THE IMPACT OF PROCESSING METHODS, SHOOTING PERIOD AND TEA GRADES ON CAFFEINE LEVELS OF TURKISH BLACK TEA

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Abstract
In the present work, Turkish black teas were analyzed to investigate some different effects on the level of caffeine. Firstly*, by the effect of shooting period and tea grades (seven grades) for teas manufactured with Orthodox method. And secondly, by the effect of the processing method for two tea types, Orthodox and Cay-kur (orthodox+ rotorvane+ orthodox) obtained from Turkish Tea Board (Cay-Kur, Turkey). After an analytical determination of caffeine levels by HPLC (High-Performance Liquid Chromatography), the obtained results were as follows: for shooting periods, the levels of caffeine had a range between 1.74 and 2.11% of dry weight. Also, levels revealed a significant difference (p<0.05) and a decrease in content from spring to autumn (May, June, to September). However, there was no significant difference (p>0.05) between the seven grades of Turkish black tea. Even the different manufacturing methods had no significant effect on caffeine levels. The amount of caffeine was similar, 2.21% in Orthodox method and 2.16 % in Cay-kur tea. Finally, it is found that shooting period had a significant effect on caffeine content, the highest caffeine levels were obtained during the first shooting period (May) of tea plucking period, while the lowest levels were in the third shooting period (September). Hence, the shooting period factor confirms its effect on the caffeine levels.*

Keywords: Black tea, caffeine, grades, manufacturing method, shooting period

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1. INTRODUCTION

Tea (*camellia sinensis*) is one of the widely consumed beverages. Hundreds of tea types can be found on market shelves. Generally, it can be classified as non-fermented (green), partially fermented and fermented (black tea). In Turkey, black tea is considered as a national drink due to its high consumption. Teas hold high organoleptic characteristics (aroma, taste) and beneficial effects on health. In order to closely understand tea, scientific researches do not cease to isolate and identify compounds of interest and determine the main factors to obtain teas with requested quality (Özdemir et al., 1993; Lin et al., 1996; Astill et al., 2001).
From an economic point of view, teas with high quality, dictate the production with paying attention to the affecting factors, namely; genetically factors, environmental factors, and manufacturing practices. Not more important than the others, manufacturing practices of black tea can be divided into many principal stages, withering, maceration, fermentation, drying and sorting (Wright, 2002). When the set parameters of the previous stages are slightly modified during tea's making, its final quality is subsequently affected.
One of the interesting compounds in tea is caffeine. Chemically, it is a methylxanthine (1, 2, 7 methylxanthine), one of the principal alkaloids in tea leaves and it occurs also in various vegetable products and beverages like coffee, cocoa, cola (Spiller, 1997). Caffeine is characterized to be responsible for the taste and briskness of tea (Panda, 2016) and recognized by the stimulative effect on nervous system (Chen et al., 2010).
Caffeine, by various researches, proved its positive effect on cardiovascular health, to enhance the physical and mental performances. Its ability to scavenge oxygen reactive species could open new horizon against cancer. In spite of that, an excessive or even an occasional consumption of caffeine could adversely exhibit some risks like impact on hypertension of individuals, influence on reproduction and sleep deprivation (Gramza, 2014). Therefore,

for the daily intake of food containing caffeine, some countries defined the recommended limits of this substance, about 400 mg/day. This limit should be less for hypertension patients and during pregnancy (Ruxton, 2009).
The aim of the current study is to compare the caffeine levels of Turkish black teas for different shooting periods to determine whether there is a seasonal variation or not, also for the different processing methods and the seven grades of black teas.

2. MATERIALS AND METHODS

Chemicals

Caffeine was bought from Carlo Erba (France). Methanol, acetic acid, and citric acid were purchased from J.T Baker (Holland). All solvents were of analytic or HPLC grade.

Materials

Black tea samples, used to determine seasonal variations, were obtained from 10 tea plants. Samples were collected at 3 different shooting period, in May (spring), July (summer) and September (autumn), as three replicates. Samples obtained from the 1st, 2nd, 3rd, 4th, 5th, 6th and 7th sieve from each plant were mixed in equal proportions and milled to pass through a 2 mm sieve. All black tea samples were manufactured by orthodox methods. In this method, the tea leaves were harvested (plucked), withered through artificial or natural methods and then rolled by rolling machines. Afterward, the rolled leaves were fermented and dried.
Secondly, concerning the determination of effects of tea grades on the caffeine levels for black tea, the seven grades (1-3 grades: high quality, 4-6 grades: low quality, 7th grade: tea dust) of black tea were collected from 4 tea plants at the first shooting period at three replicates. The tea samples were manufactured by orthodox methods.
Thirdly, to investigate the effect of the manufacturing methods, black tea samples were obtained from 4 tea plants in the first shooting period as three replicates. Two of them are processed by the Orthodox method, while the rest were produced by Cay-kur method. This latter is a modified orthodox method and consists of plucking, withering, first plain rolling, rotorvane, second plain rolling, fermentation, drying, sorting and grading. The leaf samples obtained from the 1st, 2nd, 3rd, 4th, 5th, 6th and 7th sieve from each plant were also mixed in equal proportions and milled to pass through a 2 mm sieve.

Caffeine analysis

One gram of black tea sample was extracted in 100 mL boiled distilled water for 5 min. After the extraction, the pH was adjusted to 3.2 with citric acid (Khokhar et al., 1997), and the extract was filtered through a 0.45 μm filter (Biocrom MN 718020, Phonex nylon filter 25 mm) before caffeine analysis by HPLC (High Performance Liquid Chromatography).

HPLC analysis

HPLC separation and identification were carried out according to the procedure described by Ding et al. (1999), using an HPLC system (Thermoquest, USA). The HPLC system consisted of a model P100pump, an SN4000 controller unite, a model AS 2000 autosampler and a model UV 1000 UV–Vis detector. The column used was a C18 reversed phase Luna 5u (4.6 mm x 250 mm).
The area and the retention time of the chromatographic peak were compared with pure caffeine standard. Standard solutions were injected into the HPLC, and peak area responses were obtained. A standard graph for caffeine was prepared by plotting concentration versus area. Quantification was carried out from integrated peak areas of the sample and corresponding standard graph. Twenty microliters of the analytical filtered sample were injected and analyzed. The mobile phase was aqueous of 30% methanol containing 0.1% acetic acid and ran by an isocratic elution at a flow rate of 1.0 mL/min. Detection was carried out at 270 nm (Karadeniz and Koca, 2009).

Data statistics

All results were carried out in triplicate, and the mean and standard deviation values were presented, based on the dry weight of tea leaf.

One-way analysis of variance (ANOVA) and Duncan's multiple range test was carried out to test any significant difference among the different samples. Also, differences between processing methods were determined using t-test. Statistics were carried out on the software of the SPSS for Windows (ver. 16.0).

3. RESULTS AND DISCUSSION

The Effect of different shooting period on the caffeine levels

Caffeine levels of black tea manufactured in the different shooting period are given in Table 1. Starting from the results obtained, caffeine contents for different months varied between 1.60 and 2.44 %, even more, the means were 2.11, 1.90 and 1.74, for May, June, and September, respectively.

Table 1. Caffeine levels of black tea in different plucking periods (by dry weight %)

	Plucking periods		
Samples	May	June	September
1	2.30±0.174	2.02±0.095	1.80±0.144
2	2.44±0.288	1.96±0.080	1.83±0.129
3	2.05±0.100	1.97±0.246	1.74±0.112
4	2.10±0.324	1.79±0.226	1.71±0.193
5	2.00±0.036	1.84±0.176	1.64±0.146
6	2.08±0.330	1.96±0.102	1.75±0.036
7	1.99±0.081	1.86±0.1230	1.60±0.008
8	2.04±0.137	1.79±0.245	1.78±0.127
9	2.04±0.019	2.01±0.208	1.97±0.200
10	2.02±0.065	1.79±0.416	1.60±0.372
Mean	**2.11**[a]	**1.90**[b]	**1.74**[c]

**The average values shown with different letters in the same column are different (p<0.05).*

Initially, in some previous studies and for many purposes of researches, alkaloid or especially caffeine were analyzed and determined. For instance, Naik and Nagalakshmi (1997), in a study to improve HPLC caffeine determination in black tea, the obtained percentage was in a range of 2.00 and 2.87%. Another, for Astill et al. (2001) who investigated the differences between green and black tea, they found an interesting variation (2.21-3.97 %) of caffeine in black tea. Even, Karadeniz and Koca (2009) had found content between 2.13 and 2.54%.

The research of the factors affecting the levels of polyphenols and caffeine in tea leaves, levels of caffeine for boiling water or 75% ethanol extraction for black tea were 31.95 and 35.30 mg/g, respectively (Lin et al., 2003). In addition, and in order to develop a new HPLC method for the determination of polyphenolic compounds and caffeine simultaneously, for different types of examined teas, the black tea gave a mean of 3.24 % for caffeine (Nishitani and Sagesaka, 2004). Yao et al. (2006), they have found a close level to the last mean, by 3.89%. Moreover, in the experimental analysis for Zuo et al. (2002), almost all the studied teas demonstrated high concentration of caffeine, as well as that of black tea with a concentration of 21.6 mg/g. Whereas, in the study of Khokhar and Mangnusdottir (2002), levels were between 22-28 mg/g.

Furthermore, the researches of the different periods of shooting in different processes by Özdemir et al. (1993), reported that the period of shooting even the shooting times of each period (beginning, middle, end), demonstrated a highly significant effect on caffeine contents ($p<0.01$), with means in 1.43-2.28 % of dry matter. In the same way, tea extracts content showed a decrease, from the first till the third, maybe explain a depending on shooting period. According to Turkmen and Velioğlu (2007), caffeine was the dominant alkaloid in both teas (Orthodox and Caykur), with values ranged between 17.84 and 23.79 mg/g dry weight. From a plucking season point of view, alkaloids content, for two tea types, were similar and no significant differences in their levels. However, tea major alkaloids decreased from May to September. On the other hand, a study by Lin et al. (1996), in which black tea of Assam variety (most fermented), showed among green teas and others varieties a high mean with 5.4%. Irrespective of the strains, the mean contents of caffeine and total catechins were higher in summer than spring, by 1.57 and 1.4 folds respectively.

Generally, with respect to caffeine content, our findings are close to some results of previous researches. However, our results were higher by 1.11 and 1.21 folds in May (spring) than July (summer) and September (autumn), respectively.

There are generally three seasons for plucking tea shoots in Turkey. The first, second and third crops are harvested in spring, summer, and autumn, respectively (Turkmen and Velioglu, 2007). At different shooting periods, as it is presented in Table1, manufactured black teas show a significant difference ($p<0.05$) among caffeine contents.

The first shooting in May (spring), is remarked to have significantly ($p<0.05$) the higher caffeine amounts and the lowest is in September (autumn), last month of harvesting. In a similar way, in agreement with Özdemir et al. (1993) and Turkmen and Velioğlu (2007), during shooting periods from spring to autumn, the results showed a decrease of caffeine contents. On the contrary to Lin et al. (1996), it has been seen an increase in the amount of caffeine during the same shooting periods.

In Turkey, the region of East Black sea, during the first shooting periods (May), is known to be slightly cold. Tea shoots plucked during slow growth conditions such as in winter contained high caffeine amounts.

The caffeine levels for teas, in general, are influenced by a series of factors including tea species and clone, geographical and environmental conditions, leaves age and plucking position, agricultural and processing conditions. Up to now, many researchers have studied the factors which affected the caffeine levels of tea. Gulati and Ravindranath (1996) reported that air temperature, distribution and amount of rainfall and greater sunshine hours affect the composition of green tea leaves, and higher temperatures lead to higher caffeine contents in Kangra tea. Similarly, Lee et al. (2010), reported that the chemical composition of green teas, including caffeine, has a dependence on the area of growing, precipitation, temperature and sun exposure time. In contrast, Wang et al. (2011), revealed the low influence of climate on alkaloids (caffeine) and determined that humidity is the main factor.

Yao et al. (2006), have analyzed some available black teas from Australian supermarkets, the content of caffeine for the leaf teas and tea bags, indicating that caffeine content could be affected by clone, season, harvesting time. Another, they mentioned that young tea leaves may contain more caffeine than older ones, as a result of changing of caffeine content due to the maturity, from 24% to 40%. Similar results for Lin et al. (2003), who observed that the level of caffeine in old leaves was less than that in young leaves (apical bud and the two youngest leaves).

The effects of different grades black tea on the caffeine levels

Tea leaves are usually sorted (graded) according to many criteria (quality, size, color). Generally, in Turkey in different processing plants, black manufactured teas are sorted into several particle sizes, by sieving them through different meshes. Obtaining seven grades, starting from whole leaves, broken leaves till dust size. 1st, 2nd, and 3rd grade teas do not contain any broken parts while 4th, 5th and 6th grade teas include broken leaves. 7th grade consists of small pieces of tea leaves and dust-tea. Each group of those grades represents a group of quality for processed teas, marketed in different combinations (Özdemir et al., 1999; Alasalvar et al., 2012).

Caffeine levels of different grades of black tea are shown in Table2.The content of caffeine in our findings, in tea samples, varied slightly depending on tea grade. In general, high-quality black tea (grades 1-3) contained higher caffeine content as compared to the low-quality black teas (grades 4-6). The levels of caffeine ranged from 2.21% dry weight in 5th grade tea to 2.56% dry weight in 7th grade tea (Table 2). Caffeine amounts among the seven grades of black tea were found to be insignificant ($p>0.05$).

The composition of tea beverages and final infusions are influenced by many factors; time and temperature of infusion, stirring, teabags and the size of leaves (grade) (Astill et al., 2001; Khokhar and Magnusdottir 2002).

Table 2. Caffeine levels of different grades of black tea (by dry weight %)

Grades	S_1	S_2	S_3	S_4	Mean
1	2.13±0.117	2.92±0.800	2.12±0.134	2.69±0.080	2.47
2	2.37±0.53	2.55±0.466	2.52±0.326	2.41±0.118	2.46
3	2.35±0.684	2.48±0.177	2.19±0.024	2.58±0.091	2.40
4	2.24±0.070	2.38±0.765	2.30±0.166	2.48±0.121	2.35
5	2.29±0.053	2.23±0.327	2.04±0.325	2.27±0.197	2.21
6	2.14±0.524	2.44±0.211	2.31±0.206	2.35±0.123	2.31
7	2.49±0.177	2.65±0.631	2.32±0.057	2.78±0.066	2.56

In previous study by Sun et al, (2010), which had the purpose to investigate some chemical and physical factors on extracting caffeine by a supercritical method., it is found that the size of particles has fundamental effects in extraction (mass transfer, surface area).Therefore, even if we consider our grades (1-7) with the same chemical composition, sieved particles should exhibit different contents of caffeine.

At different extents, significant variations ($p < 0.05$) were revealed, for volatile compounds and some taste-active compounds (sugars, organic and amino acids) for black tea within seven grades. For instance, volatile compounds showed a qualitative and quantitative decline from 1-3 grades to 4-7 grades which might be resulted from different processing methods and parts of tea leaves used. Therefore, 1-3 grades have been considered as high quality and the rest grades (4-7) of black tea as low quality (Alasalvar et al., 2012).

Beside antioxidant activity and polyphenols content, Erol et al (2010) have studied alkaloid levels for the seven grades of Turkish black tea processed by Cay-kur method (orthodox+ rotorvane+ orthodox) collected from the processing factory in the Eastern Black Sea region. They revealed that 1st, 2nd, 3rd and 7th grades had significantly higher content of caffeine than the rest of grades.

Our results were different when compared with the study by Erol et al. (2010) or the previous studies which demonstrated a difference in the chemical composition depending either on the size of particle or tea grade**.** The difference may result from plucking season of tea and processing method. As it is mentioned by Le Gall et al. (2004), tea chemical composition affecting parameters can be more important in some of them than others.

Effect of manufacturing methods on the caffeine levels

Several techniques are employed in the world to process black tea. The most common one is the Orthodox method. According to this technique, the tea leaves are harvested, artificially or naturally withered. Then leaves are rolled by rolling machines. they are let to be subsequently fermented, and at a specific condition, they would be finally dried. This technique is a slightly modified form of Cay-kur method applied in Turkey. The only difference between the two techniques is the application of curling and rotorvane after withering. In some factories, CTC technique is used in addition to curling and rotorvane. It is worth noting that the composition and quality of tea vary depending on the tea processing technique.

Caffeine levels of black tea manufactured by different methods are shown in Table 3.

Table 3. Caffeine levels of different process methods (by dry weight%)

Samples*	Cay-kur	Orthodox
1	2.28±0.051	2.24±0.023
2	2.05±0.038	2.19±0.130
Mean	2.16±0.130	2.21±0.087

** Two tea samples were taken from each manufacturing method (Cay-kur, Orthodox).*

For both examined processing methods (Orthodox and Cay-kur), black teas exhibited similar levels of caffeine. Also, the significance test determined that manufacturing processes have no significance at the contents of caffeine ($p>0.05$). In accordance with the research of Özdemir et al. (1993), who have found as well that processing systems do not have a significant effect on the caffeine content. The study carried out by Turkmen and Velioglu

(2007), and in order to analyze the contents of alkaloids (theobromine, caffeine) and phenolic compounds, they found that caffeine was the dominant alkaloid and no significant differences of black teas of two different processes (Orthodox and Cay-kur) in different plucking seasons.

However, next to various factors affecting teas infusion, Astill et al. (2001) investigated the compositional differences of many manufactured teas, CTC (crushing-tearing-curling) and orthodox. They reported that orthodox black teas contain slightly less caffeine than CTC manufactured teas. This difference could be explained by conditions of distinct stages or especially the least subjection of the Orthodox method for fermentation compared to the CTC method. In contrast, Carloni et al. (2013), CTC black teas were an exception for caffeine levels which were the lowest for all types including orthodox type. The unexpected findings translated to the fact that extraction time had not been sufficient.

Within the processing method, for all different stages (withering, rolling, fermentation, drying and others) and depending on the process conditions, manufactured leaves would show ultimately some physical and biochemical differentiation, in turn affects the quality of black tea (Panda, 2016). As it is reviewed by Tomlins and Mashingaidze (1997), to evaluate the effect of withering on the quality of black teas in which withering method could influence the concentrations of caffeine. Another, the content of caffeine could increase during withering step depending on time and temperature parameter (Ye et al., 2018).

4. CONCLUSION

In general, in the present study, the caffeine content of the black teas was close to that of some similar studies. Turkish black tea is manufactured by different methods, orthodox, rotorvane, CTC (crushing, tearing, and curling) and Cay-kur (orthodox + rotorvane + orthodox). The analyzed caffeine content of black tea manufactured by Cay-Kur and orthodox methods, did not show a significant effect ($p>0.05$) in manufacturing methods on the caffeine amounts. Neither for caffeine levels of seven different grades of Turkish black tea, produced by orthodox method, in spite of being considered among important factors controlling the chemical composition of tea leaves. On the other hand, we have found that the highest caffeine levels were obtained during the first shooting period (May) of tea plucking period, while the lowest levels were in the third period (September). The shooting period had a significant ($p<0.05$) effect on caffeine content and this result was similar to previous studies on Turkish black tea.

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