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# Determination of volatile and organic acid compounds in lyophilized coffee

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**ABSTRACT**: This study investigated the composition of the aroma substances and the physicochemical properties of lyophilized coffee produced from Ecuador Arabica and robusta coffee beans. In the present study, we propose a fast method of analyzing volatile compounds in coffee using the headspace solid phase extraction method (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS). According to the results, 25 aroma compounds were detected in coffee, the most important of which are 2-acetylfuran, 2-furanmethanol, 2,3-dimethylpyrazine, 2,3,5-trimethylpyrazine, and 2-methoxy-4-vinylphenol. As is known, furans and pyrazines are associated with "roasted, cocoa, coffee, and hazelnut" odours. The most important organic acids detected in the study are citric, maleic, and succinic.

**KEYWORDS**: coffee; volatile compounds; caffeine; HS-SPME; organic acids.

## 1. INTRODUCTION

Although the coffee plant belonging to the botanical genus Coffea, belonging to the Rubiaceous family, has more than 60 species, the most commonly consumed are *Coffea arabica* L. (Arabic coffee) and *Coffee canephora* P. (Robusta coffee). However, the most important factors that affect coffee consumption are the taste-related compounds that include volatile and organic acid compounds contained in coffee. Their amount varies depending on the type of coffee beans, their roasting, and the climatic conditions under which they are grown [1]. Volatile compounds, also known as odorants or flavours, are chemical substances that have a sensual property and exhibit various odours. Volatile compounds include chemical groups such as aldehydes, ketones, and terpenes, which are widely used in pharmaceuticals and food supplements [2]. More importantly, volatile compounds are promising compounds due to their health benefits [3].

Currently, volatile components in coffee can be analyzed using various extraction techniques; among them the headspace solid phase microextraction (HS-SPME) is widely used to obtain volatiles from complex matrices such as coffee. HS-SPME has attracted considerable attention due to its main advantages, such as ease of operation, being environmentally friendly, and robustness [4]. Several studies have found that HS-SPME is a sustainable extraction technique of volatiles from robusta coffee [5], and espresso [6].

Organic acids (OA) are widely found in coffee and add a different flavour to coffee depending on the preparation method. OA plays a crucial role in the sour taste of coffee brew. They increase the level of acidity of coffee, giving it a strong or soft taste. Therefore, it is important to investigate their effectiveness in the flavour of the coffee. Keeping this in mind, it is intriguing that each OA has its inherent sensory properties [7].

In the present work, a volatile composition of the lyophilized coffee was extracted using the HS-SPME extraction method and studied using GC-FID and GC-MS. In addition, organic acids and phenol compounds were also briefly evaluated using HPLC.

## 2. RESULTS and DISCUSSION

### 2.1 Measurement of total titratable acids and colour

In this study, the values of L*, a*, and b* for coffee were determined to be 48.55, 10.74, and 32.45, respectively. During the roasting of green coffee, the colour values of the coffee beans change due to

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Maillard reactions [1,8]. This colour change directly affects the coffee taste attributes. Especially in Brazil, light-coloured coffees are not preferred by coffee consumers. In a previous study, the L colour values of coffee were determined between 65# and 45# by calculation according to the SOLVER tool [9]. However, the low L* value of coffee beans has been stated to be inversely related to the amount of acrylamide. In the study, the L value in coffee beans was found to be between 24.6 and 28.1, and the value was found to be between 9.2 and 11.8l [4].

However, as seen in Table 1, the total titratable acids (TTA) value of coffee was calculated as 5.41. Previous studies have reported that robusta coffees have a lower acidity such as 3.71 [4], resulting in a significant effect on organic acid compounds and the taste of coffee [10].

**Table 1.** The chemical composition and colour properties of lyophilized coffee powder

| Parameter | Value |
|---|---|
| TTA* (mL of 0.1 N NaOH/40 mL of coffee) | 5.41±0.01 |
| L* | 48.55±0.01 |
| a* | 10.74±0.02 |
| b* | 32.45±0.02 |
| Hue | 71.68±0.01 |
| Chroma | 34.18±0.02 |
| ΔE*ab | 59,37±0.02 |
| Acetic acid (mg/L) | 164.62 |
| Malic acid (mg/L) | 144.12 |
| Succinic acid (mg/L) | 108.26 |
| Citric acid (mg/L) | 173.52 |
| Caffeine (mg/L) | 2819 |

* TTA (Total Titratable Acids)

### 2.2 Organic acids

Sourness and acidity (as well as bitterness) of coffee have always been considered important characteristics of the sensory quality of brewed coffee. Coffee has previously been reported that coffee contains organic acids such as citric, acetic, malic, succinic, fumaric and lactic [10].

In this study, citric acid and malic acid were found at 173.5 mg/L and 144.1 mg/L, respectively (Table 1). Previously, Skowron and Grzekowiak (2017) compared the organic acid profiles of various coffee brewing methods with an optimized method, and the citric acid was between 80.3 and 119.7 mg/L [11]. As is known, citric acid is generally found in fruits such as lemons and oranges. The amount of this acid, known for its sour and fruity taste, decreases with the fermentation and roasting process applied to green coffee [12]. During the roasting process, a significant portion of citric acid forms malic acid. Malic acid and citric acid found in green coffee are considered important quality indicators due to the taste of the fruit they impart [10]. On the other hand, Latin American coffees, such as Colombia, contain less citric acid than coffees of African origin.

Acetic acid, in small amounts, adds a sweet characteristic to coffee. It is formed by the breakdown of sucrose in coffee beans during post-harvest fermentation or roasting processes and contributes to the bitterness of coffee in small amounts. In our study, the amount of acetic acid was determined as 164.62 mg/L [7]. Succinic acid is an important organic acid that gives coffee a noticeably bitter taste. In previous studies, it was reported to have been formed by yeast during the fermentation phase of coffee [13].

### 2.3 Volatile Compounds

As seen in Table 2, a total of 25 aroma compounds were detected in lyophilized powdered coffee using the HS-SPME method. Pyrazines contribute significantly to coffee aroma due to their low threshold

**Table 2.** The volatile compounds in lyophilized coffee powder

| No | LRI[a] | RT | Compound | % PA[b] | Identification[c] |
|---|---|---|---|---|---|
| **1** | 702 | 5.133 | Acetic acid | 4.60 | LRI,MS,Std |
| **2** | 905 | 10.027 | 2-Acetylfuran | 6.66 | LRI,MS,Std |
| **3** | 923 | 10.930 | 2-Furanmethanol | 6.18 | LRI,MS,Std |
| **4** | 974 | 13.870 | 2,3,5-Trimetylpyrazine | 1.68 | LRI,MS,Std |

| | | | | | |
|---|---|---|---|---|---|
| **5** | 982 | 14.316 | Octanal | 1.45 | LRI,MS,Std |
| **6** | 1037 | 16.726 | 2-Acetyl-5-methylfuran | 2.02 | LRI,MS,Std |
| **7** | 1054 | 17.574 | Phenylacetate | 1.09 | LRI,MS,Std |
| **8** | 1068 | 18.593 | 2-*trans*-octenol | 0.21 | LRI,MS,Std |
| **9** | - | 18.965 | 2,3-Diethylpyrazine | 0.23 | LRI,MS,Std |
| **10** | 1084 | 19.469 | 1*H*-Pyrrole-2-carboxyaldehyde | 25.26 | LRI,MS,Std |
| **11** | 1102 | 20.430 | 2-Formyl-1-methylpyrrole | 2.24 | LRI,MS,Std |
| **12** | 1127 | 21.586 | Phenylacetaldehyde | 1.23 | LRI,MS,Std |
| **13** | 1131 | 21.955 | 3-Acetylthio-2-methylfuran | 2,55 | LRI,MS,Std |
| **14** | 1134 | 22.115 | *Cis*-Limonen oxide | 1,35 | LRI,MS,Std |
| **15** | 1140 | 22.501 | 3,5-Dimethyl-1,2-Cyclopentanedione | 2,44 | LRI,MS,Std |
| **16** | 1150 | 21.586 | 2-Acetylpyrrole | 4.15 | LRI,MS,Std |
| **17** | 1171 | 23.976 | 2-Methylthio-furoate | 2.34 | LRI,MS,Std |
| **18** | 1178 | 24.444 | *Trans*-Carveol | 2.35 | LRI,MS,Std |
| **19** | 1179 | 24.550 | 3,3,5-trimethyl-,1-cyclohexanolacetate | 2,05 | LRI,MS,Std |
| **20** | 1189 | 25.005 | Decanal | 8.07 | LRI,MS,Std |
| **21** | 1211 | 25.937 | Dodecane | 7.42 | LRI,MS,Std |
| **22** | 1310 | 31.558 | α- Terpinylformate | 2.64 | LRI,MS,Std |
| **23** | 1302 | 31.242 | Undecanal | 2.10 | LRI,MS,Std |
| **24** | 1368 | 33.271 | Maltol | 6.94 | LRI,MS,Std |
| **25** | 1674 | 39.822 | 2-Methoxy-4-vinylphenol | 0.37 | LRI,MS,Std |

[a] LRI (Retention index) values are based on Rtx-5MS capillary column.
[b] PA: %Peak Area. Average of data obtained from 3 extractions.
[c] Identification: MS (mass spectrometry library), Std (Standard chemical substance), LRI (Retention index), RT (Retention Time), Tent (Tentative Identification by MS).

values. They generally occur as a result of Maillard reactions in which sugars and amino acids are reduced during the roasting process [14]. Pyrazines are responsible for the "roasted, caramel, cocoa" odours in the coffee aroma. In the study, 2,3,5-trimethylpyrazine and 2,3-diethylpyrazine were detected. Ayseli et al. (2021) found the dilution factor (KS) values of the 2,3,5-trimethylpyrazine compound between 512 and 1024. in Turkish coffee prepared from arabica beans [1]. Although this compound gives hazelnut, cocoa, and roasted odours. The odour threshold value of this compound is stated as 9 ppm in water [15]. In previous studies, this compound was determined to give a cocoa aroma as an aroma-active compound [16].

Furan compounds can add many different aromas to coffee, such as roasted, burnt, and fruity. They are formed by the breakdown of sugar compounds such as hexose and pentose contained in the roasting of green coffee beans during the Maillard reactions[17]. The most important furan compounds detected in coffee are 2-acetylfuran (roasted, popcorn), furfuryl alcohol (burnt, fruity), 2-acetyl-5-methylfuran (roasted), and 3-acetylthio-2-methylfuran (roasted). It has recently been detected that the furfuryl alcohol compound contributes to the flavor of Turkish coffee with a 256 KS value. In the current study, the furfuryl alcohol ratio was also stated to increase in direct proportion to the increase in roasting time and temperature [1]. This compound has been detected similarly in Turkish and French Press coffee samples [18]. 2-acetyl-5-methylfuran was detected in Hainan coffee using the HS-SPME technique and confirmed as an aroma-active compound via electronic nose [19].

Trigonelline, as an important alkaloid, found in green coffee plays an important role in the formation of nitrogen-containing heterocyclic pyrrole compounds. In our study, the 1*H*-pyrrole-2-carboxyaldehyde compound was detected in coffee. This aroma compound has been reported to give a popcorn smell in Kenya [20] and Turkish coffee [1].

Phenol compounds, as the previous compounds, have a significant effect on the characteristic aroma of coffee. Among these, 2-methoxy-4-vinylphenol, also known as 4-p-vinylgaiacol, is important volatile because it gives a burned odour to the various coffee beans and brews. The amount of this compound changes positively as the roasting temperature of the coffee increases. In a recent study, this compound was

detected in roasted coffee beans of the species liberica (*Coffea liberica* var. dewevrei) [21]. As a result, 2-methoxy-4-vinylphenol is important in showing the roasting index of coffee beans [22].

Aldehydes are very important in coffee aroma as a result of their very low odour threshold. Decanal has a fruity odour and has been detected in tea obtained from coffee leaves [23].

### 2.4 Caffeine

The total caffeine content (1,3,7-trimethyl xanthine) in the lyophilized coffee powder was detected at 2819 mg/L. The most striking observation to emerge from previous research studies is the stability of caffeine during roasting [24]. Although the expected finding was a high caffeine level (1,3,7-trimethyl xanthine), this study showed. This result is consistent with the 3136 mg/L result obtained by Ayseli (2024) in instant coffee powder [4]. Therefore, the high caffeine level in our study is consistent with previous studies.

Several studies have found that caffeine (1,3,7 trimethyl xanthine) is useful for treating diabetes and neurodegenerative diseases. In addition, the correlation between the consumption of caffeine (1,3,7-trimethyl xanthine) and migraine is surprising due to its interaction with the P-450 cytochrome enzyme system P-450. What is striking here is that caffeine (1,3,7-trimethyl xanthine) has more than 82 interactions with CYP1A2, causing several side effects after drug use. It is important to note that caffeine (1,3,7-trimethyl xanthine) excretion is reduced by flavonoid consumption, resulting in reduced doses to improve their health effects [25].

## 3. CONCLUSION

This study was carried out to determine the important sensory acids, colour, and aroma substances of lyophilized coffee. The HS-SPME method was used for aroma compounds analysis and 25 aroma compounds were detected. Among these, the compounds pyrazine, furan, and pyrrole have an important effect in terms of coffee aroma. Among the furan compounds, 2-acetylfuran (roasted, popcorn), furfuryl alcohol (burnt, fruity), and 2-acetyl-5-methylfuran (roasted) are important aroma contributor. Pyrazine compounds 2,3,5-trimethylpyrazine and 2,3-dietylpyrazine are effective in the aroma of coffee. In the current study, citric, acetic, succinic, and malic acid were determined to have a significant effect on the aroma of lyophilized coffee. Surprisingly, the results, based on a literature search, showed unequivocally that the interactions between caffeine and pharmaceutical products need to be investigated in more detail.

## 4. MATERIALS AND METHODS

### 4.1 Material

The lyophilized coffee produced from arabica and robusta coffee beans grown in Ecuador was supplied by a coffee importer in Mersin (Dervisoglu Tarim San. Ve Tic. Sti.). According to the information given by the importer, the roast conditions for the green coffee blend (Arabica 20%: Robusta 80%) were 150-180 °C at the medium level for 18 min. The chemicals used in aroma analyses were provided by Merck (Darmstadt, Germany) and Sigma (St Louis, MO, USA).

### 4.2 The total titratable acids and colour analysis

For total titratable acids (TTA), a 40 mL aliquot of coffee brew was titrated with 0.1 N NaOH at 22 °C to a pH of 6.0 [4].

Colour analysis in coffee was determined using L*, a*, and b* values with a colour determination device (Konica Minolta CR-400, Tokyo, Japan). Lightness L* (100 for white and 0 for black) represents a value between yellow (+b*) and blue (-b*), while the chromatic coordinates a* and b* represent the colour space between red (+a*) and green. defines [8]. The total colour difference (ΔE*ab), Hue, and chroma values were determined using Eq. (1) and (2) respectively [4].

$$(\boldsymbol{Eq.1})\ \boldsymbol{Chroma} = (\boldsymbol{a^{*2} + b^{*2}})^{\boldsymbol{1/2}} \quad (\boldsymbol{1})$$

$$(\boldsymbol{Eq.2})\ \boldsymbol{H^{\circ}} = \boldsymbol{tan^{-1}(b^{*}/a^{*})} \quad (\boldsymbol{2})$$

### 4.3 Organic acids

The detection of organic acids was carried out with slight modifications according to the method of Ayseli et al. (2023)[26]. In the first stage, the powdered coffee was diluted with ultrapure water (1:10) and

then homogenized at 11,000 rpm (IKA Turrax T18 basic) for 45 seconds. The resulting coffee extract was kept in a water bath (Centurion Scientific Ltd, UKK2015 Series) for 5 minutes at 12,000 rpm. For complete extraction of organic acids, a microfilter (Sigma, Stenheim, Germany, CLS431225 Corning®) with a pore size of 0.45 µm was used for filtration and the process was repeated twice. The HPLC system (Shimadzu, QP2020 model) was heated at 40 °C, and the specific for the detection of organic acids was carried out with a column (coregel 87H3, Concise Separations, USA) (7.8x300mm 9um particle size).

### 4.4 Volatile compounds extraction and analysis

The study of volatile compounds in coffee was carried out using the HS-SPME technique in 50g of coffee according to the method of Ayseli and Coskun (2024). The extraction was performed in triplicate on the sample using a 50/30 µm thick and 2 cm long SPME cartridge (DVB/ CAR / PDMS: divinylbenzene/carpophore/polydimethylsiloxane) (Supelco Co., Bellefonte, PA). For this purpose, the SPME cartridge was exposed to volatile compounds coming from the coffee headspace for 40 minutes at 60°C. Subsequently, the SPME cartridge was placed in the syringe at 260 °C for 5 minutes in split mode (constant carrier pressure 18 psi) and analyzed by GC-MS [6,7]. Shimadzu brand, QP2020 NX, El Main SPL-2030 model, GC-MS device, and its associated flame ionization detector were used to identify aroma substances. Rxi-5MS column (length 30 m, inner diameter 0.25 mm, film thickness 0.25 µm; Restek Co, Bellefonte, PA, USA) was used for volatile compound analysis.

The column temperature was initially kept at 50°C for 0 minutes at a rate of 3°C/min. until 120 °C and 15°C /min, 230 °C. The sample lasted 20 minutes in spitless mode. Helium gas was selected to carry gas at 47.2 m/s and maintain velocity at 100 kPa. A 70-eV electron was used to ionize and identify the peaks of unknown samples. The mass spectrometer was scanned every 0.3 seconds in the range of 35-500 m/z in 90 minutes. GC runs. This method is modified from [27], [28] and [29].

### 4.5 Caffeine analysis

The caffeine separation was performed using an HPLC system (Shimadzu LC2050 3D model) according to the method of Ayseli and Coskun (2024)[29]. As a pretreatment, 5 g of solid samples (5 ml of liquid samples) were taken. 50 methanol is taken and stirred for 15-30 seconds and 45 m water. The sample is shaken again, cooled, and made up to 100 ml. The 0.45 ml is filtered by a syringe filter and fed into an HPLC system [31].

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