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Scientific Research

The impact of processing, time, and harvesting location on the antioxidant activity and chemical compounds of tea

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ABSTRACT

Tea, after water, is one of the most consumed beverages in the world due to its chemical compounds such as polyphenols, antioxidant compounds, and caffeine, which are beneficial. Factors such as tea leaf processing, harvest time, and cultivation region can affect the composition of tea leaves. This research was conducted in a factorial design and as a completely randomized design, with independent variables including raw green tea leaves, processed green tea, and black tea, and dependent variables in this design include measuring the amount of polyphenols, antioxidant activity, and The amount of caffeine and soil properties have been carried out in two harvest seasons: spring (May) and summer (July) and two cultivation zones in the east of Guilan (Lahijan) and west of Guilan (Fouman). According to the results, the interaction effects were, the moisture content of spring green tea in Fouman region (78.83%) was at its highest and summer green tea in Fouman region (6.22%) had the lowest value. The highest amount of solid matter was reported in summer green tea from the Fuman region (93.76%), while the lowest amount was found in spring green tea leaves from Fuman (21.20%) Additionally, the highest caffeine content was observed in spring green tea from Fuman (2.65%), and the lowest amount was in summer green tea leaves from Lahijan (1.87%) The highest and lowest levels of antioxidant compounds were also seen in both spring and summer green tea leaves from Lahijan, as well as in summer black tea from Fuman (57.27%). Furthermore, the highest amount of polyphenols was observed in the spring green tea from Fouman (12.37%), while the lowest amount was found in the summer green tea from Fouman (12.29%).

1-Introduction

Tea is among the oldest and most extensively consumed beverages globally, following water. Botanically, it is classified as a dicotyledonous and evergreen plant within the *Theaceae* family. Moreover, it constitutes a fundamental and strategic product for Iran, with the most extensive cultivated area located in the provinces of Gilan and Mazandaran. The area designated for tea cultivation in Iran encompasses approximately 32,000 hectares, primarily located in the provinces of Gilan (90%) and Mazandaran (10%). The popularity of drinking tea can be attributed to its aroma and flavor characteristics. Recently, it has garnered significant attention for its medicinal benefits, particularly its anti-cancer properties [1]. However, the poor quality of produced tea is one of the challenges of this industry in Iran [2]. Type, variety, season, weather conditions, processing method, soil type, and storage method are among the factors that generally influence tea quality [3, 4].

The tea processing stages are a critical factor that results in qualitative differences among tea products [5]. The chemical compounds of tea include polyphenols (catechins and flavonoids), alkaloids (caffeine, theobromine, theophylline, etc.), volatile oils, polysaccharides, amino acids, fats, vitamins (especially vitamin C), and minerals (aluminum, fluorine, manganese, etc.). Phenolic compounds of tea, comprising flavones, catechins, flavonols, and anthocyanins, generally account for approximately 30% of the dry matter in young shoots [6]. The compounds influencing tea quality vary with climatic conditions, leading to alterations in the qualitative characteristics of the produced dried tea [7]. Studies have shown that there are over 23 polyphenols in the leaves of green tea, which cause the antioxidant properties of tea. Free radicals are the main cause of many health-related damages, while natural antioxidants serve to protect human organisms from these threats [8]. Tea is a rich source of caffeine, with each gram of dry tea leaves generally containing 20-50 mg of caffeine [9]. The polyphenols and caffeine found in tea are critical components assessed in the quality control of this beverage at both national and international levels [10-12].

Tea is consumed in three forms: fermented (black tea), unfermented (green tea), and semi-fermented (oolong). In Iran, green tea leaf harvesting occurs from early May to early November, divided into three primary stages: spring, summer, and fall harvests. The spring tea leaf harvest typically begins in late April and extends through the end of June, accounting for approximately 44% of the total annual crop yield; the tea derived from this harvest is renowned for its delicacy and fragrance. The summer tea leaf harvest starts in July and lasts until the end of September, which produces 38% of the total annual crop yield; the tea produced from this harvest is of high quality. Finally, the fall tea harvest begins in early October and continues until mid-November. Then the harvesting process is stopped as the buds go dormant when the weather gets colder and the daylight hours decrease. Nasirirad *et al*. (2012) studied the effect of harvest season on the total phenolic compounds of Lahijan green tea and showed that the green leaves harvested in summer and spring contained more polyphenolic compounds than those harvested in fall [13].

The production of black tea involves the steps of withering, rolling, fermentation, and drying, whereas the process for green tea consists of drying and roasting and lacks the two steps of withering and fermentation. In the processing of green tea, the enzymes present in the leaves are initially inactivated using steam. Subsequently, the leaves undergo crushing, twisting, and pelletizing through rolling and heat application, followed by drying to achieve a moisture content of approximately 4% [12].

Farahmandfar and Aziminejad compared the effects of tea processing methods and brewing methods (conventional and in a microwave) on phenolic and flavonoid content and antioxidant activity of tea and reported that the fermentation process changed the antioxidant properties and phenolic content of tea. They also showed that the highest phenolic and flavonoid content and antioxidant activity were related to the tea specimen rolled and then brewed in a microwave [14]. Other important factors that affect the quality of tea are soil characteristics such as phosphorus, potassium, and pH. Mahabadi *et al*. (2019) studied the

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effect of land topographic features on soil characteristics and tea yield and quality in Lahijan and showed that the northern slope of the studied region demonstrated superior soil quality, resulting in more favorable conditions for tea cultivation, thereby enhancing tea yield and quality. They also introduced soil nitrogen and organic matter as significant parameters influencing tea quality in this region [15]. Therefore, this study aims to investigate the effects of processing (raw green leaves, green tea, and black tea), seasonal changes (spring and summer), and harvesting areas on dry matter, caffeine content, phenolic compounds, and antioxidant activity (DPPH inhibition) of tea harvested from two regions of eastern Guilan (Lahijan) and western Guilan (Fuman).

2-Materials and methods

The physicochemical properties of the soil in the two studied regions, Lahijan (37°12′15″N 50°00′17″E) and Fuman (37°13′27″N 49°18′45″E), were examined and evaluated during the spring and summer seasons (Table 1). All materials used for physicochemical tests were purchased from Merck, Germany.

Table1:Soil Tests in Spring and Summer in Two cultivation area

Location/Time	pH (1:1)H2O	Electrical conductivity (ds/m)	Organic Carbon(%)
Lahijan/spring	4.5	0.526	4.3
Fouman/spring	5.5	0.313	4.2
Lahijan/Summer	4.4	0.627	5
Fouman/Summer	5.4	0.467	5.5

Measurement of soil pH:

The soil pH was measured using the 1:1 suspension ratio method. First, 20 grams of soil was combined with 20 cc of distilled water and subsequently agitated for 30 minutes in a shaker. The beaker's contents were then emptied and allowed to rest undisturbed for 30 minutes to facilitate the separation of the solid and liquid phases. The pH meter was initially calibrated using buffers of pH 7 and 4, after which the pH of the resultant liquid was measured with the pH meter.

Measurement of soil electrical conductivity:

The samples obtained from the pH measurement test were analyzed using an EC meter (European manufactured, Milwaukee model MC310).

Measurement of soil organic carbon:

Five grams of the soil specimen were pulverized and passed through a half-millimeter sieve, then transferred into a 500-mL Erlenmeyer flask, to which 10 mL of 1N potassium bichromate was added and gently shaken. Subsequently, 20 mL of concentrated sulfuric acid was added to the solution and gently shaken for 1 minute, after which the solution was allowed to remain undisturbed for 20 minutes. Then 250 mL of distilled water was added to the solution, and upon cooling, titration with ammonium ferrous sulfate was conducted in the presence of 10 drops of orthophenanthroline until a consistent red hue was achieved.

Preparation of tea specimens:

Raw green tea leaves were obtained from tea plantations in Lahijan and Fuman and then processed into green tea and black tea at the Tea Research Institute in Lahijan.

Measurement of tea caffeine content:

The caffeine content of tea specimens was measured by extracting one gram of dry tea with chloroform in the presence of ammonia. The extract was filtered with a potash solution and dry sodium sulfate. Following dilution, the absorbance was measured at 276 nm using a

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UV/VIS spectrophotometer (manufactured in the US). The caffeine content of the tea specimens was then determined by comparing the resulting values to a standard caffeine calibration curve [16].

Measurement of tea polyphenols:

The total polyphenol content was measured using the Folin–Ciocalteu method (ISO 2004) [17]. To measure polyphenols in the tea specimens, 2 g of the specimen was weighed and transferred to a tube, followed by the addition of 5 cc of 70% methanol. The mixture was then incubated in a water bath (Arta WB150 model, manufactured in Iran) at 70℃ for 10 minutes. Following cooling, the tubes underwent vortexing for one minute and were subsequently filtered with filter paper. Subsequently, 5 cc of 70% methanol was added, followed by incubation in a water bath for 10 minutes, after which the mixture was vortexed and filtered. A 100 ml volumetric flask was filled to volume with 70% methanol, and 1 cc of the target liquid was incorporated. Then 5 cc of 10% Folin, 4 cc of sodium carbonate, and 1 cc of the specimen were added to 12 tubes and allowed to stand at room temperature for one hour. After one hour, the specimen was analyzed using a UV/VIS spectrophotometer (manufactured in the US) at 765 nm. The spectrophotometer was calibrated using a control solution consisting of 1 cc of distilled water, 5 cc of Folin, and 4 cc of sodium carbonate.

Measurement of tea antioxidants:

To measure antioxidants in tea, 0.5 cc of the prepared specimen was combined with 5 cc of DPPH in a test tube and incubated in darkness for 30 minutes. The spectrophotometer was then used to read the specimen at 517 nm.

$$p = 100 * \frac{A\,dpph0 - A\,Sample0}{Adpph0}$$

Statistical analysis:

This study employed a randomized factorial design with three replicates. Independent variables, including harvesting region (Fuman and Lahijan), processing type (green tea leaf, green tea, and black tea), and harvesting time (spring and summer), were applied to evaluate the dependent variables, including moisture, dry matter, caffeine, anticomedogenic activity, and phenolic compounds. The data obtained were analyzed using analysis of variance in SPSS-18. Moreover, all graphs were drawn in Microsoft Excel.

3-Results and discussion

Moisture

The independent effect of variables showed that harvesting region, harvesting time, and processing method significantly affected the moisture content of tea specimens. Accordingly, the highest moisture content was reported from tea specimens obtained from Fuman (29.85%), tea specimens harvested during spring (30.02%), and green leaves (73.5%) (Figure 1). The results on interactive effects of variables indicated a significant difference between the treatments, as the highest moisture was observed in the spring green leaves in Fuman (78.83%) and the lowest moisture was related to the summer green tea in Fuman (6.22%) (Figure 2). The higher moisture content in green leaves can primarily be attributed to the lack of a heating step during processing. Furthermore, the average temperature is lower in spring than in summer, which contributes to the lower moisture content observed during the summer months.

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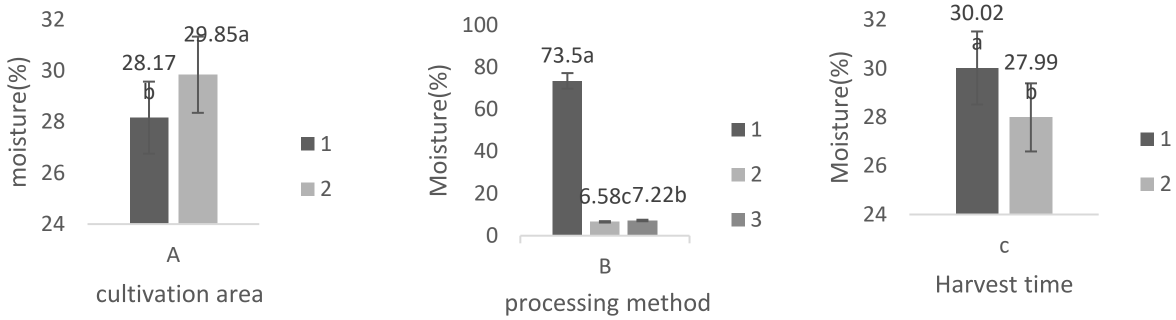

Fig1 The Effect of cultivation area (A) Processing Method (B) and Harvest Time (C) on Moisture Content

A1:Lahijan, A2:Fouman, B1:green leaves, B2:green tea, B3:black tea, C1:spring, C2:summer

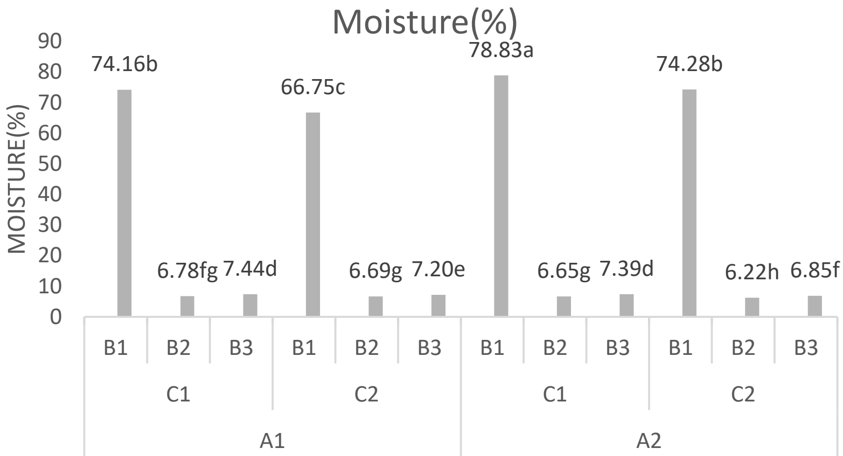

Fig2 The Effect of processing method and harvest time on Moisture content in two cultivation area (Fouman & Lahijan)

A1:Lahijan, A2:Fouman, B1:green leaves, B2:green tea, B3:black tea, C1:spring, C2:summer

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Dry matter

The independent effect of variables showed that harvesting region, harvesting time, and processing method significantly affected the dry matter of tea specimens. Accordingly, the highest dry matter was reported from tea specimens obtained from Lahijan (71.82%), tea specimens harvested during summer (71.97%), and green tea (93.4%) (Figure 2). The results on interactive effects of variables also demonstrated that the highest dry matter was reported from the summer green tea in Fuman (93.76%) and its lowest level was found in the spring green leaves in Fuman (21.20%) (Figure 4). It seems that the increased temperature and the application of heat during the plant growth and processing stages reduce the final product’s moisture content and, thereby, increase its dry matter. In other words, the moisture content is inversely correlated with dry matter; the leaf's moisture content reduces as the temperature rises during the summer, resulting in the higher dry matter of leaves. Furthermore, the rise in photosynthesis and growth rate during the summer, coupled with enhanced soil nutrients resulting from reduced rainfall, suggests that total dry matter in the summer will significantly exceed that of the spring [18].

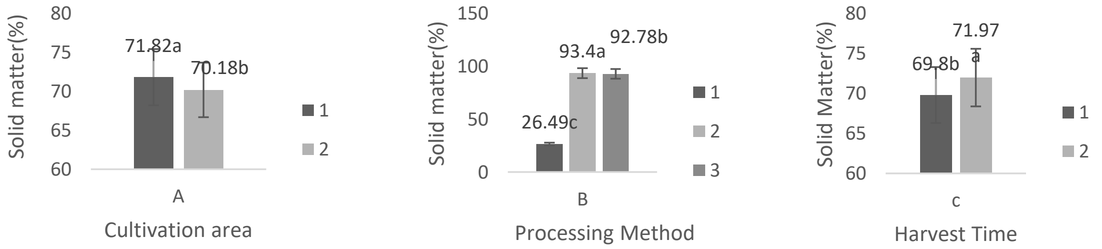

Fig3 The Effect of cultivation area (A), Processing Method (B) and Harvest Time (C) on Solid Matter Content

A1:Lahijan, A2:Fouman, B1:green leaves, B2:green tea, B3:black tea, C1:spring, C2:summer

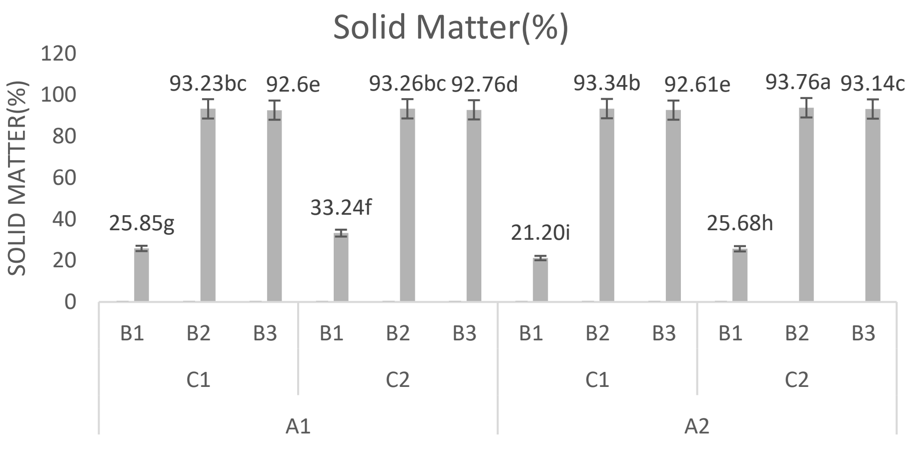

Fig4 The Effect of processing method and harvest time on Solid Matter content in two cultivation area (Fouman & Lahijan)

A1:Lahijan, A2:Fouman, B1:green leaves, B2:green tea, B3:black tea, C1:spring, C2:summer

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Caffeine

The independent effect of variables showed that harvesting region, harvesting time, and processing method significantly affected the caffeine content of tea specimens. Accordingly, the highest caffeine content was reported from tea specimens obtained from Lahijan (2.52%), tea specimens harvested during summer (2.36%), and black tea (2.41%) (Figure 5). The results on interactive effects of variables also showed a significant difference between the effect of variables, as the highest caffeine content was observed in the spring green tea harvested from Fuman (2.65%) and its lowest content was related to the summer green leaves harvested from Lahijan (1.87%). The mean temperature of May and July registered in Fuman was 19℃ and 27.4℃, respectively. These figures for Lahijan were 17.6℃ and 25.5℃. These figures can well justify the higher caffeine content of tea specimens in the fixed seasons in Fuman compared to Lahijan. Nasirirad *et al*. (2012) also showed that the intensity of sunlight directly influences the rate of photosynthesis; consequently, an increased rate of photosynthesis correlates with higher caffeine content. The increased photosynthesis during extended daylight hours with higher average temperatures, attributed to increased sunlight exposure, may contribute to the augmented biosynthesis of compounds in green leaves. This phenomenon could elucidate the rise in caffeine synthesis observed in green leaves harvested from Fuman, where the average temperature is typically higher than that of Lahijan [13, 19].

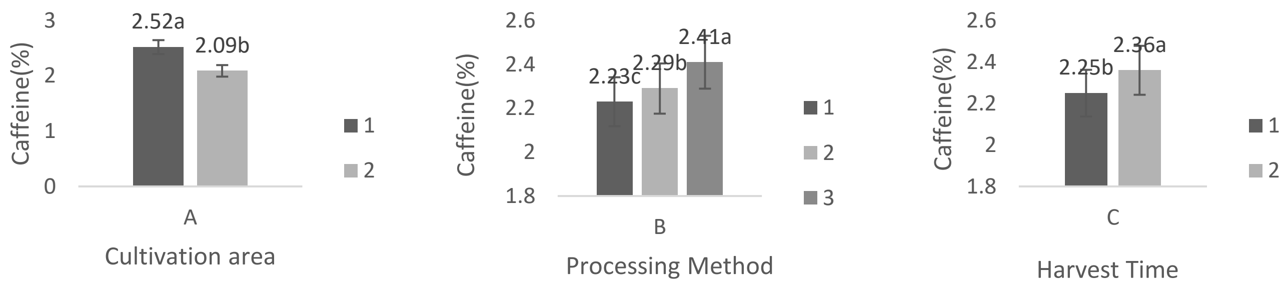

Fig5 The Effect of cultivation area (A), Processing Method (B) and Harvest Time (C) on Caffeine Content

A1:Lahijan, A2:Fouman, B1:green leaves, B2:green tea, B3:black tea, C1:spring, C2:summer

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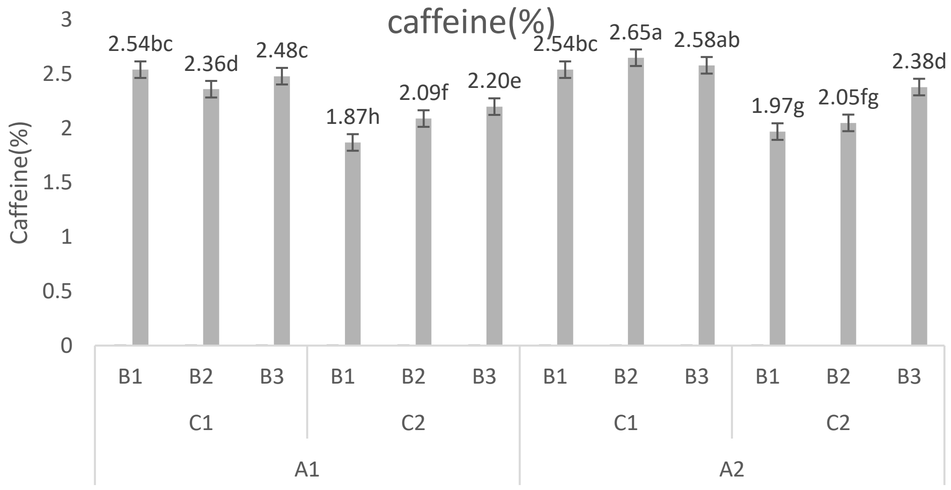

fig6 The Effect of processing method and harvest time on Caffeine content in two cultivation area (Fouman & Lahijan)

A1:Lahijan, A2:Fouman, B1:green leaves, B2:green tea, B3:black tea, C1:spring, C2:summer

Antioxidants

The independent effect of variables (harvesting region, harvesting time, and processing method) on antioxidant compounds revealed a significant difference between the tea specimens. Accordingly, the highest concentration of antioxidants was reported from tea specimens obtained from Fuman (88.43%), tea specimens harvested during spring (89.43%), and green tea (96.64%) (Figure 7). The results on interactive effects of variables also showed a significant difference between the tea specimens, as the highest content of antioxidants was observed in the spring and summer green leaves and green tea harvested from Lahijan and also the spring green tea harvested from Fuman, whereas their lowest content was found in the summer black tea in Fuman (57.27%) (Figure 8). The reduction in antioxidant activity and polyphenolic compounds observed in the second harvest during summer may be associated with the emergence of younger, fresher leaves in spring. Consequently, the compounds produced in the leaves during the summer have less time for synthesis, leading to the production of weaker compounds. Roufigeri Haghighat *et al*. (2009) stated that young leaves are collected from the tea plant during the first harvest to produce tea. Therefore, the key compounds responsible for the color and flavor of tea, particularly polyphenolic compounds, are more abundant in the buds and leaves harvested at this stage. The first harvest occurs in spring, followed by a second in summer, leading to a reduction in the strength and regenerative capacity of the fresh leaves as well as the compounds responsible for the aroma and flavor of tea. The leaves from the second harvest contain fewer polyphenolic compounds, leading to a reduced level of antioxidant activity and an anticipated increase in caffeine content [19].

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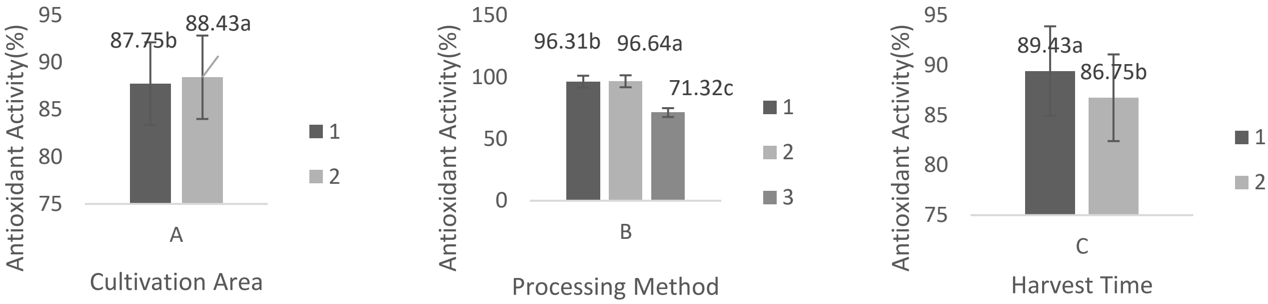

Fig7 The Effect of cultivation area (A), Processing Method (B) and Harvest Time (C) on Antioxidant Content

A1:Lahijan, A2:Fouman, B1:green leaves, B2:green tea, B3:black tea, C1:spring, C2:summer

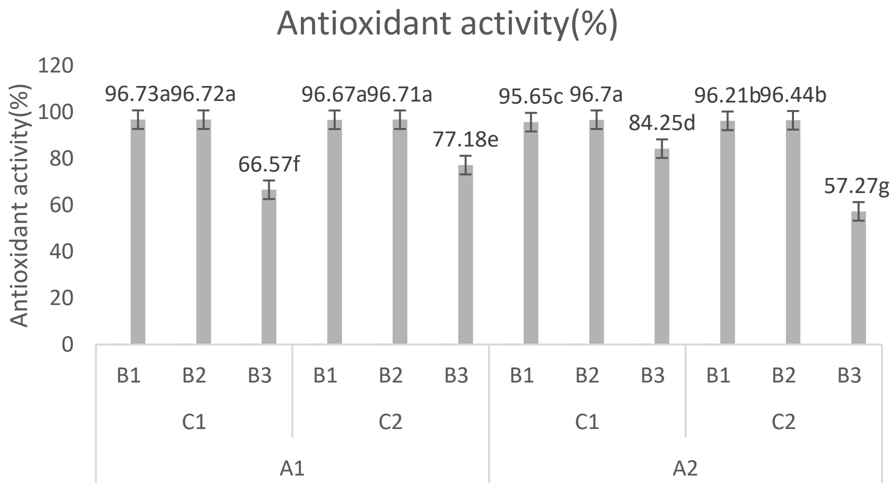

fig8 The Effect of processing method and harvest time on Antioxidant Activity content in two cultivation area (Fouman & Lahijan)

A1:Lahijan, A2:Fouman, B1:green leaves, B2:green tea, B3:black tea, C1:spring, C2:summer

Phenolic compounds

The analysis of independent effects revealed significant differences between the tea specimens in harvesting time, harvesting region, and processing method. Accordingly, the highest concentration of phenolic compounds was reported from tea specimens harvested in Fuman (10.58%), tea specimens harvested during spring (11.43%), and green tea (11.46%) (Figure 9). The results on interactive effects of variables also showed a significant difference between the tea specimens in this regard, as the highest content of polyphenols was observed in the spring green tea (12.37%) and the summer green tea (12.29%) harvested in Fuman, whereas their lowest content was related to the summer black tea harvested in Lahijan (6.06%) (Figure 10). The fermentation process in black tea processing results in a reduction of polyphenol concentration due to the activation of oxidative enzymes, which influence polyphenolic compounds and their transformation into flavor and color-enhancing compounds. Fermentation plays a crucial role in tea processing, wherein catechins are transformed into theaflavins and thearubigins through the action of oxidative enzymes such as peroxidase and polyphenol oxidase. In the subsequent reactions, thearubigins undergo polymerization, potentially contributing to a reduction in the concentration of polyphenolic compounds. The polymerization of thearubigins and the reduction of polyphenolic compounds finally reduce antioxidant activity. Amiri and Madah (2015) reported that

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fermentation reduced the phenolic compounds and increased the caffeine content of tea. Therefore, they concluded that the fermentation process in the conversion of green tea leaves into black tea influences the caffeine content and total polyphenols of the final product [20]. According to Kim *et al*. (2011), tea fermentation results in the conversion of monomeric flavonoids into polymeric theaflavins and thearubigins, leading to a reduction in the overall polyphenolic compounds in tea. The content of polyphenolic compounds generally decreased with the processing of the tea leaves harvested from both Lahijan and Fuman. However, this decrease was more pronounced in black tea than in green tea. It seems that sunlight and air temperature are important factors affecting the concentration of polyphenolic compounds. As a result, the tea leaves harvested in Fuman contained higher levels of polyphenolic compounds and, thereby, exhibited higher inhibitory effects on DPPH, as the average temperature during the harvesting time in 2022 was higher in Fuman compared to Lahijan. Hara (2001) demonstrated that the production of catechin in the tea plant is positively correlated with light intensity, which hinges on the activity of phenylalanine-ammonia lyase, a crucial enzyme in the biosynthesis of the B-ring of catechins. In shaded conditions, the activity of this enzyme diminishes significantly, leading to a reduction in the production of catechins [21]. In the second harvest (summer), the synthesis of compounds responsible for tea aroma and flavor reduces due to the weakened state of green leaves and buds. This decline can also account for the reduction in polyphenolic compounds and DPPH scavenging activity. The results of tests on the soil samples of the Fuman and Lahijan regions also confirmed the difference in phenolic compounds in green tea leaves harvested from these areas at two different times. Soil samples were collected from the low-slope, flat tea plantations in Fouman and the tea plantations in Lahijan in a steep foothill area. The effect of slope on the reduction of nutrients indicates that the polyphenol content of tea at lower altitudes is significantly greater than that at higher altitudes [22, 23].

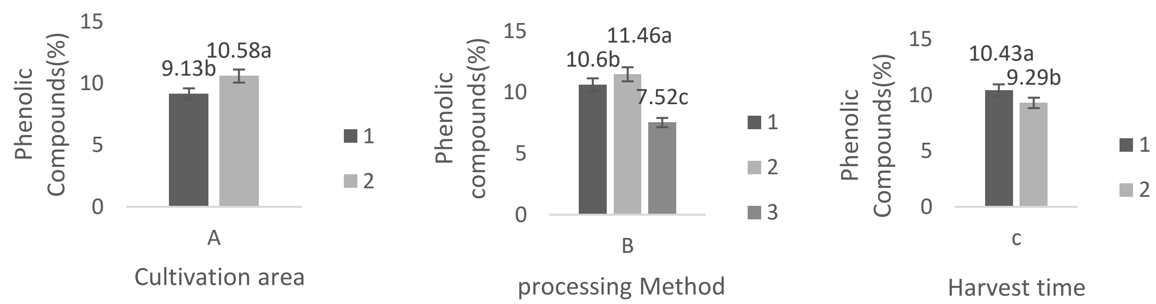

Fig9 The Effect of cultivation area (A), Processing Method (B) and Harvest Time (C) on Phenolic compounds Content

A1:Lahijan, A2:Fouman, B1:green leaves, B2:green tea, B3:black tea, C1:spring, C2:summer

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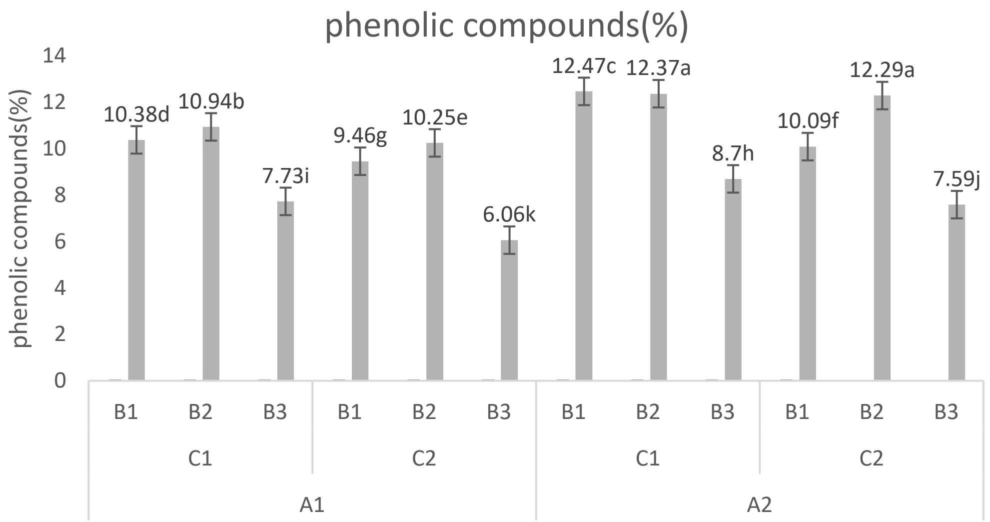

Fig10 The Effect of processing method and harvest time on Phenolic compounds content in two cultivation area (Fouman & Lahijan)

A1:Lahijan, A2:Fouman, B1:green leaves, B2:green tea, B3:black tea, C1:spring, C2:summer

4-Conclusion

Considering the importance of tea quality and the factors influencing it, this study investigated the effect of processing (raw green leaves, green tea, and black tea), harvesting time (spring and summer), and harvesting region on the chemical components of tea, including dry matter content, caffeine, phenolic compounds and, antioxidant activity (DPPH inhibition), in two regions of eastern Gilan (Lahijan) and western Gilan (Fuman). The study results showed that the highest and the lowest moisture content was measured in the spring green tea and the summer green tea harvested in Fuman, respectively. Moreover, the highest and the lowest content of polyphenols, which are responsible for tea aroma and flavor, was observed in the spring and summer green tea harvested in Fouman, respectively. Accordingly, the highest concentration of phenolic compounds was reported from tea specimens harvest in Fuman (10.58%), tea specimens harvested during spring (11.43%), and green tea (11.46%). The interactive effects of variables also showed a significant difference between the tea specimens in the concentration of polyphenols. The highest content of polyphenols was observed in the spring green tea (12.37%) and the summer green tea (12.29%) harvested in Fuman, whereas their lowest content was related to the summer black tea harvested in Lahijan (6.06%). The results also indicated that the highest caffeine content was observed in the spring green tea in Fuman and its lowest content was related to the summer green leaves in Lahijan. The concentration of phenolic compounds and corresponding antioxidant activity was found to be greater in flat areas (Fumen) compared to foothill areas (Lahijan).

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چکیده

چای پس از آب یکی از پرمصرفترین نوشیدنی‌های جهان است و به علت ترکیبات شیمیایی موجود در آن نظیر پلی‌فنل‌ها، ترکیبات آنتی‌اکسیدانی و کافئین که سودمند هستند، مورد توجه قرار گرفته است. عواملی مانند فرآوری برگ چای، زمان برداشت برگ و منطقه کشت می‌تواند بر روی ترکیبات برگ چای تاثیر بگذارد. این تحقیق در طرح فاکتوریل و در غالب طرح کاملا تصادفی شامل متغیرهای مستقل به صورت برگ سبز خام، چای سبز و چای سیاه فرآوری شده است و متغیرهای وابسته در این طرح از جمله اندازه گیری میزان پلی‌فنل، فعالیت آنتی‌اکسیدانی، میزان کافئین و ویژگی‌های خاک می‌باشد که در دو فصل برداشت بهار(اردیبهشت) و تابستان(تیر) و دو منطقه کشت شرق گیلان(لاهیجان) و غرب گیلان(فومن)، انجام شده است. بر طبق نتایج به دست آمده اثرات متقابل بدین صورت بود، میزان رطوبت در چای سبز بهاره منطقه فومن (۷۸/۸۳ ٪) در بیشترین حالت خود و چای سبز تابستانه منطقه فومن (٦/۲۲ ٪) کمترین مقدار را دارا بود. به همین ترتیب بیشترین میزان ماده جامد نیز در چای سبز تابستانه منطقه فومن (۹۳/۷٦ ٪) و کمترین میزان آن در برگ سبز بهاره فومن (۲۱/۲۰ ٪) گزارش شد. همچنین بالاترین میزان کافئین در چای سبز بهاره فومن (۲/٦٥ ٪) و کمترین مقدار آن در برگ سبز تابستانه لاهیجان(۱/۸۷ ٪) گزارش گردید و بیشترین و کمترین میزان ترکیبات آنتی‌اکسیدانی به ترتیب در برگ سبز چای لاهیجان در هردو چین بهار و تابستان و چای سیاه تابستانه فومن (۵۷/۲۷ ٪) دیده شد. علاوه بر این بیشترین میزان ترکیبات پلی‌فنلی در چای سبز بهاره فومن (۱۲/۳۷ ٪) و کمترین میزان آن در چای سبز تابستانه فومن (۱۲/۲۹ ٪) مشاهده گردید.