Determination of The Microflora, Quality and Mineral Substance Contents of Black Tea (*Camellia sinensis*)

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Abstract

The black tea is produced from different varieties of *Camellia sinensis* (Linneaus) O. Kutzeafter withering, rolling, fermantation, drying, classification and packing stages of green tea leaves, buds and the parts of fresh stems which are adjoint to them. In this study, it was planned to determine the microbiological population, quality and mineral substance values in processing stages of tea samples taken from two factories (Zihniderin and Cumhuriyet tea factories). In the study, the avarage of total microorganism for gram negative bacteria, lactic acid bacteria and yeast are found between 35.000 cfu/gr - 300.000 cfu/gr, 37.000 cfu/gr- 237.500 cfu/gr, 27.000 cfu/gr- 475.000 cfu/gr, 28.000-40.000 cfu/gr intervals, respectively at 1st, 2nd, 3rd plucking periods in 2004-2005.The avarege of quality values were measured as 5.21-6.12% total ash, 1.53-1.89% caffeine, 0.21- 0.39% theaflavin and 5.81- 14.02% polyphenol at 1st, 2nd, 3rd plucking periods in 2004-2005.The average of mineral substance values were measured as 8-22 ppm for copper, 120- 343 ppm for iron, 19- 25 ppm for zinc and 952- 1391 ppm for manganese at 1st, 2nd, 3rd plucking periods in 2004-2005.

Keywords— *Camellia sinensis*, tea processing stages, microflora, quality value, mineral substance

1 Introduction

Tea plant in botanics is situated in *Angiospermea* class, *Dicotyledonea* category of *Theaceae* family. The acceptance of *Camellia sinensis* (L) O. Kutze coincides the date in1950 [1]. Tea, which shows morfological differences, has three different kinds and these are Chinese, Assam, and Kampuchean teas. Chinese tea (*C. sinensis* var. s*inensis*); naturally 1-3 m height, has huge bush and brances are placed close together and has strong build. It is resistant to cold, diseases and drought. Assam tea (*C. sinensis* var. *assammika*) is able to grow 6-8 meters and has widely aparted branches. Under convenient growth, it has more fruitful and quality tea in comparison to Chinese and the other tea species. Kampuchean tea (*C. sinensis* var. c*ombodiensis*) is originated from Chine-Indian and able to grow 6-8 meters. It is more often like the appearance of Assam and Assam-Chinese hybrids [1, 2, 3, 4].

Black tea is made with young and fresh tea leaves and by subjecting the buds some processes like the rolling, withering, oxidation and drying. The withering stage is important for chemical changes taking place and for the reduction of the moisture levels from 70-83% to 62-64% depending on the specific manufacturing process being used. The purpose of rolling is to reduce the size of the leaf particles as well as cell disruption and contaminate it to the tea leaves that were curled. As a result of this, many biochemical events inluding respiratory that takes place in a great harmony and order in living microorganisms change fundamentally.

The heat of this stage begins increasing rapidly, it changes between 27-32 °C. During the oxidation stage, the polyphnols are oxidized to from the characteristic compounds of black tea. Thus, it is the formation phase of the manufactured tea's color, astringency, aroma, odor, flavour quality and brilliance [5, 6]. In this phase, polyphenols change and become theaflavins or thearubigins that are the best quality products. Oxidation is excellently materialized at 24-28 °C and around 60 minutes. Generally, there are alcoloids (caffeine etc.), polyphenols (catechin and flavonoids etc.), polysaccharides, readily vaporizable fats, vitamins, minerals and purinein the combination of leaves and according to the process style and the quality the ratios of those changes. After the oxidation the moist leaf is dried using heated (87–99 °C for 20-25 minutes) air and reduced of moisture to 2,5-35%. The drying process is important for some of flavour characteristics of the final product. Before drying, because of several reasons, the bacteria and fungi that contaminated the tea die wholly under high temperature [5, 6, 7, 8].

In Rize, green tea is harvested between May and October and it occurs three times a year. In this study, it was aimed the determination of microflora and mineral substances in phases of green tea prosesses.This study is first study to determine Rize tea microflora.

2 Materials and Methods

2.1 Taking samples

The research was set in association with Rize University, Faculty of Arts and Sciences, Department of Biology and Atatürk Tea and Garden Cultures Research Institute Directory connected to Tea Administrations General Directorship (ÇAY–KUR) in Rize. In the study , tea samples were supplied from two factories (Cumhuriyet and Zihni Derin Tea Factories) that belong to ÇAY–KUR to investigate microflora, quality and mineral substance concentrations. In two years (2004-2005), from each of the plucking periods, a total of 120 tea samples were taken from 10 different places which represent the processing phases of tea infactories (Table 1). The samples were taken simultaneously into sterile petri dishes for microbiological study, and into polyethylene bags to study in terms of quality. They were brought to the laboratory on the same day and tested.

Table 1. The sample stations in Cumhuriyet and Zihni Derin Tea Factories

Station No	Sample stations
1	Withering entrance
2	Withering exit
3	First curling
4	Rotervan exit
5	Second curling
6	Fermentation entrance
7	Mid-Fermentation
8	Fermentation exit
9	Fumace entrance
10	Fumace exit

2.2 The microbiological investigation of the tea samples

For microbiological analyses, 10 g of tea samples was taken into steril Erlen-meyer that includes 90 ml serum physiology under sterile conditions and shaking for 30 min.

By using macrodilution method (1:9), 10^{-4} dilution was preparated from samples. Plate count agar (PCA), Eosin methylene blue agar (EMB), MRS agar and Potato Dextrose Agar (PDA) were used to count total bacteia (aerobic and facultative anaerob mesophilic bacteria), total coliform bacteria, the bacteria that can reproduce in an acidic environment and yeasts, respectively. Serial dilutions were prepared, and 100 microliter (µl) was plated onto PCA, EMB, MRS and PDA agar and spreaded by the help of glass baguet. Then, they were incubated at 35 °C for 24 hours. For the count of yeast, from the last two dilutions to the PDA, spreading was fulfilled as 100 and 1000 µl and it was incubated at 25 °C for 7 to 10 days. The number of microorganisms that form colony in 1g tea (colony forming unit/gram= cfu/g) was determined after incubation [9].

2.3 The quality and mineral substance analyses of tea samples

The samples taken during processing phases were brought to laboratory the same day for quality analysis, and were dried at 103°C about 45-60 minutes in incubator. The dried samples were numbered by sifting with a 30 mm diametered sieve after being smashed by physical power and they were packed and made ready for the analyses. For quality analyses, the amounts of dried materials and total ash were analysed according to the method TS 1563, the theaflavin contents, according to the method which was proposed by Ellis and Cloughley[10], the amounts of the caffeine according to the spectrophotometericmethod [11] and as for the amounts of the polyphenol according to Löwental [12] were analysed and the values in one

gram tea sample were calculated as percentages. One gram sifted tea sample was taken for the determination of mineral substances (iron, copper, manganese, zinc), it was washed with nitric-perchloric acid and then their reading was done in atomic absorbation [13].

3 Results

3.1 Determination of Microbial flora

In the study, the colonies that grown in PC agar for total bacteria, in EMB agar for Gram negative bacteria, in MRS agar for the lactic acid bacteria, and in PDA agar for yeasts and moulds were counted for each station individually in each plucking periods.

The avarege of the total microorganism number in tea samples taken from Cumhuriyet and Zihniderin Tea Factories for both station and three plucking were given in Figure 1. When comparing 2014-2015 data, the number of total microorganism of 2015 is higher than 2014. The upper limits for total microorganism are 105.000 cfu/gr, 37.500 cfu/gr and 35.000 cfu/gr for first, second and third plucking of 2014, respectively. Also, the upper limits for total microorganism are 200.000 cfu/gr, 300.000 cfu/gr and 170.000 cfu/gr for first, second and third plucking of 2015, respectively. Isolation of total microorganisms were performed each station except for tenth station. In tenth station, total microorganisms were not isolated both years and each plucking.

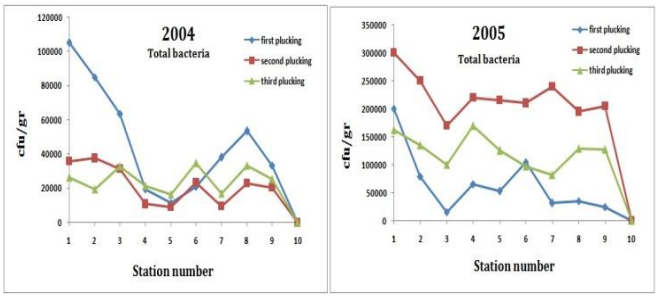

Figure 1. The avarege of the total microorganism number in tea samples taken from Cumhuriyet and Zihniderin Tea Factories according to the years and plucking periods.

The EMB agar was used to determine gram negative bacteria populations in tea samples taken from tea processing station in factories. The results was shown that gram negative bacteria populations are quite highuntil fourth station. The upper limits for gram negative bacteria are 65.000 cfu/gr, 87.500 cfu/gr and 37.000 cfu/gr for first, second and third plucking of 2014, respectively. Also, the upper limits of total microorganism are 200.000 cfu/gr, 237.500 cfu/gr and 180.000 cfu/gr for first, second and third plucking of 2015, respectively (Figure 2).

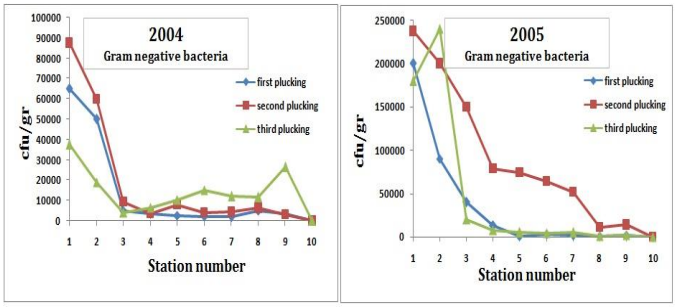

Figure 2. The avarege of the Gram negative bacteria number in tea samples taken from Cumhuriyet and Zihniderin Tea Factories according to the years and plucking periods.

The avarege of the lactic acid bacteria number in tea samples taken from Cumhuriyet and Zihniderin Tea Factories for both station and three plucking were given in Figure 3. The MRS agar was used to determine lactic acid bacteria populations in tea samples. The upper limits for lactic acid bacteria are 27.000 cfu/gr, 115.000 cfu/gr and 29.000 cfu/gr for first, second and third plucking of 2014, respectively. Also, the upper limits for lactic acid bacteria are 170.000 cfu/gr, 300.000 cfu/gr and 475.000 cfu/gr for first, second and third plucking of 2015, respectively.

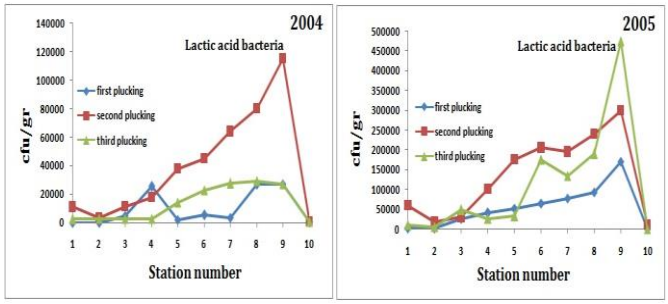

Figure 3. The avarege of the lactic acid bacteria number in tea samples taken from Cumhuriyet and Zihniderin Tea Factories according to the years and plucking periods.

The PDA agar was used to determine yeast populations in tea samples taken from tea processing station in factories. The results was shown that yeast populations begins to rise after fourth station. The upper limits for yeast are 28.000 cfu/gr, 11.000 cfu/gr and 32.500 cfu/gr for first, second and third plucking of 2014, respectively. Also, the upper limits of yeast are 35.000 cfu/gr, 40.000 cfu/gr and 11.500 cfu/gr for first, second and third plucking of 2015, respectively (Figure 4).

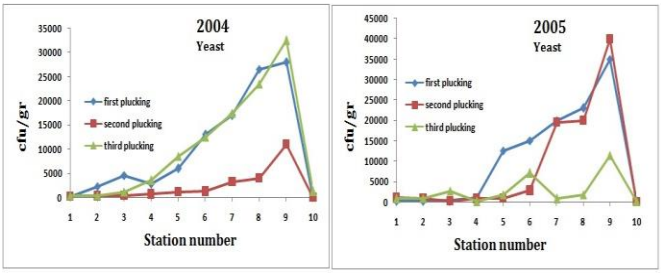

Figure 4. The avarege of the yeast number in tea samples taken from Cumhuriyet and Zihniderin Tea Factories according to the years and plucking periods.

3.2 Determination of Quality

The average analysis results of total ash, caffeine, theaflavin and polyphenolwere given in Table 2. In 2014 and 2015, the maximum and minimum values measured in sample stations were 5.21-6.12% and 5.28-5.99% for total ash, 1.53-1.89% and 1.67-1.81% for caffeine, 0.21-0.37% and 0.21-0.39% for theaflavin and 6.25-15.09% and 5.81-12.97% for polyphenol.

In the two factories, regarding to plucking periods and years, the general average quality values were shown in Table 2. The highest total ash values were as 5.99% in 2005 in the 3rd plucking periods, caffeine was as 1.89% in 2004 in the 1st plucking periods, theaflavin was as 0.39% in 2005 in the 1st plucking periods and for the polyphenol 12.97% in 2004 in the 2nd plucking periods.

Table 2. Mean percentage values of the quality variables for year and plucking periods by tea factories.

Station	2004			2005		
	1st	2nd	3rd	1st	2nd	3rd
	Plucking			Plucking		
	Total ash					
1	5,43	5,47	5,92	5,35	5,28	5,55
2	5,41	5,34	5,62	5,3	5,42	5,63
3	5,46	5,93	5,57	5,36	5,53	5,9
4	5,68	5,46	5,90	5,35	5,62	5,99
5	6,02	5,58	5,76	5,49	5,55	5,85
6	5,21	5,67	5,63	5,4	5,59	5,86
7	5,63	6,12	5,75	5,39	5,52	5,93
8	5,68	5,88	5,48	5,37	5,59	5,91
9	5,73	5,73	5,6	5,43	5,69	5,90
10	5,75	5,75	5,77	5,49	5,68	5,92
	Caffeine					
1	1,89	1,59	1,57	1,78	1,73	1,68
2	1,89	1,71	1,61	1,81	1,78	1,72
3	1,88	1,72	1,59	1,78	1,78	1,71
4	1,84	1,7	1,55	1,78	1,75	1,69
5	1,85	1,68	1,56	1,83	1,75	1,7
6	1,85	1,66	1,55	1,80	1,75	1,71
7	1,83	1,65	1,53	1,8	1,76	1,69
8	1,88	1,67	1,54	1,8	1,77	1,67
9	1,81	1,71	1,58	1,81	1,76	1,69
10	1,81	1,72	1,56	1,81	1,81	1,71
	Teaflavin					
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0,3	0,24	0,24	0,28	0,27	0,22
4	0,36	0,27	0,22	0,28	0,28	0,21
5	0,35	0,25	0,21	0,31	0,27	0,24
6	0,34	0,23	0,21	0,32	0,25	0,24
7	0,37	0,26	0,22	0,34	0,25	0,26
8	0,35	0,26	0,24	0,35	0,27	0,26
9	0,35	0,24	0,24	0,35	0,28	0,28
10	0,35	0,32	0,27	0,39	0,29	0,28
	Polyphenol					
1	13,52	15,09	12,39	12,97	11,81	10,66
2	14,02	15	11,75	12,61	11,33	11,5
3	8,76	8,93	7,68	9,11	8,92	8,57
4	8,3	8,69	7,49	8,32	8,13	7,78
5	7,95	7,6	6,92	8,19	8,02	7,35
6	6,97	6,73	6,25	7,45	7,23	6,83
7	6,74	6,98	6,69	6,34	7,68	6,7
8	6,71	6,87	6,57	5,81	7,15	6,6
9	6,8	6,82	6,49	5,98	7,15	6,52
10	7,76	7,91	6,4	6,01	7,3	6,9

3.3 Determination of mineral substances

The values of copper, zinc, iron, and manganese were investigated and the results were calculated as ppm. The lowest and highest values measured in the sample stations were8-22 ppm for copper, 120-343 ppm for iron, 19-25ppm for zinc and 849-1391 ppmfor manganese. Average values of mineral analysis results for sample stations were given in Table 3.

Table 3. Mean values (ppm) of the mineral substance variables for year and plucking periods by tea factories

Station	2004			2005		
	1st	2nd	3rd	1st	2nd	3rd
	Plucking			Plucking		
	Cu					
1	8	8	8	11	10	10
2	9	8	8	10	11	10
3	10	11	11	11	12	11
4	9	9	9	11	14	11
5	8	9	8	11	12	12
6	11	12	10	15	17	13
7	12	10	10	16	19	15
8	11	10	10	16	21	14
9	11	10	9	16	21	14
10	10	9	9	14	22	14

	Fe					
1	147	120	120	158	281	193
2	163	131	127	146	227	198
3	158	178	135	186	343	194
4	138	140	128	189	321	221
5	148	151	147	194	333	199
6	176	170	153	205	314	201
7	177	159	132	175	330	221
8	173	165	144	195	313	178
9	175	155	155	194	295	203
10	179	161	164	201	259	182
	Zn					
1	20	23	20	22	22	22
2	23	19	20	21	23	21
3	24	23	22	22	24	21
4	21	21	23	24	22	21
5	24	20	22	24	24	21
6	24	22	23	24	23	22
7	24	21	21	25	25	22
8	23	24	22	24	23	23
9	23	21	20	24	23	20
10	23	21	22	24	21	19
	Mn					
	1140	1328	1233	1120	1427	1093
2	1143	1195	1158	1080	1040	1027
3	1029	1033	1035	922	1010	1103
4	1005	981	1278	875	951	1230
5	1019	952	1343	890	1000	1293
6	998	1148	1345	849	992	1325
7	1084	1062	1203	925	1051	1391
8	1070	1278	1303	930	1098	1279
9	993	1027	1184	978	1116	1256
10	1092	1128	1221	1014	1174	1216

In the tea samples, mean values of copper content were obtained as 8-12 ppm intervals in 2004 and as10-22 ppm intervals in 2005. The highest copper content value was obtained in 2005 at the 2nd plucking period. The mean values of irron content were obtained as 120-179 ppm intervals in 2004 and as 146-343 ppm intervals in 2005. The highest irron content value was obtained in 2005 at the 2nd plucking period. The mean value of zinc content were obtained as 19-24 ppm intervals in 2004 and as19-25 ppm intervals in 2005. The highest zinc content value was obtained in 2005 at 1st and 2nd plucking periods. The mean value of manganese content were obtained as 952-1345 ppm intervals in 2004 and as 849-1391 ppm intervals in 2005. The highest manganese content value was obtained in 2005 at 3rd plucking period.

4 Discussion

The total bacteria contain aerobic and facultative anaerobic, Gram positive and all the negative bacteria which can reproduce in basic nutrition places and can be found widely extended. Gram negative bacteria contain the fermentative or the non–fermentative bacteria that can reproduce in EMB, as for the lactic acid bacteria in MRS agar media at pH 4.0-6.5, the sporeless microorganisms that are in Gram positive coccus or bacillus morphology and can reproduce in aerobic and facultative anaerobic conditions [9]. Fungi are usually filamentous, multicellular, heterotrophs and exhibit absorptive nutrition. The majority of fungi are aerobes, although a number of species, including certain types of yeast, are capable of a facultatively anaerobic existence [14].

Although tea leaves have their specific microflora, their microbial population can change proportionally with environmental factors that they were in touch with until their arrival from the garden to the factory. The tea leaves brought to factories are firstly subjected to withering and then rolling processing. During withering, plant loses water and it occurs decline of number of microflora in parallel [7]. Rolling is a required phase for the oxidative reactions to be more rapidly and in this stage, the combinations of the leaves mix with each other. Also, because of theanticeptic characteristics of oxide polyphenols, microbial population decreases during the rolling phase [15]. In this study, when looked over microorganism populations in general it is observed that the number of total bacteria and Gram negative bacteria are high, contrary to this, the number of lactic acid bacteria and yeasts are low from the first section of teas arrival to the factory (1st station) until the second rolling (4th station).

Oxidation is the most important phase of black tea processing. It is fulfilled at room temperature by providing contstant air circulation and 96-98% relative humidity. In this phase, it is indicated that the microorganisms have no role but wholly oxidation has[7].In this study, it is seen that between the stations 5-9 that are the beginning and finishing stations of oxidation, the number of yeasts and the bacteria that can reproduce in an acidic environment are becoming higher. The kampuchean tea which is a traditional fermented production is a product that was obtained from the fermentation of black tea with sugar. In this product, the presence of several symbiotic yeasts and acidic bacteria is indicated [16, 17]. At the same time, the increase in the numbers of yeasts and lactic acid bacteria in the oxidation phase shows that the microbial activity is lasting in this study.

Liu et al. [18] studied microorganisms on fermented tea samples in Thailand and they isolated acidic bacteria (*Acetobacter aceti*, *A. Pasteurianus*) and yeasts (*Saccharomyces cerevisiae*, *Zygossacharomyces bailii* and *Brettanomyces bruxellensis*). In the same research, they stated that yeasts and *Acetobacters* impeded the reproduction of other microorganisms in tea. The same

result is to be in our study. In between 5-9 stations, while lactic acid bacteria and yeast population rapidly increased, total and gram negative bacteria populations rapidly decreased.

In the present study, no microorganisms could not be determined in tenth station. On the leaves that are subjected to drying, enyzmes become inactive, biochemical reactions stop and microbiological activities end. The microorganism population in nature is affected by the environmental factors like atmospheric conditions, rain and temperature. The investigated microbiological values in 2005 were found higher in an important level as to the year 2004 and this was thought to be because of the number of the sunny days in 2005 was too many. It was observed that in the 2nd and the 3rd plucking period, generally higher population was found. The reason for this was the low temperature during May and June, but for July and September, the occurance of more convenient temperature for microbial growth.

The amount of total ash may show the differences in connection with plucking time, the growth era, the physical structure of the leaf, processing conditions, the cleanliness of the tea during plucked and processing [8, 19, 20]. Nas and Gökalp, [21] showed that the amounts of total ash in black tea samples taken from 1st, 2nd and 3th plucking periods in different tea factories were 5.6-6.64%, 5.69-6.6% and 5.71-8.02%, respectively. In this study, the amounts of total ash in tea samples taken from tea processing stations were found 5.28-6.12% during two years and six plucking periods. These values are within the limits given in the literature.

Caffeine is a purine derivative compound that forms 3-4% of dry weight of black tea and gives bitter and sharp taste to the tea. Caffeine level increases during processing and high levels of caffeine indicate a good leaf Standard [22]. Some researchers reported the amounts of caffeine in Turkish tea, 1.79% as the lowest and 4.96% as the highest[19, 23]. In this study, the amounts of caffeine in tea samples taken from tea proccessing stations were found at 1.53% as the lowest and 1.89% as the highest.

In this study, the amounts of theaflavin in tea samples taken from tea proccessing stations were found at 0% in beginning two stations (Withering entrance and Withering exit) and it was stated that the 7th, 8th, 9th and 10thstations (starting fermentation phase) had higher value in comparison to the other stations. The theaflavin and the thearubigin compounds occurs with oxidative changes from phenolic substrates during fermentation phase of tea manufacturing [24]. The theaflavin and thearubigin give the manufactured tea quality features like color, astringency, strenght and liveliness [25]. There isn't any obligation about the minimum teaflavin amounts that black teas must include. It is stressed that the theaflavin values and sensory characteristics of black tea show variation according to manufacturing method, oxidation time and temperature, the kind of tea, climate condition, the conditions of environment where the growth existed, the altitude and the plucking periods [15].

Polyphenols form the drink features of black tea like colour, odor and taste [25]. Considering of the research that carried out on Turkish teas; polyphenol content in Turkish teas samples was declared that as 6.10-9.92% and 19.6-21.1% by Kaptan [19] and Yurdagel [23], respectively. Anessini et al. [26] investigated total polyphenol content in Argentia tea, and declared that the total polyphenol concentration in black tea was 8.42-17.62%. In this study, the amounts of polyphenol content in tea samples taken from tea proccessing stations were found 5.81-15.09% as minumum and maximum value.

In this study, high average copper values were obtained in 2005 as to 2004. In two years, the mean values of cupper content between the 1st – 3rd stations are lowest than other stations. These results showed the amount of copper in the plant structure and supported that there was not any copper contamination during manufacturing. In literature, the amounts of copper content in manufactured black teas were declared at 70-80 ppm and 38.9-42.6 ppm by Kacar [27] and Ozdemir et al. [28], respectively. Lamb [29] showed that copper content changed at 16-30 ppm intervals in tea leaves.

In tea plant, the iron content increases from the young leaf towards the old leaf. Some researchers reported that the iron content of tea were as 69-577, 82-440 and 300-700 ppm, 21.99-37.64 ppm [28, 30, 31, 32].It is declared that the iron contents of the green tea leaves harvested from 30 producer tea gardens were calculated between 52-296 ppm in the Eastern Black Sea Region [27]. In this study, the iron contents of tea samples taken from tea processing stations were found between 119.5-343.0 ppm. These value shows complies with studies in literature.

The zinc content of tea plant changes dependent upon the kind of the plant, the location of the leaf in the plant and the age of the leaf. The zinc contents of young leaves are higher, but the differences in between are not very great. The zinc values in Turkish teas are declared as 31-44; 26-143; 28-56 and 30-40 ppm by [28, 31, 33, 34], respectivly.

The manganese in the tea plant is excessive in comparison to other culture plants. The mangenese content of tea increases from the young leaf towards the old leaf, and the manganese content of the old tea

reaches its highest level.The research was done byMatsushima et al. [35] showed that the content of manganese was 1440 μg/g in the case of wulong tea, 670 μg/g in green tea, and 535 μg/g in black tea. In several studies, Mn content of the Turkish teas were obtained 360-1510; 379-868 and 1053-1619 ppm by [27, 31, 34], respectively. In this study, our manganese results are within this limits.

Tea, is the most consumed beverage in the world after water. *C. sinensis* which naturally grows in India and China, first spread out to Japan and later to Asia, Arabia, Russia and Europe. Polyphenol oxidation is the most important phase of black tea processing. The increase in the numbers of symbiotic yeasts and acidic bacteria in the oxidation phase shows that the microbial activity is increasingly lasting. Moreover, this increase shows a positive correlation with theaflavin values. It is thought that the characterizations of symbiotic yeasts and acidic bacteria and its addition during the oxidation phase provides a more quality production of tea.

Acknowledgments

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