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Impacts of different brewing conditions on pyrazine and bioactive contents of Turkish coffee

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Abstract

Turkish-style brewed coffee, i.e., ground coffee boiled in special pots, is one of the most consumed traditional beverages in Turkey given its unique and delicate flavor. In the current study, the effects of brewing in different pots (automatic coffee machine (ACM), stainless steel pot (SSP), and copper pot in hot sand (CPS) on the pyrazines, total phenolic content, and antioxidant activity in Turkish coffee were elucidated for the first time. Pyrazine compounds were carried out by the application of gas chromatography-mass spectrometry (GC-MS). A total of nine pyrazine compounds were detected in the samples. According to the results, the samples brewed in a copper pot in hot sand had the highest amount of pyrazines followed by SSP and ACM. 2-Methylpyrazine was found in abundance in all three samples while 2,5-dimethylpyrazine and 3-ethyl-2,5-dimethylpyrazine were the other substantial compounds. In contrast with the pyrazine formation in samples, ACM had a considerable amount of total phenolics and antioxidant activity followed by SSP and CPS. This may result from the less boiling time in ACM (around 90 seconds) which prevented the degradation of phenolics to an extent.

Keywords: *Turkish coffee, pyrazine content, brewing effect, total phenolic content, antioxidant activity*

1.INTRODUCTION

Coffee, produced from a plant belonging to the Rubiaceae family, is the highest consumed beverage worldwide due to its unique flavor and stimulating properties (Ayseli et al., 2021). Food and Agriculture Organization (FAO, 2020) stated that global coffee production was approximately 10.7 million tons in 2020. It is mainly produced in Brazil, Vietnam, Colombia, and Indonesia. Although its plant is not cultivated in Türkiye, coffee is the second most consumed drink after tea all over the country and it represents Turkish culture with its specific brewing technique which is boiling ground coffee in copper pots generally (Yilmaz et al., 2017).

There are many various coffee brewing techniques available today that differ according to the regions all around the world. Turkish Coffee is named after the preparation technique used by the Turks and its history dates back to the 16th century (Yilmaz et al., 2017). It is a foamy drink with a special flavor as well as a unique identity and tradition. Turkish coffee makes a name for itself with its history, and it is cooked in a coffee pot and originally served in small cups with Turkish delight as an appetizer. Turkish coffee is quite popular not only all over Turkey but also in most of the Mediterranean Caucasian countries and the Balkans (Ayseli et al., 2021).

The in-depth studies on coffee over the last few decades have increased our understanding of the physicochemical, organoleptic, and health-related components (Alves et al., 2010; Ludwig et al., 2012; de Melo Pereira et al., 2019; Ayseli et al., 2021). Coffee has many benefits for human health, and they are attributed to the presence of bioactive compounds such as phenolics and caffeine, which have strong antioxidant and radical scavenging activities (de Abreu Pinheiro et al., 2021; Alves et al., 2010). Additionally, the high popularity of coffee comes from its unique and stimulating aroma which is one of the most contributing factors to consumer preference. Green coffee beans contain many chemical compounds which act as a precursor (proteins, sugar, chlorogenic acid, carbohydrates) that serves in the formation of aroma. Fisk et al. (2012) stated that the Maillard reaction, Strecker degradation and decomposition reactions of some other compounds (sulphurous and hydroxy amino acids, proline and hydroxyproline, trigonelline, quinic acid, color pigments, and lipids such as carotenoids) that occur with the application of heat treatment create the aroma of coffee. Hence, the characteristic aroma of coffee is mainly formed in the coffee roasting and brewing processes (Zhao et al., 2020). Plant varieties, growing regions and conditions, processing methods, and grinding size are the other remarkable factors that are responsible for the distinctive aroma of coffee beverages (Zhao et al., 2020).

The brewing stage, within these factors, is of particular importance, as many parameters including temperature, duration, water ratio, and pressure affect the diffusion of aroma compounds from coffee into the brew (Petisca et al., 2013).

Differently roasted and brewed coffee beans possess a volatile profile with distinctive aroma qualities. The commonality among coffee beverages is that the aroma profile is typically dominated by heat treatment-induced volatiles like pyrazines, furans, and pyridines (de Melo Pereira et al., 2019). Within these groups, pyrazines, especially, are one of the main volatiles of coffee beverages formed by the condensation of two aminocarbonyl molecules such as amino acids or amino sugar moieties, via the Maillard reaction and are known to contribute to the unique aroma production thermally processed foods (Mortzfeld, 2020). Many factors that affect the Maillard reaction can also affect the formation of pyrazines, i.e., temperature, brewing time, pH, and the composition of the product (Zhao et al., 2020). Differently roasted or brewed coffee samples can be distinguished by the presence of compounds such as 2-methylpyrazine, 2-ethyl-3-methylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, and 2,3,5-trimethylpyrazine and most of the detected pyrazines were reported to contribute to the overall aroma with roasted, nutty and sweet notes (Ayseli et al., 2021; Amanpour and Selli, 2015; Caporaso et al., 2014). Many studies regarding coffee aroma have focused on the beverages produced with different brewing techniques but there has been no detailed study on the effect of brewing using different pots (stainless steel, copper pot in hot sand, and automatic coffee machine) on the quality of Turkish coffee. Therefore, the aim of this study was to investigate the effects of different brewing pots on the total phenolic content, antioxidant activity and to characterize the pyrazine content of brews by using GC-MS.

2. MATERIAL AND METHOD

2.1. Chemicals and Reagents

Distilled water was obtained by double purification using a Millipore-Q system (Millipore, Billerica, MA, USA). Dichloromethane (≥99.8 GC purity, CAS#: 75-09-2), and 4-nonanol (CAS#: 5932-79-6) were provided by Merck (Darmstadt, Germany). Folin–Ciocalteu (CAS#: 12111-13-6), gallic acid (CAS#: 149-91-7), 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS, CAS#: 28752-68-3), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, CAS#: 53188-07-1), 2,2-diphenyl-1-picrylhydrazyl (DPPH, CAS#: 1898-66-4) and all pyrazine standards used in this work were acquired from Sigma-Aldrich (St Louis, MO, USA).

2.2. Preparation of Turkish coffee brews

Arabica coffee (*Coffea arabica* L.) beans (2 kgs) of Brazilian origin obtained from a coffee merchant located in Adana, Türkiye were used in the preparation of Turkish coffee. The beans were roasted at 190°C for 17 min equally and ground. Ground coffee was brewed using three different pots: An automatic coffee machine (ACM) (Arçelik Mini Telve, Türkiye), stainless steel pot (SSP), and a copper pot in hot sand (CPS). Turkish coffee samples were prepared traditionally in the same conditions. 5 g of ground-roasted coffee and 75 mL water were brewed and the brew was heated until it had foamed twice, and allowed to settle (5 min) (Amanpour and Selli, 2015). The temperature of brews was constantly measured with a type K thermocouple (Verth RS232, Taiwan). The boiling time of samplless was in the order of ACM (90 sec), SSP (3 min), and CPS (4 min), while the highest average temperature was recorded in the reverse order, i.e., CPS (99,8°C) followed by SSP (99,2°C) and ACM (98,8°C). All of the brews were directly used in the analysis.

2.3. Analysis of Turkish Coffee Brews

2.3.1 Total phenolic content

Determination of total phenolic content was carried out by the Folin-Ciocalteu method (Caporaso et al., 2014). Appropriate dilutions were made to the coffee samples, 0.1 ml of each sample was taken from the diluted coffees and 0.5 ml of Folin Ciocalteu solution, 1.5 ml of 20% sodium bicarbonate, and 8 ml of distilled water were added to the coffee samples for 2 hours in the dark. After waiting, a reading was made in the spectrophotometer (Shimadzu UV–Vis 1201, Shimadzu Corporation, Tokyo-Japan) at 765 nm. Data are calculated as milligram gallic acid equivalents per kilogram (mg GAE/kg). All measurements were repeated three times and the results were expressed as the mean ± std deviation.

2.3.2. Antioxidant activity analyses

Antioxidant activities of coffee samples were determined by two different methods, DPPH and ABTS (Ludwig et al., 2012). After phenolic extraction, 3.9 ml of DPPH solution was added to 0.1 ml of phenolic extract (coffee extract). The same process was performed for ABTS (at 734 nm wavelength). The solutions were kept in the dark at room temperature for 30 minutes. Absorbance measurement was performed by reading against the blank in 10 mm cuvettes in a UV-Vis spectrophotometer (Shimadzu UV–Vis 1201, Shimadzu Corporation, Tokyo-Japan) at a wavelength of 515 nm. A Trolox calibration curve was used to calculate the antioxidant capacity of each sample.

2.3.3. Extraction of aroma compounds

Extraction of aroma compounds was carried out by purge and trap method according to Hinds et al. (2021) with some modification. The principle of this method is the attachment of aroma compounds in sorbent with the help of carrier nitrogen gas. 6 ml of the cooled and filtered coffee samples were taken to the vials and added 41,5 µg of 4-nonanol as an internal standard. Volatiles were extracted for 90 min under a nitrogen flow (50 mL min-1) after pre-incubation at 60 °C for 10 min. A detailed explanation of the extraction process was given in our previous paper (Hinds et al., 2021).

2.3.4. GC-MS analysis

Agilent 6890N gas chromatography (GC) (Agilent, DE, USA) and associated 5975B mass spectrophotometer (MS) were used for the amount and identification of aroma compounds. Separation of aroma compounds was carried out using a DB-Wax column (30 m x 0.25mm i.d x 0.5 µm, J&W Scientific-Folsom, USA). The injection temperature was 250°C, column temperature was adjusted to 220 °C by increasing 4 °C per minute after 10 minutes of holding at 40 °C. Helium was used as the carrier gas with a flow rate of 3.2 ml/min. The electron energy is 70 eV and the mass range is 35-425 m/z. The volatile compounds were identified by injecting the standard solutions for the compounds and assessed by comparison with the mass spectra of the aroma libraries available on the software (Wiley 11.0, NIST-11, and Flavor.2L). The internal standard method was used to quantify the volatile compounds. 4-nonanol was used as internal standard in the extractions because it fulfilled all necessary criteria as internal standards (Ayseli et al., 2021) and behaved similarly with the main groups of analytes in studied samples. A quantitative method based on a combination of experimental calibration by internal standards and FID response factors was employed. Each analysis was performed with 3 replications.

2.3.5 Statistical analysis

All the analyses were carried out in triplicate and mean values were reported. Analysis of variance (ANOVA) and Duncan's test was carried out using IBM (Version 24) and the results were considered statistically significant at $p < 0.05$. Multivariate analysis was performed using principal component analysis which was processed using an online tool reported by Metsalu and Vilo (2015).

3. RESULTS AND DISCUSSION

3.1. Total phenolic content and antioxidant activity of Turkish coffee brews

Due to its high content of phenolics including mainly chlorogenic acids and some important volatiles, coffee is known to be a significant source of antioxidant chemicals (Farah et al., 2005). Three colorimetric tests were used to determine the total phenolic and antioxidant content of coffee brews produced by different coffee makers. Antioxidant activity values of Turkish coffee samples were determined by two different spectrophotometric radical scavenging methods namely DPPH and ABTS. The antioxidant activities and total phenolic contents of the examined samples are shown in Table 1. In the current study, the total amount of phenolic compounds was determined as 1558 mg GAE/L, 1900 mg GAE/L, and 1413 mg GAE/L in SSP (stainless steel pot), ACM (automatic coffee machine), and CPS (copper pot in hot sand) Turkish coffee samples, respectively. As seen in Table 1, the highest phenolic content was determined in the ACM sample, in which the boiling process was the fastest of all brewing techniques. The antioxidant activities of Turkish coffee samples were found in the same order as total phenolics. The results changed between 4493-4892 μmol Trolox/L for DPPH and 6354-6839 μmol Trolox/L for ABTS analysis.

Table 1. *Total phenolic and antioxidant activity values of Turkish coffee samples brewed with different pot*

Analysis	SSP*	ACM	CPS
Total phenolic content (mg GAE/L)	1558±2,5^{b}	1900±3,1^{a}	1413±4,6^{c}
Antioxidant activity			
DPPH (μmol Trolox/L)	4763±0,5^{b}	4892±0,8^{a}	4493±1,2^{c}
ABTS (μmol Trolox/L)	6534±0,8^{b}	6839±4,7^{a}	6354±3,8^{c}

Similar results were reported by Ludwig et al. (2012) in the study investigating the differences between the filter and espresso coffee samples from Guatemala and Vietnam. The authors reported a remarkable decrease (around 58%) in the antioxidant activity of espresso coffee samples with brewing time. The compounds responsible for the antioxidant activity of coffee brews were reported to be extracted more in the early stages of brewing and then they get diluted. This goes in line with the study by Alves et al. (2010) for DPPH results in espresso coffees with different brewing times, i.e., from short to long. Similarly in the current study, the brewing technique with the shortest boiling time (ACM) had the highest total phenolic and antioxidant activity values. Additionally, it was declared in the literature that technical conditions such as coffee/water ratio, water temperature, and water pressure are substantial contributors to the extraction of bioactive compounds and volatiles in coffee brewing (Andueza et al., 2002; 2003; 2007). As the coffee/water ratio was the same for all Turkish coffee samples used in this study, the other parameters are expected to be effective on the total phenolic content and antioxidant activity. The water pressure was implied to enhance the extraction of bioactive compounds that are responsible for the antioxidant activity in previous studies (Andueza et al., 2002; Pérez-Martínez et al., 2010). As the automatic coffee machine uses more water pressure than the other two pots, higher values of total phenolics and antioxidant activity can be supported by this information.

3.2. Pyrazine content of Turkish coffee samples

This study is focused on the pyrazine content of Turkish coffee samples out of all volatiles. A total of 9 pyrazines were determined by GC-MS in each sample including pyrazine, 2-methyl-pyrazine, 2,5-dimethylpyrazine, 2,6-dimethyl-pyrazine, 2,3-dimethylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-5-methylpyrazine, 2-ethyl-3-methylpyrazine, 3-ethyl-2,5-dimethylpyrazine. These compounds were previously reported in various papers investigating Turkish and French press coffee (Ayseli et al., 2021; Amanpour and Selli, 2015), espresso coffee (Angeloni et al., 2001), decaffeinated and regular coffee (Zou et al., 2022).

A heat map was produced to display the variation of pyrazine compounds in Turkish coffee samples according to the brewing technique. As demonstrated in Figure 1, Turkish coffee brews were located horizontally while the pyrazine compounds were positioned vertically. The average concentration of each pyrazine was marked by a different color on the heat map changing between blue and red. The blue color represents the volatiles lower than the mean level while the red colors show the ones higher than the mean. Turkish coffee brews were divided into two clusters based on the brewing techniques while CPS and ACM were grouped into the same category. Samples clustered in the same category show a high degree of similarity and correlation.

The highest total pyrazine content was determined in CPS (2957 µg/L) followed by SSP (2534 µg/L) and ACM (2027 µg/L). The data demonstrated that the brewing technique had a significant effect on the concentration of these compounds. 2-Methylpyrazine was found as the most abundant compound with a concentration changing between 467-793 µg/L with CPS having the highest amount. This compound was previously reported to be the dominant pyrazine compound in Turkish coffee and French press coffee (Amanpour and Selli, 2016), roasted ground coffee (Pickard et al., 2013), medium and dark roasted Turkish coffee (Ayseli et al., 2021) and freshly brewed espresso coffee (Akiyama et al., 2009). According to Pérez-Martínez et al. (2008), the most prevalent pyrazine in coffee brews is 2-methylpyrazine, which exhibits behavior resembling that of pyridine. This compound was also reported as a key odorant contributing to the roasted, chocolate, earthy, and nutty odor notes of coffee samples (Laukaleja and Kruma, 2019; Ayseli et al., 2021). According to Lee et al. (2017), pyrazine compounds such as 2-methylpyrazine, with chocolate and nutty notes, and 2-ethyl-6-methylpyrazine, with roasted hazelnut note, have a positive effect on the aroma of coffee beans when they are lightly roasted. As the roasting degree increases, these compounds may be linked to "black roast defect," which is defined as overripe, harsh, and fermented flavor in dark roasted coffees (Yang et al., 2016). The aroma compounds of coffee are mainly produced in the process of roasting and brewing (de Melo Pereira et al., 2019). Depending on the degree of these thermal applications, the formation of pyrazine, pyridine, furan, and furanone-like compounds changes drastically (Moon and Shibamoto, 2009). Among them pyrazines are mainly aromatic compounds determined in a wide variety of foodstuff other than coffee including cocoa, meat, bread, boiled potatoes, roasted peanuts, roasted sesame seeds, corn chips, popcorn, dairy products, fresh tomatoes, spinach, asparagus, peppers, and peas (Buchbauer et al. 2000). Since pyrazines have a low detection threshold value, they have a crucial impact in the formation of the unique aroma of coffee beverages. These compounds are generally formed as a result of the Maillard reaction by pyrolysis of hydroxyamino acids and the reduction of sugars (Mottram, 2007).

The structure of the food matrix is one of many variables that could affect pyrazine production.

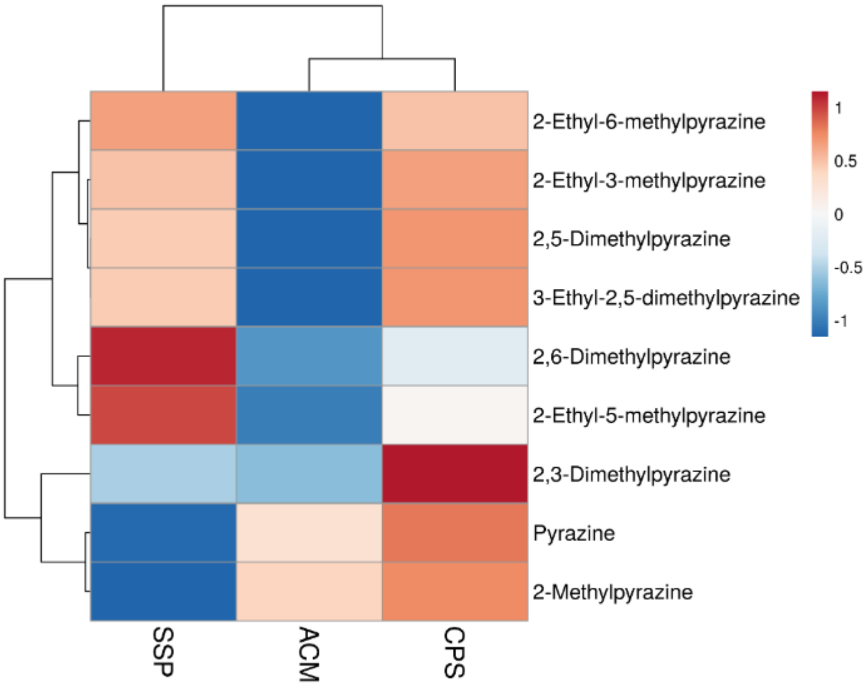

Figure 1. Heatmap of the volatile compounds of the Turkish coffee samples. Rows (9 rows; independent variables) and columns (3 columns; coffee samples) are grouped based on correlation distance and average linkage

Additionally, parameters like temperature and duration in brewing are likely the most significant variables (Asikin et al., 2016). Ayseli et al. (2021), investigating the aroma compounds of coffee produced from beans roasted at different degrees, emphasized that especially pyrazines play an important role in the aroma of coffee and that the heat treatment applied to the formation of these compounds has a substantial effect. In another study by Andueza et al. (2003), the pyrazine content of espresso coffee was related to the brewing water temperature. The authors reported that the higher the water temperature, the higher amount of pyrazines formed. This goes in line with our study as the highest amount of pyrazines was determined in Turkish coffee brewed with copper pot in the sand (CPS) which had the highest recorded temperature followed by SSP. The ACM sample with lower brewing temperature and shorter time had the lowest total of pyrazines. Following 2-methylpyrazine, the compounds found in abundance in Turkish coffee samples were 2,5-dimethylpyrazine, 3-ethyl-2,5-dimethylpyrazine, and 2,6-dimethylpyrazine (Figure 1). The order of concentration of these compounds similar to 2-methylpyrazine is as follows: CPS>SSP>ACM. Within these, 2,5-dimethylpyrazine was reported to be formed by thermal degradation of L-threonine (Guo et al., 2018). Additionally, all of the pyrazines determined in this study were reported to be produced by the reaction of sugars and amines resulting in α-aminocarbonyl intermediates which condense to form a broad range of pyrazines (Moon and Shibamoto, 2009). All these pyrazine compounds were reported previously in coffee-based beverages including Turkish coffee (Ayseli et al., 2021), ultrasonicated blended coffee (Zamanipoor et al., 2020), French press coffee (Amanpour and Selli, 2016), and freshly brewed espresso (Akiyama et al., 2009).

4. CONCLUSION

This research aimed to examine the variations in the pyrazine composition, total phenolic content, and antioxidant activity of Turkish coffee brewed in three different pots. The findings displayed that the difference in brewing had a statistically important impact on these properties of coffee samples. According to GC-MS results, nine pyrazines were determined in all samples while CPS had the highest concentration and was followed by SSP and ACM. 2-Methylpyrazine was found as the most prevalent pyrazine in coffee brews. The higher temperature and the longer brewing time applied in CPS resulted in a higher amount of pyrazine compounds than in the other methods. Contrary to volatiles, the lowest total phenolic content and antioxidant activity were recorded in the CPS sample, while ACM had the highest values. In conclusion, ACM is recommended for better preservation of antioxidant activity while CPS provides higher pyrazine content which affects the aroma quality of Turkish coffee.

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