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Effect of pH and Brewing Methods on Volatile Nitrogenous Compounds in Turkish Coffee

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Abstract

In this study, the changes in volatile nitrogenous compounds of Turkish coffee samples obtained using water with four different pH values (6.5, 7.5, 8.5, and 9.5) and three different brewing methods (cold brewing, traditional brewing, and machine brewing) were investigated. The extraction of volatile nitrogenous compounds was performed using the liquid-liquid extraction method. GC-MS technique was used for the quantification and identification of volatile nitrogenous compounds in Turkish coffee. These compounds were examined under three groups: pyrazines, pyrroles, and pyridines. In all samples of Turkish coffee obtained by all brewing methods, a total of 29 volatile nitrogenous compounds were identified, and it was found that 17 of them were pyrazines, 7 consisted of pyrroles, and 5 consisted of pyridines. Therise in pH values of the water used to Turkish coffee generally increased the total amount of volatile nitrogenous compounds. Statistical analysis of the volatile nitrogenous compound data showed that both the change in pH value and the different brewing methods had a significant effect on Turkish coffee samples.

Keywords: Coffee, pyrazines, pyridines, pyrroles, GC-MS

1.INTRODUCTION

Coffee is a beverage very rich in aroma compounds, phenolic and antioxidant components. Although green coffee beans are rich in phenolic and antioxidant components, they are very poor in aroma (Buffo and Cardelli-Freire, 2004; Zellner et al. 2008; Fisk et al. 2012). For this reason, complex pyrolytic reactions occurring by roasting green coffee beans at different temperatures and times are essential to obtain the desired coffee aroma and taste (Oosterveld et al. 2003). Unroasted coffee beans contain many precursor chemical compounds (proteins, sugar, chlorogenic acid, carbohydrates) that serve in aroma formation. Fisk et al. (2012) stated that the Maillard reaction, Strecker decomposition, and the decomposition reactions observed in some other compounds (color pigments and lipids such as sulfur and hydroxy amino acids, proline and hydroxy proline, trigonelline, quinic acid, carotenoids) that occur with the application of heat treatment create the coffee aroma. Depending on the time and temperature applied in the roasting process, coffee; is classified as light roasted, medium-roasted, and dark-roasted (Buffo and Cardelli-Freire, 2004; Mondello et al. 2005; Zellner et al. 2008; Bröhan et al. 2009).

To date, more than 1000 aroma compounds of different chemical classes and characters have been identified in coffee. Of these, 350 were detected in green coffee and approximately 650 in roasted/ground coffee beans and cooked coffee (Blank et al. 1992; Lee and Shibamoto 2001; Marin et al. 2008; Zellner et al. 2008; Miyazoto et al., 2013). 50% of the aroma compounds found in

coffee consist of furans, pyranones, thiophenes, pyrazines, pyrroles, oxazoles, thiazoles, pyridines, amines, and sulfur compounds. The remaining aroma compounds are ketones (9.5%), phenols (9.2%), hydrocarbons (7.8%), acids (6.9%), esters (5.8%), alcohols (4%), aldehydes (4.4%), and lactones (1.5%) (Hashim and Chaveron, 1996; Buffo and Cardelli-Freire, 2004; Mondello et al. 2005; Zellner et al. 2008; Fisk et al. .2012). Among these compounds, pyrazines, pyrroles and pyridines, which are in the group of volatile nitrogenous compounds, are responsible for the unique aroma and flavor of coffee. These compounds are formed during the roasting process and contribute to the overall sensory experience of the beverage. Understanding the composition and behavior of these volatile compounds is crucial for coffee producers and researchers who want to optimize the quality of their products. Additionally, studying these compounds can provide valuable information about the chemistry of coffee and its interactions with other substances (Flament, 2002).

In this study, changes in volatile nitrogenous compounds of Turkish coffee samples obtained using water with four different pH values (6.5, 7.5, 8.5, and 9.5) and three different brewing methods (cold brewing, traditional brewing, and machine brewing) have been investigated.

2. MATERIALS AND METHOD

2.1. Materials

In this study, Turkish Coffee, obtained from Arabica coffee beans and ground to a particle size of 75-125 µm, was used due to its unique aroma. All chemical solvents and standard compounds were obtained from Sigma Aldrich (St. Louis, Missouri, USA) and Merck (Darmstadt, Germany).

2.2. Method

2.2.1. Adjusting the pH Value of Water and Cooking Turkish Coffee

According to the "Regulation on Water for Human Consumption" issued by the Ministry of Health, it is stipulated that the pH value of drinking water should be between ≥6.5 and ≤9.5 (Anonymous, 2005). In adherence to this regulation set forth by the Ministry of Health, the pH values of pure water in the cooking of Turkish coffee were adjusted to 6.5, 7.5, 8.5, and 9.5 by using 0.01 N citric acid and 0.01 N NaOH buffer solutions. Following the pH adjustment, Turkish coffees were prepared using both a traditional copper coffee pot and a Turkish coffee machine (Kısmet, King, production place). In the preparation of the coffee samples for analysis, 5 g of ground coffee sample was measured, and the coffee-water mixture was prepared for brewing, ensuring a total volume of 65 ml, including the added ground coffee. In preliminary tests, it was determined that the outlet temperature of Turkish coffee brewed from the machine was 93 °C. Consequently, in the traditional brewing method, the process was monitored using a thermometer, and it was concluded when the temperature reached 93 °C. Turkish coffees prepared using both methods were rapidly cooled in an ice water bath and prepared for aroma extraction. Additionally, Turkish coffees were cold brewed at 20 oC by stirring for 1 hour, taking into account the particle size of the coffee. Each of the Turkish coffee samples is coded as given in Table 1.

2.2.2. Extraction of free volatile nitrogenous compounds

For each aroma extraction, 65 ml of Turkish coffee was utilized. The processes applied in the extraction of free volatile nitrogenous compounds are given in Figure 3.1. A total of 65 ml of Turkish coffee was placed in a 500 ml conical flask and 40 ml of dichloromethane and 40 µg of 4-nonanol as internal standard were added. The extraction process was conducted by stirring the

mixture in the conical flask under nitrogenous gas, at 4-5 °C, in a magnetic stirrer for 30 minutes (Blanch et al., 1991; Priser et al., 1997). Following this procedure, the solvent phase containing volatile nitrogenous compounds was taken from the contents of the flask, which was divided into two phases, and concentrated in the "Vigreux" distillation column at 40 °C until 1 ml remained. The concentrated liquid was directly injected into the GC-MS system and free volatile nitrogenous compounds were determined. Extractions were performed in triplicate.

2.2.3. GC-MS conditions

The quantification and identification of volatile nitrogenous compounds were carried out using the "AOC 6000 Plus Combi PAL" system connected to the "OP-2020 NX" model gas chromatography-mass spectrometer (Shimadzu).

The separation of volatile nitrogenous compounds was conducted using a DB-WAX capillary column (60 m x 0.25 mm x 0.4 µm). The injector temperature was 220 °C, the detector temperature was 250 °C, the column temperature was increased by 2 °C per minute to 220 °C and then by 3 °C per minute to 245 °C after waiting for 3 minutes at 60 °C. The temperature was then programmed to remain constant for 20 minutes. The amount to be injected into the device is 1 µl. Helium (He) was used as the carrier gas. The flow rate of He was set at 1.5 ml/min. Detector and injector temperatures are fixed at 250 °C.

By keeping the ionization energy of the mass spectrometer at 70 eV, the ion source temperature at 250 °C, and the quadrupole temperature at 120 °C, scanning was performed between 29-350 mass/charge (m/e) at 1-second intervals. Identification of the peaks was made by injecting standard solution for compounds with standards and comparing the mass spectra for compounds without standards with the mass spectra in the computer memory. After the identification of the peaks, the concentrations of volatile nitrogenous compounds were calculated by the internal standard method (Schneider et al., 1998; 2001).

2.2.4. Calculation of amounts of volatile nitrogenous compounds

To calculate the amounts of volatile nitrogenous compounds after identifying the peaks, calibration curves were obtained from standard compounds, and the amounts were calculated using the following formula with the internal standard method. The response factor of each compound was taken into account in the calculation.

$$Ci = (Ai / Ast) \times Cst \times RF \times HF$$

Ci: Concentration of the compound
Ai: Peak area of the compound
Ast: Peak area of internal standard
Cst: Concentration of internal standard (40 µg/100 ml)
RF: Response factor
HF: Calculation factor (factor for converting the sample amount to kg: 10).

2.2.5 Statistical Analyses

The data obtained as a result of this study were analyzed with 95% confidence interval and analysis of variance (One-way ANOVA) using the statistical program (SPSS 23.0.0, SPSS Inc., USA). Whether the difference between the averages of the experimental groups was significant or not was determined by the Duncan multiple comparison tests performed after analysis of variance ($p<0.05$). Results were presented as mean ± standard deviation. (Bek and Efe, 1988; Özdamar, 1999; Landau and Everitt, 2004).

3. RESULTS AND DISCUSSION

The number and amounts of volatile nitrogenous compounds detected in Turkish coffee samples are given in Table 2. When Table 2 is examined, it is seen that 29 volatile nitrogenous compounds were detected in Turkish coffee samples. Volatile nitrogenous compounds consist of three different subgroups: pyrazines, pyrroles, and pyridines. Cheong et al. (2013) reported in a study that ketones, lactones, volatile phenols, pyrazines, pyridines, pyrroles, and sulfur-containing compounds are the main contributors to Arabica coffee aroma. Similarly, Petisca et al. (2013) reported that furans, pyrroles, pyridines, pyrazines, ketones, hydrocarbons, and aldehydes are among the groups of compounds that contribute significantly to ground coffee aroma and espresso coffee. In another study, some of the main classes of compounds identified in roasted coffee beans were sulfur compounds, pyrazines, pyridines, pyrroles, oxazoles, furans, aldehydes, ketones, and volatile phenols (Flament 2002). Ayseli et al. (2021) in their study, in a Turkish coffee sample obtained from medium roasted coffee beans, 4 aldehydes, 4 alcohols, 5 acids, 9 volatile phenols, 9 furans and furanones, 10 ketones, 3 esters, 2 lactones, 1 pyran, 4 pyridines, 16 pyrazines. They identified a total of 74 aroma compounds, including four pyrroles, 2 thiazoles and 1 thiophene. On the other hand, researchers found that in the Turkish coffee sample obtained from double-roasted coffee beans, there were 3 aldehydes, 4 alcohols, 1 acid, 7 volatile phenols, 6 furans and furanones, 6 ketones, 1 ester, 1 lactone, 2 pyranes, 4 pyridines, 16 pyrazines. They detected a total of 58 aroma compounds, including 4 pyrroles, 2 thiazoles, and 1 thiophene. In another study, a total of 60 and 58 aroma compounds, including furans, lactones, volatile phenols, pyridines, pyrazines, acids, cyclopentenes, pyrroles, furanones, ketones, alcohols, aldehydes, thiols, and other compounds, were detected in Turkish coffee and French Press coffee samples respectively have been defined (Amanpour and Selli, 2016).

Volatile nitrogenous compounds are the major group of aroma compounds found in coffee (Flament and Bessiere-Thomas 2002). During the coffee roasting process, Maillard reactions occur with the reduction of reducing sugars and amino acids naturally found in green coffee, and this reaction chain is responsible for the formation of many heterocyclic compounds, including pyrazines, pyridines and pyrroles (Lopez-Galilea et al. 2006). Therefore, the formation of this group of aroma compounds caused by roasting conditions plays an important role in the flavor of brewed coffees (Moon and Shibamoto 2009). In this study, volatile nitrogenous compounds were examined under three different subgroups: pyrazines, pyrroles, and pyridines.

3.1. Pyrazines

Pyrazines, which are among the basic aroma components of coffee, are products of Maillard condensation reactions, and more than 80 such compounds have been previously detected in coffee (Grosch 2001). Many pyrazines are characterized by a roasted odor in cooked foods, and these compounds are considered volatile substances that characterize the aromas of heat-treated foods (Maarse and Visscher 1989; Shimoda and Shibamoto 1990). Pyrazines are associated with roasted and earthy/musty flavors, especially in ground coffee and brewed coffees (Blank et al., 1991; Maeztu et al., 2001; Sanz et al., 2002).

A total of 17 pyrazine compounds were identified in all Turkish coffee samples considered within the scope of this study. In their study, Amanpour and Selli (2016) identified nine and eight pyrazine compounds in Turkish coffee and coffee brewed with the French press method, respectively. If examined in terms of total pyrazine content, the highest

amount of pyrazine was detected in the M85 sample (6227.9 μg/l), and remarkably, the lowest amount of pyrazine was detected in the T85 sample (3424.7 μg/l) ($p < 0.05$). In cold-brewed samples, the pH increase generally caused a qualitative increase in the total pyrazine amount. There was a decrease of 1.8% to 6.2% in Turkish coffee samples cooked with the traditional method and with a pH value of up to 8.5 ($p<0.05$). It was found that the highest amount of pyrazine (4255.3 μg/l) in Turkish coffees cooked with the traditional method was in the T95 sample ($p<0.05$). In machine-cooked Turkish coffee samples, the increase in pH increased the amount of pyrazine in all samples except the M95 sample.

The most dominant pyrazine in Turkish coffee samples is 2-methyl-pyrazine. While this compound was detected in the highest amount in the C85 sample (1724.8 μg/l), the lowest amount was found in the T75 sample (850.4 μg/l) ($p<0.05$). The amount of 2-methyl-pyrazine increased in C75 and C85 samples (1688.1 μg/l and 1724.8 μg/l), and a similar situation was observed in M75 and M85 samples (1667.2 μg/l and 1673.5 μg/l).) was observed ($p<0.05$). On the other hand, in general, increasing pH increased the amount of 2-methyl-pyrazine in Turkish coffee samples cooked with the traditional method. Amanpour and Selli (2016) determined 2-methyl-pyrazine as the most dominant pyrazine compound in Turkish coffee and coffee brewed with the French press method. Researchers reported that the amount of 2-methyl-pyrazine was higher in coffee brewed with the French press method (1.129 μg/kg) than in Turkish coffee (1.032 μg/kg). In another study, 2-methyl-pyrazine was also detected in roasted and ground Arabica coffee and was identified as the most dominant pyrazine among 20 other pyrazines detected (Sanz et al. 2002). Martinez et al. (2008) reported in their study that 2-methyl pyrazine, which has a similar behavior to pyridine, is the pyrazine found in the highest amount in brewed coffees.

The second dominant pyrazine compound detected in coffee samples is 2,6-dimethyl-pyrazine. This compound was detected in the highest amounts in cold-brewed Turkish coffee samples. In terms of cooking methods, it was determined that the amount of 2,6-dimethyl-pyrazine was higher in Turkish coffee samples obtained by machine cooking than in those obtained by traditional methods. This compound was detected in the highest amount in the M75 sample (945.0 μg/l) and in the lowest amount in the T85 sample (568.8 μg/l) ($p<0.05$). In a study conducted on Turkish coffee, it was reported that 2,6-dimethyl-pyrazine compound was the second most dominant pyrazine compound after 2-methyl-pyrazine, and the amount of this compound in Turkish coffee was determined to be 658 μg/kg (Amanpour and Selli, 2016). Similarly, Ayseli et al. (2021), the 2,6-dimethyl-pyrazine compound was the second most dominant pyrazine compound. Researchers found that the amount of this compound was 362 μg/l in Turkish coffee obtained from medium-roasted coffee beans and 236 μg/l in the Turkish coffee sample obtained from double-roasted coffee beans.

The third pyrazine compound dominant in Turkish coffee samples is 2,5-dimethyl-pyrazine. Except for the M95 sample, increasing pH qualitatively increased the amount of this compound in all other samples ($p<0.05$). In terms of brewing methods, it was determined that the highest amounts of this compound were in Turkish coffee samples obtained by the cold brewing method ($p < 0.05$). It was determined that the amount of 2,5-dimethyl-pyrazine in Turkish coffee samples cooked with the traditional method was lower than in the samples obtained with other methods ($p < 0.05$). While the highest amount of 2,5-dimethyl-pyrazine was detected in the C85 sample with 746.5 μg/l, the lowest amount of

this compound was found in the M95 sample with 465.3 μg/l ($p<0.05$). Amanpour et al. (2016) reported that the third most dominant pyrazine compound in Turkish coffee was 2,5-dimethyl-pyrazine and they determined the amount of this compound as 321 μg/kg. Ayseli et al. (2021) reported that 2,5-dimethyl-pyrazine was the third dominant pyrazine compound in Turkish coffee samples obtained from both medium-roasted and double-roasted coffee beans, and they determined the amount of this compound as 374 μg/kg and 538 μg/kg, respectively.

3.2. Pyrroles

Pyrroles are common compounds of the amino acid-sugar model system. They are closely related to furan compounds and probably result from the reaction of a 3-deoxyketose with ammonia and/or amino compound followed by dehydration and ring closure. Some pyrroles are desirable aroma compounds in foods and may contribute to the characteristic odor of food, but alkyl and acetyl pyrroles have been reported to have adverse odors (Mottram 2007). All of these compounds have also been detected in brewed coffees and espresso coffee aromas, as well as in-ground Arabica coffee (Martinez et al. 2008; Maeztu et al. 2001; Sanz et al. 2002).

A total of 7 pyrrole compounds were determined in all Turkish coffee samples considered in this study. When Turkish coffee samples were examined in terms of total pyrrole compounds, it was determined that the amount of total pyrrole compounds increased due to the increase in pH value in the samples obtained by the cold brewing method ($p<0.05$). On the other hand, a decrease in the total pyrrole amounts was detected in Turkish coffee samples cooked both traditionally and machine-cooked with water with pH values of 7.5 and 8.5 ($p<0.05$).

The two most dominant pyrrole compounds in terms of quantity in Turkish coffee samples are 2-formylpyrrole and 2-acetylpyrrole, respectively. The highest amount of 2-formylpyrrole compound was detected in the C85 sample (698.7 μg/l) and the lowest amount was detected in the T75 sample (508.5 μg/l) ($p<0.05$). It was observed that the amount of this compound in all Turkish coffee samples increased relatively when the increase in pH value was taken into account ($p<0.05$). On the other hand, the amount of 2-acetylpyrrole increased in Turkish coffee samples obtained by cold brewing due to the increase in pH value ($p<0.05$). However, except for T95 and M95 samples, the increase in pH partially reduced the amount of 2-acetyl pyrrole in both conventional and machine-cooked coffee samples ($p<0.05$). While the highest amount of 2-acetylpyrrole was detected in the M95 sample with 567.7 μg/l, the lowest amount of this compound was found in the T75 sample with 457.8 μg/l ($p<0.05$). Ayseli et al. (2021) detected 278 μg/l and 486 μg/l 2-acetyl pyrrole in Turkish coffee samples obtained from medium and double-roasted coffee beans, respectively.

Table 1. Codes of Turkish coffee samples

Brewing Methods	**pH 6.5**	**pH 7.5**	**pH 8.5**	**pH 9.5**
Cold Brewing	C65	C75	C85	C95
Traditional Brewing	T65	T75	T85	T95
Automatic Brewing in the Machine	M65	M75	M85	M95

Table 2. Aroma composition of Turkish coffee samples brewed with different methods

	Volatile Nitrogenous Compounds	LRI	C65	C75	C85	C95	T65	T75	T85	T95	M65	M75	M85	M95	Identification
	Pyrazine Compounds														
1	Pyrazine	1266	94.2^{bc}	104.8^{b}	59.5^{d}	113.4^{b}	59.1^{d}	145.2^{a}	60.1^{d}	86.6^{c}	61.1^{d}	101.8^{b}	56.8^{de}	50^{e}	LRI,MS,Std
2	2-methyl-Pyrazine	1306	1469.2^{b}	1688.1^{a}	1724.8^{a}	1529.6^{ab}	879.8^{e}	850.4^{e}	1014.5^{d}	1317.8^{c}	938.5^{de}	1667.2^{a}	1673.5^{a}	911.6^{de}	LRI,MS,Std
3	2,5-dimethyl-Pyrazine	1346	652.7^{b}	725.6^{a}	746.5^{a}	696.8^{ab}	468.1^{d}	509.2^{c}	476.3^{cd}	571.6^{bc}	520.7^{c}	724.7^{a}	686.1^{ab}	465.3^{d}	LRI,MS,Tent
4	2,6-dimethyl-Pyrazine	1352	875.6^{ab}	942.4^{a}	855.1^{b}	922.5^{a}	590.5^{c}	585.5^{c}	568.8^{c}	750.5^{bc}	611.2^{c}	945^{a}	850.9^{b}	586.9^{c}	LRI,MS,Tent
5	2-ethyl-Pyrazine	1358	319.2^{ab}	356.7^{a}	317.6^{ab}	350.4^{a}	229.5^{c}	234.1^{c}	195^{d}	262.1^{b}	252.6^{bc}	361.5^{a}	288.7^{b}	222.5^{c}	LRI,MS,Std
6	2,3-dimethyl-Pyrazine	1364	131.6^{b}	156^{a}	142.4^{ab}	156.5^{a}	106.9^{d}	109.7^{d}	112.8^{d}	124.6^{bc}	120.4^{c}	156.2^{a}	143^{ab}	106.5^{d}	LRI,MS,Std
7	2-ethyl-6-methyl-Pyrazine	1398	303.7^{b}	341.2^{a}	354.5^{a}	336.4^{a}	231.9^{c}	249.2^{c}	209.2^{d}	252^{c}	268.4^{bc}	319.7^{ab}	340.8^{a}	238.7^{c}	LRI,MS,Tent
8	2-ethyl-5-methyl-Pyrazine	1402	171.7^{b}	194.5^{a}	204^{a}	192.1^{a}	132.3^{cd}	140.7^{c}	119.6^{d}	143.8^{c}	151.3^{bc}	182.4^{ab}	207.3^{a}	139^{c}	LRI,MS,Tent
9	2,3,5-trimethyl-Pyrazine	1411	195.8^{a}	214.7^{a}	214.4^{a}	216.4^{a}	162.6^{bc}	162.2^{bc}	147.5^{c}	174.5^{b}	181.1^{ab}	212.3^{a}	219.1^{a}	161.5^{bc}	LRI,MS,Std
10	2-ethyl-3-methyl-Pyrazine	1413	110.6^{ab}	123.4^{a}	121.1^{a}	125.2^{a}	87.6^{bc}	76^{c}	74.2^{c}	93.8^{b}	101.3^{b}	122.8^{a}	107.3^{b}	94.8^{b}	LRI,MS,Std
11	2-Ethyl-3,5-dimethylpyrazine	1440	22.8^{b}	24.3^{ab}	17.8^{cd}	24.1^{ab}	20.9^{bc}	19.9^{c}	15.3^{d}	19.8^{c}	26.8^{a}	25.2^{a}	22.4^{b}	25.7^{a}	LRI,MS,Tent
12	2-Ethyl-3,6-dimethylpyrazine	1448	40.4^{b}	35.1^{c}	37.9^{bc}	67.8^{a}	36^{c}	38.9^{bc}	24.5^{e}	29.5^{d}	43.3^{b}	36.1^{c}	29.9^{d}	34.2^{cd}	LRI,MS,Tent
13	2,3-diethyl-5-methyl-Pyrazine	1491	25de	27.7^{d}	30.3^{c}	33.1^{c}	38^{ab}	36.1^{b}	23.7^{e}	27.7^{d}	42.6^{a}	41.7^{a}	44.6^{a}	39.8^{ab}	LRI,MS,Tent
14	6,7-Dihydro-5-methyl-5(H)-cyclopentapyrazine	1596	29.8^{bc}	27.7^{c}	27.4^{c}	57.9^{a}	43.6^{ab}	22.3^{e}	22.1^{e}	24.7^{d}	55.5^{a}	27.3^{c}	34.8^{b}	48.4^{ab}	LRI,MS,Tent
15	2-acetyl-Pyrazine	1613	97.1^{c}	108^{b}	115.2^{b}	139.9^{ab}	108.8^{b}	102.2^{bc}	76.2^{d}	85.8^{c}	112.5^{b}	109.6^{b}	150.4^{a}	100.4^{bc}	LRI,MS,Std
16	2-acetyl-3-methyl-Pyrazine	1630	180.3^{c}	190.8^{c}	189.7^{c}	256.4^{b}	278.7^{b}	161.1^{d}	158.9^{d}	170.2^{cd}	360.6^{a}	232.3^{bc}	63.7^{e}	329.9^{a}	LRI,MS,Tent
17	Pyrazine carboxylamide	1742	141.9^{ab}	97.2^{d}	106.7^{d}	154.5^{a}	162.5^{a}	126.9^{c}	125.8^{c}	120.5^{c}	163.5^{a}	131.4^{bc}	122.5^{c}	115.6^{cd}	LRI,MS,Std
	Total		**4861.6^{b}**	**5358.2^{a}**	**5264.9^{a}**	**5373^{a}**	**3636.8^{d}**	**3569.6^{d}**	**3424.5^{d}**	**4255.5^{c}**	**4011.4^{c}**	**5397.2^{a}**	**5041.8^{ab}**	**3640.8^{d}**	

(Continued from Table 2)

	Volatile Nitrogenous Compounds	LRI	C65	C75	C85	C95	T65	T75	T85	T95	M65	M75	M85	M95	Identification
	Pyrrole Compounds														
1	Pyrrole	1534	44.8^{a}	38.2^{b}	31.8^{c}	25.9^{d}	29.2^{c}	21.2^{e}	25.9^{d}	17.9^{f}	40.8^{ab}	24.3^{de}	35.9^{bc}	26^{d}	LRI,MS,Std
2	2-ethyl-Pyrrole	1607	35.7^{c}	35.8^{c}	30^{d}	28.6^{d}	86^{a}	37.1^{c}	30.5^{d}	24.4^{ef}	67^{b}	33.0^{cd}	26.9^{e}	20.8^{f}	LRI,MS,Std
3	2-acetyl-1-methyl-Pyrrole	1641	46.9^{d}	58.9^{c}	61.3^{c}	92.1^{a}	84^{ab}	48.2^{d}	46.5^{d}	75.5^{b}	94.6^{a}	102.6^{a}	84.8^{ab}	82.1^{ab}	LRI,MS,Tent
4	Furfuryl pyrrole	1819	65.7^{c}	28.8^{f}	27.7^{f}	83.6^{a}	70.3^{b}	56.3^{cd}	51.3^{d}	47.3^{e}	70.6^{b}	61.5^{c}	54.2^{d}	42.4^{e}	LRI,MS,Tent
5	2-acetyl pyrrole	1969	480.7^{b}	537.8^{a}	529.8^{a}	522.2^{a}	493.9^{b}	457.8^{b}	465.2^{b}	494.3^{b}	538^{a}	521.4^{a}	535.1^{a}	567.7^{a}	LRI,MS,Std
6	2-pyrrolidon	2013	24^{f}	61^{c}	43.6^{d}	66.3^{c}	115.4^{a}	24.3^{f}	34.9^{e}	96.5^{b}	105.8^{ab}	61^{c}	52.7^{cd}	131.8^{a}	LRI,MS,Std
7	2-Formylpyrrole	2031	600.6^{ab}	661^{a}	698.7^{a}	679.7^{a}	520.5^{b}	508.5^{b}	550.4^{b}	615.5^{a}	622.2^{a}	648.7^{a}	628.2^{a}	693.8^{a}	LRI,MS,Std
	Total		**1298.4^{d}**	**1421.5^{b}**	**1422.9^{b}**	**1498.4^{ab}**	**1399.3^{bc}**	**1153.4^{e}**	**1204.7^{d}**	**1371.4^{c}**	**1539^{a}**	**1452.5^{b}**	**1417.8^{b}**	**1564.6^{a}**	
	Pyridine Compounds														
1	Pyridine	1239	940.3^{a}	982.7^{a}	1019.8^{a}	973.6^{a}	516.1^{b}	575.6^{b}	545.6^{b}	793.8^{ab}	562.5^{b}	1010.2^{a}	1090.1^{a}	475^{c}	LRI,MS,Std
2	5-Ethyl-2-methylpyridine	1443	26.4^{b}	19.6^{d}	23.3^{c}	30.5^{a}	23.9^{c}	22.7^{cd}	20.1^{d}	22.2^{cd}	27^{b}	26.3^{b}	28^{ab}	25.3^{b}	LRI,MS,Std
3	2-acetyl-Pyridine	1589	14.1^{cd}	15.9^{c}	16.5^{c}	16.2^{c}	16.4^{c}	16.8^{c}	10.4^{f}	11.1^{e}	23.7^{a}	18.2^{b}	19.3^{ab}	13.3^{d}	LRI,MS,Std
4	2-(3-Phenylpropyl)Pyridine	2075	40.8^{a}	32.5^{ab}	27^{b}	17.8^{c}	25.6^{b}	23.4^{b}	16.7^{c}	14.2^{cd}	23.7^{b}	18.5^{c}	13.6^{d}	10.2^{e}	LRI,MS,Tent
5	2-Pyridine methanol	2110	173^{c}	175.4^{c}	179.3^{c}	210.9^{b}	209.1^{b}	171.6^{cd}	166.6^{d}	183.7^{bc}	215^{b}	174.8^{c}	177.4^{c}	240.4^{a}	LRI,MS,Std
	Total		**1194.6^{b}**	**1193.6^{b}**	**1265.9^{ab}**	**1249^{b}**	**791.1^{e}**	**810.1^{de}**	**759.4^{e}**	**1025^{c}**	**851.9^{d}**	**1248^{b}**	**1328.4^{a}**	**764.2^{e}**	
	General Total		**7354.6^{a}**	**7973.3^{a}**	**7953.7^{a}**	**8120.4^{a}**	**5827.2^{c}**	**5533.1^{c}**	**5388.6^{c}**	**6651.9^{b}**	**6402.3^{b}**	**8097.7^{a}**	**7788^{a}**	**5999.6^{bc}**	

LRI: Linear retention index calculated on DB-WAX capillary column; Concentration: average of 3 different extraction results in µg/l; Identification: LRI (Linear retention index), MS (Mass spectrometry library), Std (Standard chemical substance), MS tent.(tentative identification by MS); S: not detected; The standard deviation values of aroma substances are below 10%. a, b, c, c, d, g, h: The difference between the values shown with different letters in the same column is statistically significant (p<0.05).

3.3. Pyridines

Pyridines are found in relatively few foods that have undergone some heat treatment (Maga 1981). The presence of high amounts of pyridine is often associated with off-flavors (Sanz et al. 2002). In a study on the effect of time-temperature conditions on aroma formation during roasting of coffee, high temperature was needed to initiate the reactions that lead to the formation of pyridine, and the concentration of these compounds increased continuously during roasting. Thus, roasting time is more effective than temperature (Baggenstoss et al. 2008).

In this study, a total of 5 pyridine compounds were detected in Turkish coffee samples. When Turkish coffee samples were examined in terms of total pyridine compounds, it was determined that the amount of total pyrrole compounds increased in the samples obtained by the cold brewing method depending on the increase in pH value ($p < 0.05$). On the other hand, total pyridine amounts increased due to the increase in pH value in both traditionally and machine-cooked Turkish coffee samples, except for T85 and M95 samples ($p<0.05$).

In terms of quantity, the most dominant pyridine compounds in Turkish coffee samples are pyridine and 2-pyridine methanol compounds. The amount of pyridine in all Turkish coffee samples except the M95 sample increased depending on the increase in pH value. While the highest amount of pyridine was detected in the M85 sample with 1090.1 μg/l, the lowest amount of pyridine was found in the M95 sample with 475 μg/l ($p<0.05$). When the amounts of 2-pyridine methanol compound were examined, the amount of this compound increased in Turkish coffee samples obtained by cold brewing due to the increase in pH value ($p < 0.05$). On the other hand, the increase in pH value, except for the M95 sample, caused a decrease in the amount of 2-pyridine methanol in both traditionally cooked and machine-cooked Turkish coffee samples ($p<0.05$). While the highest amount of 2-pyridine methanol was detected in the M95 sample with 240.4 μg/l, the lowest amount of this compound was detected in the T75 sample with 171.6 μg/l ($p<0.05$). Ayseli et al. (2021) determined the amount of pyridine compound in Turkish coffee samples obtained from medium and double-roasted coffee beans as 840 μg/l and 6494 μg/l, respectively.

4. CONCLUSION

It was determined that 17 of the volatile nitrogenous compounds detected in Turkish coffee samples consisted of pyrazine, 7 consisted of pyrroles, and 5 consisted of pyridines. As can be seen, most of the volatile nitrogenous compounds are composed of pyrazines. The presence of pyrazines in coffee is a well-known factor that contributes to its aroma and flavor profile. Pyrazines are a class of organic compounds that are responsible for producing the nutty, toasty, and earthy notes often associated with coffee. These compounds are formed during the roasting process as a result of the Maillard reaction, which occurs between amino acids and reducing sugars. The specific types and concentrations of pyrazines present in coffee can vary depending on the bean variety, roast level, and brewing method. Understanding the role of pyrazines in coffee can provide valuable insight for coffee producers and enthusiasts seeking to optimize the sensory experience of their brew.

If a general evaluation is made, it has been observed that Turkish coffee samples obtained by heat treatment have more acceptable properties than samples obtained by cold brewing. Considering the effects of waters with different pH values used during the preparation of Turkish coffee on volatile nitrogenous compounds, it has been revealed that the use of water with low pH values in Turkish coffee brewing causes a relative increase in the amount

of volatile nitrogenous compounds. Based on these results, both the use of copper coffee pots and the use of automatic coffee machines did not make a significant difference in the preparation of Turkish coffee. Accordingly, both methods can be used in cooking Turkish coffee. On the other hand, it is thought that the use of highly alkaline water in the cooking of Turkish coffee will improve the flavor properties of the coffee. However, it would be beneficial to expand this study to include other non-volatile coffee components such as phenolic compounds, carotenoids, and glycosidic compounds, in addition to aroma compounds, and re-plan future studies in this direction.

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