) reported that the concentration of different elements in black tea leaves are divided into three groups, the first group (mg/g) consists of Ca, Na, K, Mg and Mn, the second group (mg/s) is comprised of Cr, Fe, Co, Ni, Cu, Zn and Cd, and the third group consists of rare elements present at mg/g level. Tea could be beneficial for hypertensive patients due to its manganese, potassium, Chromium, selenium, and zinc contents that are important in human metabolism (Pelus *et al.,* 1994; Fennema 2000). On the other hand, the presence of heavy metal concentration in food and beverages has been restricted by several countries according to food laws (Bosque *et al.,* 1986).

Setia *et al.* (1989) showed that, the concentration of heavy metals varied depending on agro inputs, soil, pesticides, nutrient, fertilizers and the ability of plants to accumulate some of these elements. Powell *et al.* (1998) confirmed that different parameters such as country of origin and the manufacturing process are important. Studies have shown that black tea quality is being affected by many factors such as season and altitude, genetic make-up of the plant (Owuor & Obanda, 1995) and the region of production and the climate (Owuor *et al.,* 2008).

Iran's population is about 1% of the world population, but account for over 5% of the world's total tea consumption. The amount of tea producing area in Iran is about 35,000 acres of land in north of the country mainly in Gilan. Information based on chemical standardization, parameters and biological assays are quality indicators of tea. Presently, no studies have been reported on the chemical composition and phenolic compounds of tea from north of Iran. Thus, the aim of this study is to analyse the chemical composition, phenolic compounds and the level of elements (Mn, Cu, Ni and Pb) in black tea from north of Iran to provide useful information to estimate black tea quality.

Materials and Methods

- *Samples*

A set of 5 black tea samples from different tea factories in north regions of Iran (5 Region: Rasht, Lahijan, Langerud, Rudsar and Tonkabon) were analysed in triplicate order.

- *Extraction of polyphenols*

The method was carried out according to the instructions of International Organization for Standardization (ISO) 14502-1. In brief, 0.20 g of each sample and 5 mL of 70% methanol at 70 °C were transferred into an extraction tube. The resulting solution was mixed and heated at 70 °C via gentle vortexing for 10 min. The mixture was centrifuged at 200*g* for 10 min after cooling at room temperature. The aqueous phase was decanted in a graduated tube, and the procedure was repeated twice. Both extracts were adjusted to 10 mL with cold 70% methanol. For dilution, the volume of each milliliter of the extract increased to 100 mL with distilled water.

- *Determination of total polyphenol content*

Total polyphenol content was determined using Folin-Ciocalteu reagent, carried out according to the following instruction presented by International Organization for Standardization (ISO) 14502-1. Briefly, 1.0 mL of the diluted sample extract was transferred in duplicate to separate tubes followed by the addition of 5.0 ml of Folin-Ciocalteu's reagent (1/10 dilution) and 4.0 ml of sodium carbonate solution (7.5% w/v). The tubes were allowed to stand in the dark at room temperature for 60 min before the absorbance was measured at 765 nm. The TPC was expressed as gallic acid equivalents (GAE) in g/100 g of the sample. The calibration equation for gallic acid was $y = 0.0111\upsilon + 0.0148$ ($R^2 = 0.9998$).

- *Determination of catechins, theaflavins and thearubigins*

The preparation consisted of the addition of the boiling distilled water (150 ml) to leaf tea samples (3 g) in boiling water bath for 10 min. Then, the tea solution was filtered through ''Double-ring'' No.102 filter paper (Xinhua Paper Industry Co. Ltd, Hangzhou, China) and 0.2μm Milipore filter and injected into the HPLC. HPLC was carried out according to the procedure of Liang, Ma, Lu & Wu (2001). A high-performance liquid chromatography system (Milford, MA, USA) equipped with a 5μ-DiamonsilTM-C18 column (4.6 mm, 250 mm) and Shimadzu SPD ultraviolet detector was employed to carry out the analysis. Acetonitrile, acetic acid and water was used as a mobile phase with a flow rate of 1 mL/min at 25 °C.

- *Determination of caffeine, water extract, moisture and ash*

The methods used for the analysis of caffeine, water extract and moisture of the tea solution were based on the international standards (ISO 1839, 1980; ISO 9768, 1994; ISO 1573, 1981). The ash contents of tea samples were determined according to ISO 1575, 1987 by using furnace at 500-600°C for 5 to 6 hours.

- *Determination of metal concentration*

An atomic absorption spectrophotometer (AAS) (shimadzu, Japan) with flame and graphite furnace was employed for simultaneous determinations of Cu, Mn, Ni, and Pb contents in the samples of black tea leaves.

Total samples of black tea were accurately weighed, Exactly 1 g of the dried sample of tea leaf was taken in a 100 ml beaker and after adding 10 ml nitric acid and perchloric acid (5:1)sample was heated up to completely combine insoluble particles with acid. Again half of the previous mixed acids were applied and the resulting solution vigorously mixed to eliminate total fumes. While heating continued, a mixture of white gelatinous was produced and the mixture with adding 10 ml of water and further heating decreased to 2 ml. Following this, 10 ml of water was added to the mixture and to eliminate the turbidity or suspended matter Whatman No.42 filter paper was employed to filter the solution. After adding 2-3 drops of HCl, the volume of solution was adjusted to 50 ml with distilled water. Finally, the obtained solutions were kept in polythene bottles. Every sample of black tea was prepared and examined in triplicate order.

- *Statistical analysis*

Analysis of variance (ANOVA) and two-tailed independent-sample T-test in SPSS 16.0 software (SPSS Inc., Chicago, IL, USA) were employed to determine the statistical significances among the different treatments. To approve the statistical significance of the results, the data are presented as mean ± standard error (S.E.).

Results and Discussion

Total phenols, total catechins, theaflavins and thearubigins contents of five black tea leaf samples are given in Table 1.

The results show that the total polyphenol content of black tea samples varied from 17.10 to 12.20 g/100g. Significant differences were found between polyphenol concentrations in tea samples. The differences found between regions could be due to a post maturation process in the fermentation of black tea. The oxidation of phenolic compounds in all types of teas during the storage period has been determined in research carried out by Hara *et al.* (1995). The values obtained in this study for Iranian tea were comparatively higher than those reported for different samples in Malaysia, that indicated polyphenol contents of 19.13 and 11.37 for green tea and 8.49 and 6.06 for black tea (Chan *et al.,* 2007).

The results of the catechins content in black tea from the 5 tea cultivars assayed in this study (Table 1) showed that significant ($p \leq 0.05$) differences were present between the samples. Lahijan samples contained significantly ($p \leq 0.05$) higher amounts of catechins 17.20 mg/g than Rudsar samples which had 15.80 mg/g. These results confirmed that the degree of auto-oxidation ''fermentation'' during the manufacturing process had an effect on the catechin level of the final product. Lin *et al*. (2003) reported 1.23-2.01% of catechins in ethanolic extract of black tea samples.

In black tea production, after the tea leaves are rolled, it will disrupts the cellular compartment and brings phenolic compounds in contact with polyphenol oxidases, and the young *C. Sinensis* leaves undergo oxidation for 90–120 min. During this phase, the catechins are changed to complex condensation products (Ravichandran, 2002). The theaflavins contents in tea samples were found in the range of 10.30 and 12.30 mg/ g with significant ($p \leq 0.05$) differences between the samples. The highest and the lowest level of theaflavins belonged to Lahijan and Tonekabon regions respectively. According to Table 1, thearubigins content in black tea

Table 1. Total contents of polyphenol, catechins, theaflavins and thearubigins in black tea samples

Region	Total polyphenol (g/100g)	Total catechins (mg g^{-1})	Theaflavins (mg g^{-1})	Thearubigins (g/100 g)
Rasht	14.63 ± 0.20^{b}	16.20 ± 0.4^{c}	10.79 ± 0.10^{bc}	9.6 ± 0.01^{c}
Lahijan	17.10 ± 0.20^{a}	17.20 ± 0.3^{a}	12.30 ± 0.12^{a}	11.2 ± 0.05^{a}
Langerud	13.10 ± 0.12^{c}	16.50 ± 0.5^{bc}	10.70 ± 0.09^{bc}	10.7 ± 0.02^{b}
Rudsar	13.80 ± 0.20^{c}	15.80 ± 0.6^{cd}	11.30 ± 0.15^{b}	9.8 ± 0.05^{c}
onekabon	12.20 ± 0.15^{d}	16.70 ± 0.3^{b}	10.30 ± 0.15^{c}	10.8 ± 0.02^{b}

Data are means ± S.D of triplicate measurements. Values with different superscript upper case letters in a column are statistically significant at $P < 0.05$.

samples varied from 9.6 g/100 g in Rasht to 11.2 g/100 g in Lahijan. Significant differences were found between thearubigins concentrations in tea samples from different regions in north of Iran. Yao *et al.* (2006) observed variations in theaflavin and thearubigins contents from 2-5 and 15-23%, respectively. They found that the amount of theaflavin and thearubigins were influenced by the temperature, extraction time and fermentation stage.

The EGC concentration was found in the range of 0.98 and 1.52 mg/g in different black tea samples. ECG was measured in the range of 0.98 and 2.69 mg/ g and in the range of 0.90 and 1.41 mg/g for EC content in tea samples from different regions of north Iran. The average concentration of EGCG in the black teas shown in Table 2 was 2.51mg/g. The EGCG concentration in Rasht black tea (1.31) was minimum while EGCG level was at its maximum concentration in Lahijan tea (2.96).

EGCG, EGC, ECG and EC were the individual polyphenols measured. Longo *et al.* (2008) applied reverse phase HPLC to measure EGCG and ECG in 25 major Indian cultivars. EGCG varied from 1.36 to 68.35 mg/g and ECG varied from 0.32 to 27.39 mg/g in tea leaves.

Moisture content is considered to be one of the important parameters for tea quality (Roberts & Smith, 1963).The results of the studies for tea (Othieno & Owuor, 1984; Robinson & Owuor, 1993) have shown that the moisture content of tea should be controlled and be present under 6.5% for black teas, although Millin (1987) believed that tea had a moisture level of 7–8% during retailing. Moisture contents in the 5 region were significantly (p>0:05) different. In this study moisture contents were found in the range of 4.27% (Lahijan) and 5.83 % (Langerud).

The average concentration of ash in the black tea samples are shown in Table 3, significant differences were found between ash contents in tea samples. This finding is consistent with the results reported by other researcher such as Mohammed and Sulaiman (2009), they reported the ash content of 4.90 to 7.20, while Heong *et al.* (2011) analysed black tea samples and reported values around 5.45% for the ash content.

According to ISO, water extract of a tea is ‘‘the soluble matter extracted from a test portion by boiling water under the conditions specified in the international standard expressed as a percentage by mass on a dry basis’’ (ISO 9768, 1994). Water extract in tea should be more than or equal to 32% of the dry mass (ISO 3720, 1986). The water extract obtained in this study varied, from 37.2% to 41.07% and it was the highest in Lahijan and the lowest in Tonekabon. Owuor *et al.* (1986) estimated the water extract of black leaf teas in other countries, China 36.79%, India 36.89–41.95%, Kenya 44.12% and Sri Lank 36.72–46.90%.

Table 2. Contents of most abundant tea catechins in black tea samples (mg/g dry weight)

Region	Epigallocatechin (EGC)	Epicatechin (EC)	Epigallocatechin gallate (EGCG)	Epicatechin gallate (ECG)
Rasht	$1.09±0.05^{c}$	$1.05±0.03^{c}$	$1.31±0.03^{c}$	$2.15±0.01^{b}$
Lahijan	$1.52±0.03^{a}$	$1.41±0.05^{a}$	$2.96±0.05^{a}$	$2.69±0.07^{a}$
Langerud	$1.21±0.01^{b}$	$1.08±0.04^{c}$	$2.23±0.04^{b}$	$1.63±0.05^{c}$
Rudsar	$0.98±0.06^{d}$	$0.90±0.06^{d}$	$1.38±0.09^{c}$	$0.98±0.06^{d}$
Tonekabon	$1.48±0.04^{a}$	$1.20±0.02^{b}$	$2.87±0.04^{a}$	$2.20±0.02^{b}$

Data are means ± S.D of triplicate measurements. Values with different superscript upper case letters in a column are statistically significant at P < 0.05.

In the five black leaf teas, caffeine content ranged from 3.10% to 3.31% (w/w) with the mean of 3.19% (w/w) (Table 3). The results of other studies indicated that clone, season, and stage of plucking were influenced on caffeine content of black teas. (Hara *et al.,* 1995; Harbowy & Balentine, 1997).The caffeine level in the black teas from Australian regions could be affected by the tea clone, geographical locations, harvest time and infusion conditions (Owuor *et al.,* 1987; Hicks *et al.,* 1996; Yao *et al.,* 1992).Caffeine has been analyzed to be present in dry black tea at 3-4% (Milin, 1987), depending upon the type of leaf used (e.g., there is more in fresh shoots). Detailed information concerned with this matter is available from Cloughley (1983). In UK, Kazi (1985) determined the caffeine contents in five samples of commercial blended black tea that ranged between 2.7 and 3.2% using high-performance liquid chromatography procedure.

The results of the elements contents (Mn, Cu, Pb, and Ni) of tea leaves are presented in Table 4.

The level of Mn in experimental samples of black tea showed that Mn content in black tea varied from 702 mg kg^{-1} in Lahijan to 715 mg kg^{-1} in Rudsar. Significant differences were found between Mn concentrations in tea samples from 5 different regions in north of Iran. Okamoto and Fuwa (1987) determined the Mn content in level 700 ± 25 mg kg^{-1} according to International standards in Japan. High Mn concentrations (390-900 mg kg-1) in the tea plants were determined by AL-Oud (2003).

Table 3. Determination of moisture, ash, water extract and caffeine in various tea samples

Region	Moisture (%)	Total ash (%)	Water extract (%)	Caffeine (%)
Rasht	$5.63{\pm}0.10^{b}$	$6.20{\pm}0.04^{b}$	$38.84{\pm}0.10^{b}$	$3.10{\pm}0.02^{c}$
Lahijan	$4.27{\pm}0.20^{de}$	$5.90{\pm}0.03^{d}$	$41.07{\pm}0.10^{a}$	$3.31{\pm}0.54^{a}$
Langerud	$5.83{\pm}0.15^{a}$	$5.50{\pm}0.05^{e}$	$38.10{\pm}0.20^{b}$	$3.15{\pm}0.43^{b}$
Rudsar	$5.26{\pm}0.10^{c}$	$6.30{\pm}0.06^{a}$	$38.90{\pm}0.10^{b}$	$3.30{\pm}0.35^{a}$
Tekabon	$4.30{\pm}0.15^{de}$	$6.10{\pm}0.03^{c}$	$37.20{\pm}0.20^{c}$	$3.10{\pm}0.47^{c}$

Data are means ± S.D of triplicate measurements. Values with different superscript upper case letters in a column are statistically significant at P < 0.05.

Table 4. Mineral concentration in black teas from the north of Iran

Region	Mn (mg kg^{-1})	Cu (mg kg^{-1})	Pb (mg kg^{-1})	Ni (mg kg^{-1})
Rasht	$705{\pm}8.54^{a}$	$28.50{\pm}0.51^{c}$	$0.22{\pm}0.02^{a}$	$2.70{\pm}0.06^{b}$
Lahijan	$702{\pm}10.15^{a}$	$29.30{\pm}0.71^{c}$	$0.32{\pm}0.01^{bc}$	$3.67{\pm}0.05^{c}$
Langerud	$709{\pm}6.81^{b}$	$32.60{\pm}0.54^{d}$	$0.30{\pm}0.02^{b}$	$2.62{\pm}0.08^{b}$
Rudsar	$715{\pm}5.00^{c}$	$24.30{\pm}0.43^{a}$	$0.30{\pm}0.01^{b}$	$2.31{\pm}0.03^{a}$
Tonekabon	$713{\pm}12.58^{bc}$	$25.68{\pm}0.59^{b}$	$0.35{\pm}0.02^{c}$	$2.35{\pm}0.01^{a}$

Data are means ± S.D of triplicate measurements. Values with different superscript upper case letters in a column are statistically significant at P < 0.05.

Table 4 indicates the mean concentrations of Cu measured for tea samples from north of Iran that is 28.08 mg kg^{-1}. Analysis of variance of the data showed statistically significant differences in mean Cu content among the different regions in north of Ian (p > 0.05), where tea samples from Langerud had the highest Cu content while the samples from Rudsar had the lowest Cu concentration. Xie *et al.* (1998) and Ferrara *et al*. (2001) found the Cu content of black tea samples and showed that the Cu level in all of the tea samples were less than 30 mg kg^{-1} (Ferrara *et al.,* 2001; Xie *et al.,* 1998), Although Cu was reported below 150 mg kg^{-1} in United kingdom, India and Kenya (Subbiah *et al.,* 2008), Wang *et al.* (1993), reported the Cu content in Chinese tea samples in the range of 9.6 and 20.9 mg kg^{-1}.

According to Table 4, the lead contents in the experimental samples varied between 0.22–0.35 mg kg^{-1}. The highest was indicated in Tonekabon sample and the lowest in Rasht sample. Xie *et al.* (1998) determined the concentrations of lead in tea leaves of china in the average range of 1.42 mg kg^{-1}. Franklin *et al.* (2005) reported that some elements in the form of salts are required for growing tea plants. Various sources with lead impurities are potassic fertilizers, phosphatic fertilizers, N–P–K blends, manganese, zinc, boron and magnesium. According to Semu & Singh (1996), by using copper fungicides and copper oxychloride with heavy metal contamination, the accumulation of Pb and Cd in tea might be increased. Lagerwerff (1972) have found that another lead contamination source is minute particles of inorganic Pb mixtures in the atmosphere, hence fields in the roadside have higher amount of pb content.

The concentration of Ni in the black tea samples are presented in Table 4. Significant differences were observed in Ni concentrations in different Iranian tea samples. The nickle content in the samples was found in the range of 2.31 and 3.67 mg/kg. The highest and the lowest level of Ni belonged to Lahijan (sample 2) and Rudsar (sample 4) regions respectively. According to the research carried out by Marcos *et al.* (1998) nickle in the sample of tea leaves varied between 2.89 and 22.6 mg kg^{-1}.

In a research carried out by Chand *et al.* (2011), it was indicated that variations among mineral contents in tea might be a function of climate, soil and agronomic practices.

Conclusion

Five different black tea samples from different regions in north of Iran were evaluated to determine their quality perception. The data obtained in this study showed that the quality and biochemical contents in the black tea from different regions could be affected by geographical locations, soil and agronomic practices. Furthermore the results confirmed that the contents of elements were significantly different in tea leaves from different regions in north of Iran. The results showed that the levels of Mn, Cu, Pb and Ni were less than the acceptable limit according the international standards.

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