

Original Article

Effects of the Residue Types, Harvest Seasons, and Factories on the Bioactive Compounds of Black Tea (*Camellia sinensis*) Residue

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Article History

Received: 16 February 2022
Accepted: 29 October 2023
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Keywords

Black tea
Factories residue
Plucking time
Bioactive compounds

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ABSTRACT

Annually, lots of useless black tea [*Camellia sinensis* (L.) Kuntze] residue is produced in Iranian factories; nonetheless, their bioactive compounds can be extracted and used in the pharmaceutical and food industries. The investigation, which looked at the extraction of all compounds from tea residue, was conducted from 2006 to 2008 to solve environmental problems related to the disposal of tea residues. Extracting one compound to build a lateral products factory is unprofitable, but extracting all bioactive compounds can increase productivity. Four bioactive substances (caffeine, polyphenol, protein, and fiber) were extracted from four tea residues types (dust, fluff, footstalk, and stalk) that nine tea companies generated in the spring, summer, and autumn, assessed in this study. Caffeine was measured by spectrophotometer, followed by cellulose and polyphenol by weight technique, and protein by micro-Kjeldal. Statistical analysis was done on a split plot in randomized complete blocks with three replications (sampling places were randomly selected, and treatments were fixed). The triple effect of residue types, harvest seasons, and factories were significant at a 0.01 level on the levels of caffeine, protein, and fiber, according to the variance analysis findings. The highest amount of caffeine in the dust × summer × Moein factory, followed by protein in the dust × spring × Poltan factory, and fiber in the stalk × autumn × Tohied and Setareh Shomal factories, was observed. Finally, the result demonstrated that consideration must be given to the types of residue, harvest seasons, and factories when using tea factory residues for industrial purposes.

INTRODUCTION

Tea, known as *Camellia sinensis* (L.) Kuntze, is a species of plant whose leaves and buds are used to produce tea. *Camellia* is a genus of plants in the family Theaceae that contains nearly 4,000 bioactive compounds, of which one-third are contributed by polyphenols (tannins) [1, 2].

Other compounds are alkaloids, amino acids, carbohydrates, proteins, chlorophyll, volatile organic compounds, fluoride, aluminum, minerals, and trace elements. Polyphenols in tea are mostly flavonoids [3, 4]. Polyphenols are a large group of plant compounds that comprise catechins. They offer several health benefits [5]. Tea's major catechins are the epicatechin, the epicatechin gallate, the epigallocatechin, and the epigallocatechin gallate in tea, epigallocatechin gallate is the most active catechin, acting as an antioxidant, antibacterial, antiviral, lipid-lowering,

hypoglycemic, hypotensive, and inhibiting DNA hypermethylation, telomerase, metastasis, caspase, and metalloproteinase [6].

Caffeine is a bioactive chemical found in tea as well as coffee, guarana, and cacao. It usually tastes bitter and is often physiologically active in humans. They act as stimulants of the central nervous systems, muscles, and circular systems and are consumed in pharmaceuticals and beverages. It creates health risks for children, pregnant women, and some patients [7].

The protein found in tea has many biological functions in many aspects, such as antitumor, anti-inflammation, antiviral, lowering blood sugar, anticaducity, and anticoagulant [8, 9].

Fiber is a group of tea polysaccharides that have been used in the pharmaceutical and food industries for a long time due to biological activities. It is one of the main bioactive components of tea that has

activities of immunologic, anti-oxidant, anti-irradiation, anti-coagulation, anti-cancer, anti-HIV, and hypoglycemic [10].

In a research, the sequential extraction of bioactive compounds was reported from tea residues. The caffeine content (1.5–3.5%), polyphenol content (8–10%), protein content (10–15%), and fiber content (15–20%) were extracted, respectively, by a sequential model [11]. Also, the residue of tea factories was studied as an edible color [12].

Tea stalk and fiber residue have no economic value, so cheap raw materials make the investigation of the extraction of bioactive compounds from tea residue a promising program for technological applications, which is necessary [7].

The purpose of this project was to investigate the amount of bioactive compounds in residue types, harvest seasons, and factories.

MATERIALS AND METHODS

In this study, nine factories in Iran's tea-growing areas (three in each of the eastern, central, and western regions) processed black tea wastes following the typical procedure over the three harvest seasons (spring, summer, and autumn) [13]. The factories were called Moein, Poltan, Saba, Shokoh Chay, Mersad, Liseh Road, Tohid, Setareh Shomal, and Sabzineh Gilan, in that order. The factory residues were blended of fluff, footstalk, and stalk; therefore, they were separated and dried at $105 \pm 2^\circ\text{C}$ for 2 hours, then ground using a cutting mill and sieved. The main components of caffeine - polyphenols, protein, and fiber were extracted in replicates dust, fluff, footstalk, and stalk.

Statistical analysis is used on a split plot in randomized complete blocks with three replications (sampling places were randomly selected, and treatments were stabilized). The comparison of means is based on Duncan's multiple-range test.

Determination of Main Components Content

Caffeine: 1 g of each tea residue was put in a separator funnel, and 0.88N ammonium solution, equivalent to 42%, was added. During the four stages, 25 ml chloroform was added to the separator funnel and stirred slowly. 5 ml was removed and diluted with 100 volumes of chloroform after four steps of chloroform extraction. The standard caffeine solution was analyzed at different concentrations by spectrophotometer, and the results

were used as a calibration graph (Fig. 1). The absorption of caffeine was determined at the 276 nm wavelength [14].

Polyphenol: 2 g of each tea residue was added to 200 ml of boiling distilled water and heated for about 30 minutes. The solution was filtered, and the remaining was washed several times with boiling water. Then, as a precipitator, 50 ml of copper acetate (4%) was added to the diluted tea extract. Precipitates were passed through filter paper and washed several times with cold water. They were burned in an electric furnace after drying in an oven at 103 to 105 °C. Then, the remainder weighed. The polyphenol amount was obtained by calculations [15].

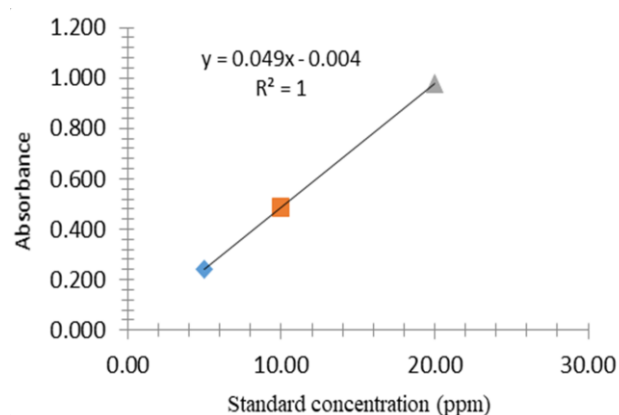


Fig. 1 Calibration curve of caffeine standard

Protein: 0.3 g of each tea residue was weighed into the balloons, and then 3.5 ml of salicylic acid, 3% w/v sulfuric acid, was added. About 24 hours later, digestion was performed on a heater at 350 °C for 2 hours, and 2 ml of hydrogen peroxide was added. This addition was continued until completely bleached and then reached 100 ml with distilled water. The amount of protein was determined by micro-Kjeldal [16].

Fiber: 1 g of each tea residue was refluxed in 200 ml of sulfuric acid (0.255 M) for 30 min, then filtered. The obtained substance was subsequently refluxed in 200 ml of sodium hydroxide (0.313 M), then filtered and washed. The remaining was collected as fiber [17].

RESULTS AND DISCUSSION

The ANOVA results are given in Table 1. All simple, dual, and triple effects were significant on the amounts of caffeine and protein. The fiber results indicated that the all-simple effects, the dual effects of the residue types \times factories, and the triple

effect of the residue types \times harvest seasons \times factories were significant for the amounts of fiber. The polyphenol findings showed that only the simple effect of the residue types was significant on amount of polyphenol.

The mean comparison of the bioactive components for the residue types has been indicated at Table 2. The dust treatment had the highest amount of caffeine, polyphenols, and protein and the lowest amount of fiber.

The fluff treatment was ranked second for all bioactive combinations. Footstalk treatment was ranked third for caffeine and polyphenol, followed by second for protein and fiber. The stalk had the highest amount of fiber and the lowest amount of caffeine, protein, and polyphenol.

The mean comparison of the bioactive components for the harvest seasons is demonstrated in Table 3. The highest amounts of caffeine, protein, and fiber were measured, respectively, in the summer, spring, and autumn.

The mean comparison of the bioactive components for the factories has been indicated in Table 4. The highest amounts of caffeine, protein, and fiber were measured, respectively, in factories No. 1, No. 2, and No. 4.

The mean comparison of the bioactive components for the residue types \times harvest seasons has been indicated in Table 5. The highest amounts were measured for caffeine in the dust \times summer and protein in the dust \times spring.

The mean comparison of the bioactive blends for the residue types \times factories has been represented in Table 6. The highest amount of caffeine was measured in the dust \times No. 1 factory, followed by protein in the dust \times No. 2 factories, and fiber in the stalk \times No. 3, No. 7, and No. 8 factories.

The mean comparison of the bioactive components for the harvest seasons \times factories has been indicated in Table 7. The highest amount of caffeine in the spring and summer \times No.1 and No.4 factories, followed by protein in the spring \times No.1 factory, and fiber in the summer and autumn \times No.4 and No.8 factories, were measured.

The means comparison of the bioactive components for the triple effect includes the residue types \times harvest seasons \times factories had 108 means comparing among them was difficult, so the 30 means were selected, including maximums and minimums, and then ranked (Table 8). The test to

measure specific differences between pairs of means was performed by the Duncan Multiple Range Test (DMRT). The highest amount of caffeine in the dust \times summer \times No. 1 factory, followed by protein in the dust \times spring \times No. 2 factory, and fiber in the stalk \times autumn \times No. 7 and No.8 factories, were measured.

Caffeine

In this examination, caffeine amount was measured in the tea residue from 0.60 to 3.58% (table 8). The research results on the tea residue of Sri Lanka showed that the caffeine amount was 1.5 to 3.6% [18]. The means comparison of the caffeine for the residue types showed that the highest amount of caffeine (2.37%) was in the dust, and the lowest (1.28%) was in the stalk (Table 2). Caffeine is synthesized in the leaves, and the dust is part of the leaf, so the amount of caffeine is high, and then caffeine from the leaves is entered into other parts of the plant. Fluff is the back hair of the leaf, and its caffeine amount is close to the dust. Footstalk, as leaf veins, was ranked third, and the stalk had the lowest caffeine amount.

The means comparison of the caffeine for the harvest seasons showed that the highest amount of caffeine (2.39%) was in the summer, and the lowest (1.27%) was in the autumn (Table 3). The harvest seasons affects on the amount and type of chemical components of black tea and residue due to climatic conditions [19]. In a research, the effect of tea plucking kinds including one leaf and a bud (1 + 1), 2 + 1, 3 + 1, 4 + 1 \times the harvest seasons on the quality factors showed that the highest amount of caffeine was in 1 + 1 > 2 + 1 > 3 + 1 = 4 + 1 \times summer [20].

The means comparison of the caffeine for the factories indicates the highest amount of caffeine in the No. 1 factory and the lowest amount in the No. 7 and No. 8 factories (Table 4). Eastern factories (No. 1, 2, 3), Central factories (No. 4, 5, 6), and Western factories (No. 7, 8, 9) are rendered in Table 4. The outcomes of this table illustrate that the highest amount of caffeine was in the eastern factories and the lowest amount of caffeine was in the western factories due to the ratio of 1st-degree leaves to 2nd-degree leaves; this ratio in eastern factories was higher than in central, and western factories (Tables 9 and 10). The conditions and stages of black (orthodox) tea making in all factories were the same and had no effect on the amount of caffeine, and its

amount was directly related to the tea plucking patterns; the caffeine amount in standard plucking was higher than non-standard plucking [21].

The means comparison of the caffeine for the residue types \times harvest seasons indicated the highest amount of caffeine was in the dust \times summer and the lowest content of caffeine in the stalk \times autumn (Table 5). In a research, the caffeine amount in black tea residue types (residue was assigned by the Kashef Research factory in Lahijan Tea Institute) \times harvest seasons, was measured and results showed that the highest amount of caffeine %2.618 was in the dust \times summer and the lowest amount of caffeine % 1.66 in the stalk \times autumn [22].

The means comparison of the caffeine for residue types in the factories showed that the highest amount of caffeine was in the dust \times No. 1 factory and the lowest amount of caffeine in the stalk \times No. 8 factory (Table 6). The results of this research showed that the amount of caffeine in the residue types from the eastern factories was higher than in the central and western factories.

The means comparison of the caffeine for the harvest seasons \times factories indicated the highest amount of caffeine was in the spring and summer \times No.1 and No.4 factories. The lowest amount of caffeine was in the autumn \times No. 5 factory (Table 7). The findings of this research showed that the highest amount of caffeine in all factories was in the summer and the lowest amount in the autumn. The means comparison of the caffeine for the residue types \times harvest seasons \times factories showed that the highest amount of caffeine was in the dust \times summer \times No. 1 factory and the lowest amount of caffeine was in the stalk \times autumn \times No. 9 factory (Table 8). The results of this table showed that the caffeine amount in the dust was higher than in fluff, footstalk, and stalk and increased in the summer ratio to the spring and autumn. Also, the caffeine amount in the eastern factories was higher than in the western and central factories due to the high proportion of the 1st to 2nd degrees leaves (Table 10).

Reducing Polyphenols (Antioxidants)

In this analysis, the measured amount of polyphenol was in tea residues ranging from 5.80 to 11.53% (Table 8). Regarding polyphenols, the results indicated that the simple effect of residue types was significant on the amount of polyphenol. The highest amount of polyphenol was measured in the

dust because it was synthesized in leaves, and the lowest amount was in the stalk (Table 2). In a research, the amount of polyphenols in tea residues was reported 11.8% [23]. The simple effect of harvest seasons and factories and the dual and triple effect of residue types \times harvest seasons \times factories were insignificant on the polyphenol amount. The amount of polyphenols in the tea plant depends on the tea variety and environmental factors such as climate, light, rainfall, temperature, soil (as concerns nutrient availability), and leaf age [24]. The results of this research showed that tea plant genetics had the most influence on the amount of the polyphenol, of which all the bushes were seedlings.

Protein

The nutritional value of tea residues is related to the amount of protein that can be used in the diet of Livestock and poultry [11]. In this investigation, the measurement range of protein amount in tea residues was between 9.17 and 29.49% (Table 8).

The means comparison of the protein percentages in residue types revealed that the highest amount of protein, 24.87 %, was observed in the dust because it was synthesized in leaves and the lowest content 11.28% in the stalk (Table 2). In a research, the maximum amount of protein in residues was reported 29.3% [23]. Also the highest amount of protein was measured in tea leaves from 21 to 28% [25]. In a study, the highest protein amount in black tea residue types (residues represented by the Kashef Research factory in Lahijan Tea Institute) was measured in the dust with 28.14%, and the lowest in the stalk with 11.93% [22].

The means comparison of the protein amounts in harvest seasons showed that the highest protein amount was in the spring and the lowest in the summer and autumn (Table 3). Harvesting seasons affect the quality factors of tea. Different seasons change the amount and type of chemical compounds in the leaves, as well as the chemical compounds of black tea and residues [19]. The means comparison for factories showed the highest and lowest protein amount were measured in No. 2 and No. 7 factories, respectively (Table 4). The highest amount of protein was assigned to eastern factories and the lowest to western and central factories because there were more than 1st-degree leaves in the Eastern factories (Table 10)

Table 1 ANOVA of the bioactive compounds in tea waste from nine factories in three seasons.

Source of Variation	Df	Mean squares			
		Caffeine	Protein	Fiber	Polyphenol
Factories	8	1.677 **	27.876 **	62.34 **	9.252 ^{ns}
E ₁ (first error)	18	0.001	6.241	0.437	5.43
Residues	3	17.113 **	2947.883 **	8537.125**	33.193 **
Residues × Places	24	0.23 **	9.049 **	43.687**	4.888 ^{ns}
E ₂ (second error)	54	0.003	2.907	0.768	5.542
Harvest seasons	2	34.563 **	167.879 **	350.307 **	7.226 ^{ns}
Residues × Harvest seasons	6	0.497 **	9.919 **	27.086 ^{ns}	8.909 ^{ns}
Places × Harvest seasons	16	0.73 **	24.113 **	46.444 **	8.158 ^{ns}
Places × Residues× Harvest seasons	48	0.114 **	8.199 **	29.517 **	5.817 ^{ns}
E ₃ (third error)	144	0.003	3.385	0.899	4.982
Coefficient of variation	-	2.98	11.47	3.62	24.77

** Significance at the 1% probability level

ns: NOT significant

Table 2 The comparison of bioactive components of the residue types.

Residue	Caffeine (%)	Protein (%)	Fiber (%)	Polyphenol (%)
Dust	2.37 a	24.87 a	12.38 c	9.82 a
Fluff	2.04 b	13.56 b	26.99 b	9.06 b
Footstalk	1.85 c	14.46 b	28.41 b	8.88 bc
Stalk	1.28 d	11.28 c	37.13a	8.27 c

The values with similar superscript letters are in a statistical column statistically in one class.

Table 3 The comparison of bioactive components of the harvest seasons.

Harvest residue	Caffeine (%)	Protein (%)	Fiber (%)	Polyphenol (%)
Spring	2.00 b	17.48 a	24.77 c	9.28 a
Summer	2.39 a	15.34 b	25.67 b	8.99 a
Autumn	1.27 c	15.30 b	28.24 a	8.76 a

The values with have similar superscript letters are at a column statistically in one class.

Table 4 The comparison of the bioactive components of the factories.

No. of factories	Caffeine (%)	Protein (%)	Fiber (%)	Polyphenol (%)
F ₁	2.38 a	17.25 ab	25.36 e	8.91 a
F ₂	1.87 c	17.35 a	23.93 f	8.92 a
F ₃	1.97 b	15.60 abc	26.61 c	8.28 a
F ₄	1.99 b	16.21 abc	28.62 a	8.76 a
F ₅	1.80 e	15.26 dc	25.42 e	8.83 a
F ₆	1.83 d	15.26 dc	26.50 c	9.52 a
F ₇	1.68 g	14.67 d	25.77 d	8.97 a
F ₈	1.68 g	15.59 bc	27.10 b	8.93 a
F ₉	1.75 f	16.39 abc	26.74 c	8.96 a

F₁, 2, 3, ... 9, respectively, factory No. 1–No. 9.

The values with similar superscript letters are in a statistical column statistically in one class.

In a research, the amino acid content was measured in different parts of tea bushes, the results showed that the amount of amino acids in the buds and first leaf is higher than in older leaves and tea stalks [11].

The means comparison of the protein amount for the residue types × harvest seasons indicated the highest amount of protein in the dust × spring and the lowest amount in the stalk × summer and autumn (Table 5).

The means comparison of the protein for the residue types × factories showed that the highest amount of protein in the dust × No. 2 factory and the lowest in the stalk × No. 6 factory (Table 6). The means comparison of the protein for the harvest seasons × factories indicated the highest amount of protein in the spring × No.1 factory and the lowest in the autumn × No.7 factories (Table 7).

Table 5 The comparison of bioactive components of the residue types × harvest seasons.

TB	Caffeine (%)	Protein (%)	Fiber (%)	Polyphenol (%)
T ₁ B ₁	2.447 bc	26.083 a	11.22 a	10.47 a
T ₁ B ₂	3.023 a	24.889 b	12.30 a	9.86 a
T ₁ B ₃	1.658 e	23.626 c	13.60 a	9.15 a
T ₂ B ₁	2.120 d	15.654 d	25.76 a	9.47 a
T ₂ B ₂	2.612 b	12.122 g	26.60 a	9.20 a
T ₂ B ₃	1.403 fg	12.892 f	28.61 a	8.53 a
T ₃ B ₁	1.938 d	15.953 d	27.78 a	8.96 a
T ₃ B ₂	2.349 c	13.640 e	26.71 a	9.26 a
T ₃ B ₃	1.261 g	13.790 e	30.74 a	8.43 a
T ₄ B ₁	1.501 ef	12.235 g	34.34 a	8.22 a
T ₄ B ₂	1.567 ef	10.718 h	37.04 a	7.66 a
T ₄ B ₃	0.7719 h	10.900 h	40.01 a	8.94 a

T₁, T₂, T₃, and T₄, respectively, are dust, fluff, footstalk, and stalk.

B₁, B₂, and B₃, respectively, are spring, summer, and autumn.

The values with similar superscript letters are in a statistical column statistically in one class.

Table 6 The comparison of bioactive components of the residue types × the factories.

TF	Caffeine (%)	Protein (%)	Fiber (%)	Polyphenol (%)
T ₁ F ₁	2.904 a	26.668 abc	8.749 r	9.25 a
T ₂ F ₁	2.589 bc	14.658 def	26.80 k	9.47 a
T ₃ F ₁	2.496 d	14.896 def	29.78 ef	9.11 a
T ₄ F ₁	1.538 q	12.798 defghij	36.13 c	7.80 a
T ₁ F ₂	2.542 cd	27.859 a	8.997 r	10.31 a
T ₂ F ₂	1.962 j	15.293 d	26.78 k	8.78 a
T ₃ F ₂	1.756 no	14.721 def	23.53 m	9.08 a
T ₄ F ₂	1.250 s	11.527 fghij	36.42 bc	11.95 a
T ₁ F ₃	2.329 e	25.062 abc	12.10 p	9.49 a
T ₂ F ₃	2.198 g	11.918 defghij	27.77 hij	8.41 a
T ₃ F ₃	1.983 ij	14.630 def	28.16 ghi	7.61 a
T ₄ F ₃	1.397 r	10.810 hij	38.42 a	7.58 a
T ₁ F ₄	2.123 h	24.471 abc	19.19 n	9.98 a
T ₂ F ₄	2.259 f	14.780 def	30.13 e	8.85 a
T ₃ F ₄	2.021 i	14.381 defghi	30.47 e	8.41 a
T ₄ F ₄	1.557 q	11.237 fghij	34.70 d	7.45 a
T ₁ F ₅	2.620 b	24.053 bc	12.51 p	9.79 a
T ₂ F ₅	1.799 mn	13.796 defghi	24.02 m	8.80 a
T ₃ F ₅	1.656 p	14.559 defg	28.60 gh	8.75 a
T ₄ F ₅	1.154 t	11.666 fghij	36.57 bc	7.85 a
T ₁ F ₆	2.510 d	24.863 abc	14.71 o	9.06 a
T ₂ F ₆	1.866 kl	13.448 defghi	25.15 l	9.79 a
T ₃ F ₆	1.747 no	13.482 defghi	28.99 fg	9.91 a
T ₄ F ₆	1.207 s	9.258 j	37.13 b	8.42 a
T ₁ F ₇	2.086 h	22.711 c	10.32 q	10.00 a
T ₂ F ₇	1.829 lm	10.839 hij	24.90 l	9.07 a
T ₃ F ₇	1.588 q	14.194 defgh	28.76 g	8.94 a
T ₄ F ₇	1.224 s	10.961 ghij	39.10 a	7.88 a
T ₁ F ₈	2.023 i	22.740 c	12.24 p	9.44 a
T ₂ F ₈	2.010 ij	12.787 defghij	26.93 jk	9.35 a
T ₃ F ₈	1.669 p	15.146 de	30.08 e	9.10 a
T ₄ F ₈	1.047 u	11.724 defghij	39.14 a	7.79 a
T ₁ F ₉	2.244 fg	25.367 abc	12.57 p	10.07 a
T ₂ F ₉	1.896 k	14.487 defgh	30.45 e	9.05 a
T ₃ F ₉	1.728 o	14.141 defghi	27.37 ijk	9.02 a
T ₄ F ₉	1.147 t	11.577 defghij	36.589 bc	7.70 a

T₁, T₂, T₃, and T₄, respectively, dust, fluff, footstalk, and stalk.

F₁, F₂, F₃, ..., respectively, factory No. 1–No. 9.

The values with similar superscript letters are in a statistical column statistically in one class.

The outcomes displayed that the amount of protein varied in various seasons and decreased from the spring to the autumn. Results of a research showed that harvest season's effects on amino acids content, which diminished from the spring to the summer [26]. The means comparison of the protein for the residue types \times harvest seasons \times factories demonstrated the highest amount of protein was in the dust \times spring \times No. 2 factory, and the lowest recorded in the stalk \times autumn \times No. 7 factory (Table 8). The consequences exhibit that the protein amount in the dust was higher than fluff, footstalk, and stalk and increased in the spring compared to the summer and autumn. Also, the protein amount in the eastern factories was more elevated than in the central and western factories because there were more 1st-degree leaves in the Eastern factories (Table 10).

Fiber

The crude fiber of the plants originates from four distinct structural units, including the cell wall,

sclerenchyma, calcium, and transporter tissues. Due to the growth of transitional tissues, the stem had the highest increase. The young tea leaves have the lowest percentage of fiber. The fiber amount was measured in tea residues from 5.31 to 45.06 percent (Table 8). The means comparison of the residue types fiber displayed the highest percentage (37.13%) in the stalk, and the lowest percentage (12.38%) was in the dust (Table 2). In a research, the fiber content in tea residues was reported from 5.83 to 43.85 percent [15]. The means comparison of fiber for harvest seasons showed the highest and the lowest percentages were in the autumn and the spring, with 28.24% and 24.77%, respectively (Table 3). In a study, fiber content in black tea residue types \times harvest season, (prepared by the Kashef Research factory in Lahijan Tea Institute), was measured, results showed that the highest fiber amount was 25.86% in the autumn season [22].

Table 7 The comparison of bioactive components of the factories \times the harvest seasons.

FB	Caffeine (%)	Protein (%)	Fiber (%)	Polyphenol (%)
B ₁ F ₁	2.819 a	19.75 a	22.74 ghi	8.99 a
B ₂₁	3.063 a	14.97 efg	25.91 fg	9.28 a
B ₃ F ₁	1.263 jkl	17.05 bcde	27.44 bce	8.45 a
B ₁ F ₂	1.97 cdefgh	19.09 ab	21.99 j	9.67 a
B ₂ F ₂	2.254 bc	15.16 defg	22.01 j	8.62 a
B ₃ F ₂	1.408 ij	17.81 abcde	27.78 cde	11.81 a
B ₁ F ₃	2.003 cdefg	16.81 bcdef	24.60 ghi	8.05 a
B ₂ F ₃	2.493 b	14.19 fg	26.80 cdef	8.75 a
B ₃ F ₃	1.434 ij	15.81 cdefg	28.42 acb	8.01 a
B ₁ F ₄	1.753 gh	15.91 cdefg	29.22 ab	8.09 a
B ₂ F ₄	2.86 a	16.22 cdefg	30.22 a	8.62 a
B ₃ F ₄	1.357 jk	16.52 bcdefg	26.41 ef	8.31 a
B ₁ F ₅	2.189 bcde	18.60 abc	23.27 ghi	9.63 a
B ₂ F ₅	2.227 bcd	15.34 defg	25.22 gh	9.17 a
B ₃ F ₅	1.006 l	14.11 fg	27.77 bce	17.58 a
B ₁ F ₆	1.870 efgh	15.27 defg	25.94 fg	9.52 a
B ₂ F ₆	2.199 bcd	15.41 defg	26.73 def	9.05 a
B ₃ F ₆	1.428 ij	15.11 efg	26.80 cdef	10.06 a
B ₁ F ₇	1.831 fgh	16.40 bcdefg	25.58 fgh	9.44 a
B ₂ F ₇	2.133 cdef	16.13 cdefg	22.14 j	9.21 a
B ₃ F ₇	1.081 kl	11.50 h	29.58 ab	8.26 a
B ₁ F ₈	1.668 hi	17.51 abcde	23.91 ghi	9.26 a
B ₂ F ₈	2.098 cdef	15.47 defg	26.63 def	9.11 a
B ₃ F ₈	1.295 jkl	13.82 gh	30.74 a	8.37 a
B ₁ F ₉	1.913 defgh	17.99 abcd	25.71 fgh	9.81 a
B ₂ F ₉	2.160 cde	15.19 defg	25.28 gh	9.10 a
B ₃ F ₉	1.188 jkl	15.99 cdefg	29.22 ab	7.96 a

F_{1, 2, 3, ...,} respectively, factory No. 1–No. 9.

B₁, B₂, and B₃, respectively, are spring, summer, and autumn.

The values with similar superscript letters are in a statistical column statistically in one class.

Table 8 The comparison of bioactive components of the types of residues × the harvest seasons × the factories place.

No.	Caffeine (%)	Protein (%)	Fiber (%)	Polyphenol (%)
1	3.44 ab (T1B1F1)	27.5 b (T1B1F1)	5.31 vw (T1B1F1)	9.90 a (T1B1F1)
2	3.58 a (T1B2F1)	19.09 d (T2B1F1)	6.00 uv (T1B2F1)	9.93 a (T1B2F1)
3	3.24 bc (T3B2F1)	15.27 gh (T4B1F1)	29.80 jk (T3B2F1)	7.93 a (T1B3F1)
4	1.97 hij (T4B2F1)	10.86 klm (T4B2F1)	36.60 edf (T4B3F1)	9.96 a (T2B2F1)
5	2.72 d (T1B1F2)	12.26 ijk (T4B3F1)	7.62 u (T1B1F2)	8.46 a (T3B3F1)
6	2.01 ghi (T2B1F2)	29.49 a (T1B1F2)	23.16 nop (T2B1F2))	7.58 a (T4B1F1)
7	1.57 mno (T3B1F2)	28.16 b (T1B3F2)	29.86 jk (T2B3F2)	11.53 a (T1B1F2)
8	3.08 c (T1B2F3)	16.28 def (T3B1F2)	41.10 b (T4B3F2)	7.85 a (T4B1F2)
9	2.26 fg (T2B1F3)	15.68 defg (T3B3F2)	10.23 t(T1B1F3)	8.63 a (T2B1F3)
10	1.58 klm (T4B2F3)	25.06 bc (T1B1F3)	11.76 ts (T1B2F3)	5.80 a (T3B1F3)
11	1.09 qpr (T4B3F3)	14.14 hi (T2B1F3)	14.30 r (T1B3F3)	7.45 a (T4B1F3)
12	2.93 c (T3B2F4)	25.99 bc (T1B1F4)	30.66 ij (T2B3F3)	10.35 a (T1B1F4)
13	1.27 oqd (T3B3F4)	23.50 c (T1B3F4)	20.88 p (T1B1F4)	9.78 a (T1B2F4)
14	3.00 c (T1B1F5)	14.84 gh (T2B2F4)	22.50 op (T2B3F4)	6.77 a(T4B3F4)
15	2.43 ef (T1B1F6)	13.18 ijk (T4B1F4)	31.56 hi (T3B1F4)	9.48 a (T3B1F5)
16	1.60 kl(T1B3F6)	26.62 bc (T1B2F5)	12.43 ts (T1B3F5)	8.53 a (T4B1F5)
17	1.71 jkl (T3B1F6)	16.64 def(T3B1F5)	32.50 ghi (T4B1F5)	7.75 a (T4B2F5)
18	0.99 qpr (T4B3F6)	10.21 x (T4B3F5)	38.50 cde (T4B2F5)	9.76 a (T1B2F6)
19	1.49 noq (T1B3F7)	24.44 c (T1B1F6)	33.60 fgh (T4B2F6)	10.04 a (T2B3F6)
20	1.37 noq (T4B2F7)	25.68 bc (T1B3F6)	39.50 bcd (T4B3F6)	8.36 a (T2B3F7)
21	0.77 rs (T4B3F7)	13.48 hij (T3B1F6)	19.40 p (T2B2F7)	8.88 a (T4B1F7)
22	1.82 ijk (T1B1F8)	9.56 lm (T2B3F7)	44.83 a (T4B3F7)	7.74 a (T4B2F7)
23	2.57 d (T1B2F8)	12.67 ijk (T2B1F7)	13.50 rs (T1B2F8)	7.02 a (T4B3F7)
24	1.17 qpr (T3B3F8)	9.17 m (T4B3F7)	15.46 q (T1B3F8)	9.64 a (T1B1F8)
25	1.14 qpr (T4B1F8)	24.00 c (T1B2F8)	25.50 mxo (T3B1F8)	8.48 a (T3B3F8)
26	1.33 noq (T4B2F8)	11.90 ijk (T4B2F8)	34.56 feg (T3B3F8)	7.33 a (T4B3F8)
27	0.66 rs (T4B3F8)	17.44 de (T2B1F9)	35.56 feg (T4B2F8)	10.84 a (T1B1F9)
28	2.61 d (T1B2F9)	12.79 ijk (T4B1F9)	45.06 a (T4B3F8)	8.08 a (T2B3F9)
29	2.11 fgh (T3B2F9)	10.46 lm (T4B2F9)	28.56 kl (T3B1F9)	8.79 a (T4B1F9)
30*	0.60 rs (T4B3F9)	11.47 k (T4B3F9)	27.60 lm (T3B2F9)	6.71 a (T4B3F9)

* 30 means were selected from 108 means, including maximums and minimums, and then ranked.

T₁, T₂, T₃, and T₄, respectively, dust, fluff, footstalk, and stalk.

B₁, B₂, and B₃, respectively, are spring, summer, and autumn.

F_{1, 2, 3}, respectively, factory No. 1–No. 9.

The values with similar superscript letters are in a statistical column statistically in one class.

Table 9 Grading of plucking tea leaves

components	Premium	1st degree	2end degree
Two leaves and one bud	75	60	20
Three leaves and one bud	20	25	50
Four leaves and one bud	-	-	10
Single tender leaf	5	5	10
Single banjee leaf	-	5	5
Two banjee leaf	-	5	5

Table 10 The weight statistics of one and two degrees leaves for nine selected factories in 2006.

Factory name	1st-degree leaves (Kg)	2end-degree leaves (Kg)	Ratio 1 to 2	Total (Kg)
Moein	256467	513651	50	770118
Poltan (Shariat)	316458	161393	51	477851
Saba	237115	790600	30	1017715
Shokoh Chay	165392	526976	31	692368
Mersad	232239	1171208	20	1403447
Liseh road	336817	851362	40	1188179
Tohid	294716	1439976	20	1734629
Setareh Shomal	340649	1354591	25	1695240
Sabzineh Gilan	264965	1082222	24	1347187

The tea plant grows slowly, after two stages of plucking in the spring and summer seasons, so its harvest intervals are prolonged, and its structure becomes woody. Therefore, the percentage of insoluble solids and fiber increases in the autumn. The means comparison of fiber for factories showed that the highest fiber amount and the lowest was 28.62% in the No. 4 factory and 23.93% in the No. 2 factory, respectively (Table 4). The eastern factories had a lower fiber amount than the central and western because they purchased high-quality leaves (1st-degree leaves) to 2nd-degree leaves (Table 10). The consequences offered that there was an inverse relationship between the amount of caffeine and fiber. If the leaf-plucking pattern was nonstandard, the fiber amount increased, and the caffeine amount decreased, as well.

The means comparison of fiber for the residue types × harvest season was insignificant (Table 5).

The means comparison of fiber for the residue types × factories reveals the highest amount was in the stalk × No. 3, No. 7, and No. 8 factories. The lowest amount of fiber and the highest amount of caffeine were observed in the dust × No. 1 and No. 2 factories (Table 6). The means comparison of fiber for the harvest seasons × factories shows the highest amount in the summer and autumn × No. 4 and No. 8, and the lowest amount in the spring and summer × No. 2 and No. 7 manufacturers recorded (Table 7). The fiber amount grew from the spring to autumn because of the woody leaves in the autumn season.

The means comparison of fiber for the residue types × harvest season × factories exhibit the highest fiber amount was in the stalk × autumn × No. 7 and No. 8 factories, and the lowest fiber amount in the dust × spring × No. 1 factory (Table 8). The results disclose that the fiber amount in the dust was less than fluff, footstalk, and stalk and enhanced in the autumn compared to the spring and summer. Also, residue percentage and the amount of fiber in western and central factories was higher than in eastern, an effect of the low proportion of the 1st to 2nd degrees of leaves (Table 10).

CONCLUSION

This paper revealed in the use of tea factory residue in the industrial, paying attention to the types of residues, harvest seasons, and factories is necessary because they effect on the amount of bioactive components. In this research, the best places for the

bioactive compounds extraction from residue were western and central factories due to the high percentage of residue and more bioactive compounds.

ACKNOWLEDGEMENTS

We are grateful to acknowledge the Tea Research Center, Agricultural Research, Education, and Extension Organization, Lahijan, Iran, for the financial support of this investigation.

REFERENCES

1. Parmer N., Rawat M., Kumar J.V. *Camellia Sinensis* (Green Tea): A Review. *The Global J. Pharma.* 2012; 6(2): 52-59.
2. Tariq M., Naveed A., Barkat Ali K. The morphology, the characteristics and the medicinal properties of '*Camellia sinensis*' tea. *J. Med. Plants Res.* 2010; 4(19): 2028-33.
3. Sumpio B.E., Cordova A.C., Berke-Schlessel D.W., Levites Y., Weinreb O., Maor G., Youdim M.B., Qin F., Chen QH. Green tea, the Asian Paradox and cardiovascular disease. *J. Am. Coll. Surg.* 2006; 202: 813-20.
4. Cabrera C., Gimenez R., Lopez M.C. determination of tea components with antioxidant activity. *J. Agric. Food Chemistry.* 2003; 51(15): 4427-4435.
5. Cabrera C., Artacho R., Gimenez R. Beneficial effects of green tea-a review. *J. Am. Coll. Nutrition.* 2006; 25(2): 79-99.
6. Granja A., Pinheiro M., Reis S. Epigallocatechin gallate nanodelivery systems for cancer therapy. *Nutrients.* 2016; 8, 307.
7. Icen H., Guru M. Extraction of caffeine from tea stalk and fiber residues using super critical carbon dioxide. *J. of Supercritical Fluids.* 2009; 50: 225-228.
8. Marcel W.L, Chi H. Pharmacological effects of green tea on the gastrointestinal system. *European J. Pharma.* 2004; 500: 177-185.
9. Richard B., Denis G. Green tea: prevention and treatment of cancer by nutraceutical. *Lancet.* 2004; 364(9349): 1021-1022.
10. Nie ShP., Xie M.Y. A review on the isolation and structure of tea polysaccharides and their bioactivities. *Food Hydrocolloids.* 2011; 25(2): 144-149.
11. Wickremasinghe R.L. The Monographs on tea products in Sri Lanka. Tea Research Institute of Sri Lanka. 1978; 7: 45-51.
12. Roofigari haghigat Sh., Shokrgozar S., Shirinfekr A., Azadi R., Serajie A., Cheraghi K., mohebbian S., Jalali M. Extraction of edible color from tea residue and its stability assessment. Tea Research Institute. Agric. Res. Education and Extension Organization, Lahijan, Iran. 2017.

13. Institute of Standards and Industrial Research of Iran. The black tea-properties, standard number 624. 1992.
14. Lakin A. Food Analysis, Practical Handout. Reading Uni. UK. 1989.
15. Smiechowska M., Dmowski P. Crude fiber as a parameter in the quality evaluation of tea. Food Chem. 2006; 94: 366-368.
16. Emami A. Protein Measurement Method. Journal of Plant Breeding Methods, Organization for Research, Education and Promotion of Agriculture, Water and Dust Res. Institute. 1996; 982(1): 28.
17. Institute of Standards and Industrial Research of Iran. Fiber measurement method. Standard number: 3394. 1992.
18. Modder W.W.D. Tea research in Srilanka, Talawakelle: The tea research institute of srilanka. 2003.
19. Hilton P., Palmer R., Ellis R.T. Effects of seasons and nitrogen fertilizer upon the flavanol composition and tea making quality of fresh shoots of tea in central Africa. J. Sci. Food Agric. 1973; 24: 819-826.
20. Roofigari Haghghat Sh., Cheraghi K., Adib A., Ali Naghipour B., Motavi Jalali S.M. Investigation of the effect of leaf picking on green leaf yield and quality of black tea during leaf picking periods. Final report of the research project. Final report of the research project. Agricultural Research, Education and Extension Organization, National Tea Research Center, Lahijan. 2007; 974/86.
21. Keegel E.L. Tea manufacture in Ceylon. Tea Research Institute of Ceylon. Colombo. 1983.
22. Parsa F., Azadi R., Shokrgzar S.M.T., Hosseini S.M., Cheraghi K., Motavi Jalali S.M. Investigation and measurement of important constituents of tea dust and three common types of tea plant wastes. Final report of the research project. Agricultural Research, Education and Extension Organization, National Tea Research Center, Lahijan. 2009; 87:207.
23. Kunjikutt N., Ramachandran P., Devasia P.A., Thomas C.T., Nandakumaran M. Studies on goat nutrition. J. Vet. Sci. 1978; 9(2): 206-14.
24. Mulky M.J., Sharma V.S. Tea Culture: processing and marketing. Bombay: Oxford and IBH. 1993.
25. Liaqing S., Xiangyang W., Zhongyang W., Yuanfeng W. Studies on tea protein extraction using alkaline and enzyme methods. China. Gongshang University. College of food science, Bio technology and Environmental Engineering, Hang Zhou. 2007.
26. Takayanaji H., Anan T., Ikegawa K., Nakagawa M. Chemical composition of Chinese and the preference testing of these teas. Tea Res. J. 1984; 60: 59-65.