

Caffeine content in Nepalese coffee: Influence of brewing methods and storage time

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ABSTRACT

Coffee's global appeal, attributed to its caffeine content impacting flavor and health, lacks thorough exploration of Nepalese coffee despite its growing coffee sector. The lack of comprehensive studies inhibits informed choices on caffeine consumption and coffee selection. Here, we aimed to bridge this gap by assessing caffeine content and pH levels in Nepalese coffee beans using common extraction methods - boiling, French Press, and Moka pot. We compared ten locally sourced coffee brands from Pokhara, Nepal, conducting the experiment upon unsealing the coffee packets, designated as day 0, and repeated it after 21 days with the same samples, labeled as day 21. An Ultra Performance Liquid Chromatography (UPLC) with a UV/Visible detector was used to estimate caffeine concentration. The Moka pot maintained the highest average caffeine concentration at 14.39 ± 1.87 mg/g (day 0) and 13.83 ± 1.24 mg/g (day 21), followed by boiling at 11.63 ± 1.17 mg/g (day 0) and 10.16 ± 1.63 mg/g (day 21), with the French press yielding the lowest concentration at 10.11 ± 0.96 mg/g (day 0) and 8.90 ± 2.09 mg/g (day 21). pH values varied, with the boiling method resulting in the highest pH (5.51 ± 0.22). Coffee pH increased from 5.41 ± 0.20 to 5.45 ± 0.21 over 21 days. Our findings underscore the importance of considering extraction methods when evaluating coffee's caffeine content and pH levels. This study provides valuable insights for consumers to make informed choices about caffeine intake and coffee preferences. Further research could explore additional factors influencing caffeine content and pH levels, such as regional differences, cultivation practices, titratable acidity, sensory evaluations, and chemical profiling.

Key words: UPLC analysis; pH; moka pot; french press; boiling.

1 INTRODUCTION

Coffee is one of the most popular beverages and the primary source of caffeine consumption. *Coffea*, a member of the Rubiaceae family, is one of the most prominent angiosperm families and consists of 124 species found in two genera, *Coffea* and *Psilanthus* (Maghuly; Jankowicz-Cieslak; Bado, 2020). Out of the 103 known coffee varieties, only two of them, *C. arabica* (Arabica) and *C. canephora* (Robusta), make up 99% of the coffee consumed worldwide (Alshaw, 2020). According to a report published by the International Coffee Organization, the global consumption of coffee has been steadily increasing, with a compound annual growth rate (CAGR) of 1.0 % reported in 2020/2021 compared to 2017/2018 (International Coffee Organization World Coffee Consumption, 2021). Within the same period, global coffee production also increased by 1.0%. Arabica and Robusta account for 60% and 40% of global production, respectively (International Coffee Organization World Coffee Production, 2021). In Nepal, commercial coffee farming spans 32 districts of mid-hilly regions situated within an altitude range of 800 m to 1600 m. According to a report published by the National Tea and Coffee Development Board of Nepal, annual coffee production in Nepal is 1574 tons (Survey of Commercial Coffee Production 2076, 2019). Nepal exported coffee worth USD 1 million in 2022 (Coffee Trade for Fiscal Year 2022, 2022).

'Arabica' (*Coffea arabica*) is Nepal's primary coffee variety (Tuladhar; Khanal, 2020). The major components of green and roasted Arabica coffee beans, respectively, on dry weight basis are polysaccharides (50-55% and 24-39%), lipids (12-18% and 14.5-20%), proteins (11-13% and 13-15%), oligosaccharides (6-8% and 0-3.5%), chlorogenic acids (5.5-8% and 1.2-2.3%), minerals (3-4.2% and 3.5-4.5%), trigonelline (1-1.2% and 0.5-1%), aliphatic acids (1.5-2% and 1-1.5%), amino acids (2% and 0%), humic acids (0 and 16-17%), and caffeine (0.9-1.2% and 1%) (Clarke; Macrae, 1985). While coffee enthusiasts drink the beverage because of its flavor and aroma, the primary reason is its stimulating effect, predominantly attributed to caffeine (Nehlig; Daval; Debry, 1992). Caffeine (1,3,7-trimethylxanthine), a purine alkaloid obtained from xanthosine, is one of the essential constituents of coffee beans (Simon-Gruita et al., 2019). It is a secondary metabolite that holds significant importance in coffee due to its influence on the quality of the beverage. When it accumulates in green coffee beans, it enhances the bitterness of the drink (McCusker; Goldberger; Cone, 2003). Caffeine is known to affect coffee's taste, aroma, and physiological effects. Caffeine is a potent stimulant for the central nervous system. It has various effects, such as increasing heart rate and blood pressure, acting as a bronchodilator, promoting diuresis (increased urine production), and stimulating intestinal peristalsis (contractions of the intestines) (Simon-Gruita et al., 2019).

The caffeine content in coffee beans can vary significantly, depending on various factors such as the type of coffee bean (species), geographical location, processing of the beans, storage conditions, roasting method, and extraction method (Olechno *et al.*, 2021). The differences reported in the literature may also be caused by the measurement method. Recent advancements in analytical methodologies have enhanced the precision and throughput of caffeine quantification in coffee. Lee *et al.* employed liquid chromatography coupled with high resolution orbitrap mass spectrometry (LC–HR-OMS) for metabolomic profiling of brewed coffee, enabling simultaneous detection of 163 non-volatile and 93 volatile compounds (Lee *et al.*, 2025). Similarly, Jan-Smith *et al.* utilized LC–MS system consisting of a ‘Vanquish Flex’ U-HPLC-PDA, and an ‘Orbitrap Fusion’ mass spectrometer fitted with an “Ion Max NG” heated electrospray source and applied metabolomics approaches to compare chemical profiles related to flavor (Jan-Smith *et al.*, 2025). While these high-resolution techniques offer powerful tools for broad chemical characterization, high-performance liquid chromatography (HPLC) and its variants, such as ultra-performance liquid chromatography (UPLC), remain widely accepted and reliable methods for targeted caffeine quantification, particularly in quality control and routine analytical settings.

There are various methods of brewing coffee. The most common ones are aeropress, drip, moka, espresso, French press, and simple infusion or boiling (Janda *et al.*, 2020). In addition, newer techniques such as cold brew and nitro coffee have gained both market and research attention for their distinct chemical profiles and sensory characteristics. Angeloni *et al.* (2019) and Pan *et al.* (2023) compared hot and cold brews and found that cold brew typically contains lower acidity and altered caffeine extraction efficiency due to prolonged extraction at low temperatures (Angeloni *et al.*, 2019; Pan *et al.*, 2023). Moka, French press, and boiling methods are common in Nepal, and coffee can be brewed at home without any expensive machines. Moka pot, an iconic Italian stove-top coffee maker, comprises three parts: a bottom resembling a pressure cooker, a middle funnel for ground coffee, and a top coffee collector with an inner sprout and a handle (Binder; Scheidle, 2020). When the water placed on the bottom unit is heated, it rises through the funnel containing ground coffee and collects on the top unit. Modern French press consists of a cylindrical container (glass or plastic), a plunger that fits tightly to the cylindrical container with a fine wire or nylon mesh filter, and a metal or plastic lid. Hot water is poured over the ground coffee in the cylindrical container and left to brew for 3-5 minutes. After brewing, the plunger is pressed down, and the brewed coffee is poured out. Simple infusion or boiling is the simplest method of all. Ground coffee and water are brought to a boil in a pot and served by filtering the coffee grounds or without filtering (Turkish coffee). The various methods of brewing coffee provide different tastes and chemical profiles (Angeloni *et al.*, 2019).

Coffee beverages are acidic due to their acidic contents, including chlorogenic acids, aliphatic acids, amino acids, humic acids, and quinic acids, as well as melanoidins containing chlorogenic acids (Clarke; Macrae, 1985), (Farah, 2012; Gloess *et al.*, 2013; Santos *et al.*, 2017). The pH level and acidity of coffee are other crucial factors that contribute to its overall flavor profile and consumer perception. pH measurements quantify the concentration of hydrogen ions in coffee, providing insights into its acidic content. Total titratable acidity encompasses all acidic protons in coffee, including those that can be neutralized by adding a strong base (Rao; Fuller, 2018). Batali *et al.* (2021) demonstrated that titratable acidity correlates more strongly with perceived sourness than pH alone, emphasizing the importance of comprehensive acidity measurements in coffee profiling (Batali *et al.*, 2021). While some studies have successfully correlated titratable acidity with sourness intensity (Fuse; Kusu; Takamura, 1997; Batali *et al.*, 2021), others have found no clear relationship between pH, titratable acidity, and perceived acidity (Andueza *et al.*, 2007; Gloess *et al.*, 2013). Recent research has also focused on the relationship between chemical changes and sensory outcomes (Gutiérrez-Guzmán; Cortés-Cabezas; Chambers, 2018; Yoon *et al.*, 2023; Nascimento; Ombredane; Oliveira, 2024). Šeremet *et al.* (2022) analyzed the chemical and bioactive composition of Arabica and Robusta coffee beans. They found Robusta brews more astringent and recognized as more sensorily attractive than Arabica brews, which were evaluated as bitter (Šeremet *et al.*, 2022).

Storage conditions of green and roasted coffee significantly affect its quality, particularly in terms of aroma, flavor, and chemical stability (Cortés-Macías *et al.*, 2022; Smrke *et al.*, 2022; Błaszkiwicz *et al.*, 2023; Borém *et al.*, 2023). A recent study by Barrera-López *et al.* (2024) suggests that optimizing roasting and storage can improve shelf life and sensory outcomes, emphasizing the need to understand interactions over practical durations (Barrera-López *et al.*, 2024). While extended storage studies have been conducted, short-term storage and its immediate impacts on sensory perception remain underexplored.

Nepalese coffee has gained recognition for its highland Arabica quality, but only a few scientific studies have addressed the regional agronomic factors affecting caffeine content. A few studies have evaluated the effect of altitude and climatic variations on the 1000 cherry weight, wet parchment weight, dry parchment weight, green bean weight, green bean density, and caffeine content of coffee from selected districts of Nepal and its value chain (Kamal *et al.*, 2021; Paudel *et al.*, 2021; Khatri *et al.*, 2023). Shrestha *et al.* (2016) reported caffeine content in 12 tea samples, 8 roasted coffee beans, 3 coffee powder, and 7 green tea samples collected from the retail store of Kathmandu and Kaski (Shrestha *et al.*, 2016). However, only one extraction procedure was used, and the method was only optimum for laboratory procedures and did not mimic the household

method of brewing coffee. Moreover, the study was limited to measuring caffeine concentration and did not examine the pH. Pokharel et al. (2023) determined the antioxidant activity and measured the total phenolic content (TPC) of coffee varieties cultivated in Nepal (Pokharel et al., 2023). Other studies have reported the caffeine content in tea samples consumed in Nepal (Sharma et al., 2019; Timsina, 2020). However, none of the previous studies have looked at the temporal dynamics. Our study aimed to provide useful information for coffee consumers interested in their coffee's caffeine content and pH. Therefore, we determined the caffeine concentration of coffee samples from the retail market of Pokhara, Nepal using three different household methods: boiling, French press, and moka (Navarini et al., 2009). Moreover, we measured the differences in caffeine concentration and pH of coffee samples twice (21-day interval) to determine the changes over time. Caffeine consumption can have positive and negative health effects, depending on the amount consumed (Mejia; Ramirez-Mares, 2014). A 2017 systematic review found that consuming up to 400 milligrams of caffeine daily is safe for most adults (Wikoff et al., 2017). By providing information on the caffeine content and pH in coffee samples, the study helps to inform individuals about their caffeine intake and its potential health effects. Consumers can use this information to make informed decisions about the type of coffee they consume and how much caffeine they consume.

2 MATERIAL AND METHODS

2.1 Sample collection and storage

We conducted a comprehensive survey of coffee offerings across several supermarkets to ensure a diverse representation of coffee brands in Pokhara. Following this selection process, we purchased 10 different brands of coffee powder, each originating from distinct varieties of coffee beans cultivated in various regions of Nepal. None of the samples mentioned the roast level, date, or particle size distribution. The samples were labeled with randomized identifiers ranging from 1 to 10. We stored these samples under standard laboratory conditions. Upon unsealing the coffee packets, we immediately conducted the experiment, considering this as day 0 for analysis. Following experimentation, the coffee samples were securely sealed with rubber bands and stored under standard laboratory conditions at room temperature without any temperature or humidity control. After 21 days, we repeated the experiments with the same samples, designating them as day 21 for analysis.

2.2 Preparation of the extract

We used three different extraction methods (constant coffee to water ratio) to extract caffeine from the samples: i) boiling, ii) French press, and iii) Moka pot. First, to extract caffeine by boiling, we immediately weighed 4 g of coffee

powder in a conical flask and poured 50 ml of boiled distilled water (Type 1) from the water boiler. The sample was stirred for 3 min using a magnetic stirrer at medium speed. To obtain the coffee solution, the sample was extracted using vacuum filtration (stainless steel vacuum filter with a 47mm disk and Grade 1 Whatman filter paper). The volume was made up to 50 ml using distilled water. Second, to extract caffeine from the French press, we added 10 g of coffee powder to a French press device (French Press BH 5V - 600ml, Daraz Nepal) and poured 125 ml of boiled distilled water. The plunger was pressed to extract the coffee solution and poured into a clean beaker immediately. For both boiling and French press, boiled water was used without any temperature control in successive steps. Third, to extract caffeine by the Moka pot, we added 20 g of coffee powder in a Moka pot (Moka Pot 3 Cups, Daraz Nepal) and poured 250 ml of distilled water. The sample was heated at 96°C for 12 min. The solution was poured into a conical flask, and the volume was made up to 250 ml using distilled water. We conducted each experiment in triplicate.

2.3 pH measurement

pH was measured by a pH meter (Professional Benchtop pH meter BP3001, TTBH Pte Ltd, Jalan Kilang Barat, Singapore) at room temperature immediately after extraction. Before use, the pH meter was calibrated using standard pH 4.0, 7.0, and 10.0 (supplied by Nepal Scientific and Instrument Solutions, Kathmandu). We did all measurements in triplicate.

2.4 Estimation of caffeine

Extracted coffee samples (1 ml) were filtered into HPLC vials using Bio-Plus syringe filters (nylon, pore size: 0.20 µm, 25 mm, Bioplus Industries SDN.BHD, Malaysia). Finally, 5 µl of the extract was injected into an Agilent 1260 Infinity II 1260 MCT series HPLC (Agilent Technologies, Santa Clara, US), and compounds were separated on a C18/150x4.6 mm (5 µm, Agilent Technologies) column. Caffeine was eluted at a constant flow rate of 1 ml/min at 30°C using 0.5% Phosphate buffer (in HPLC grade water) - acetonitrile in the ratio of 85:15 for 4.5 min, followed by 0.5 min reconditioning. We interpreted peaks with symmetrical absorption maxima at 275 nm as caffeine (Shrestha et al., 2016) using the Agilent OpenLab Data Analysis software 2.206.0.780. Caffeine concentrations were calculated using an external caffeine (supplied by Nepal Scientific and Instrument Solutions, Kathmandu) calibration curve at 0.125, 0.25, 0.5, and 1 mg/ml. All measurements were done in triplicate.

2.5 Statistical analysis

Statistical analyses were computed using JMP® Pro 17 statistical software (SAS Institute, Cary, NC, US). We evaluated the dataset for normal distribution and homogeneity of variances by visual inspection of the q-q and residual plots, respectively. To compare the amount of caffeine, we constructed

a linear model using least squares, including caffeine amount as a continuous variable, and extraction methods, different samples, time points, and number of measurements in HPLC as fixed effects. We used ANOVA followed by LSMeans Tukey HSD test to assess the differences between extraction methods. Timepoint data were analyzed by ANOVA, followed by LSMeans differences Student's t-test. Similarly, to compare the pH value, we constructed a linear model using least squares, including pH value as a continuous variable, and extraction methods, different samples, and time points as fixed effects. To assess the effect of extraction methods on the pH, we used ANOVA followed by LSMeans Tukey HSD test, and time point data were analyzed by ANOVA followed by LSMeans differences Student's t-test. Probability values < 0.05 were considered statistically significant.

3 RESULTS

3.1 Caffeine content varies among different coffee samples, brewing methods, and time points

We observed statistical differences between different samples [$F(9, 221.98) = 19.8$, $p = < .0001$], extraction methods [$F(2, 669.31) = 268.64$, $p = < .0001$], and two time points [$F(1, 52.47) = 42.12$, $p = < .0001$]. The caffeine content in coffee powder varied across different extraction methods. On day 0, the Moka, boiling and French press method yielded

caffeine in the range of 10.14 to 17.78 mg/g, 10.06 to 13.24 mg/g and 8.82 to 11.95 mg/g, respectively whereas on day 21, the Moka, boiling and French press method yielded caffeine in the range 11.59 to 15.38, 7.52 to 12.65 and 7.30 to 14.30 mg/g, respectively (Figure 1a, Table 1).

The Moka pot yielded the highest average concentration at 14.39 mg/g (Day 0) and 13.83 mg/g (Day 21), followed by boiling at 11.63 mg/g (Day 0) and 10.16 mg/g (Day 21), and the French press extracting the lowest amount at 10.11 mg/g (Day 0) and 8.90 mg/g (Day 21). The average caffeine content was significantly reduced for all extraction methods from 12.04 mg/g on day 0 to 10.96 mg/g on day 21 (Figure 1b).

3.2 pH varies among different coffee samples, brewing methods, and time points

We observed statistical differences between different samples [$F(9, 2.1) = 62.41$, $p = < .0001$], extraction methods [$F(2, 0.18) = 24.37$, $p = < .0001$], and two time points [$F(1, 0.04) = 9.89$, $p = 0.003$]. Among all extraction methods, the boiling method resulted in the highest average pH value of 5.50 on day 0 and 5.52 on day 21 ($p = < .0001$). In contrast, coffee extracted from the Moka pot (pH 5.40 on day 0 and pH 5.39 on day 21) and French press (pH 5.31 on day 0 and pH 5.46 on day 21) showed similar average pH values ($p = 0.81$) (Figure 2a, Table 1). The average pH in coffee samples for all extraction methods increased from 5.41 on day 0 to 5.45 on day 21 (Figure 2b).

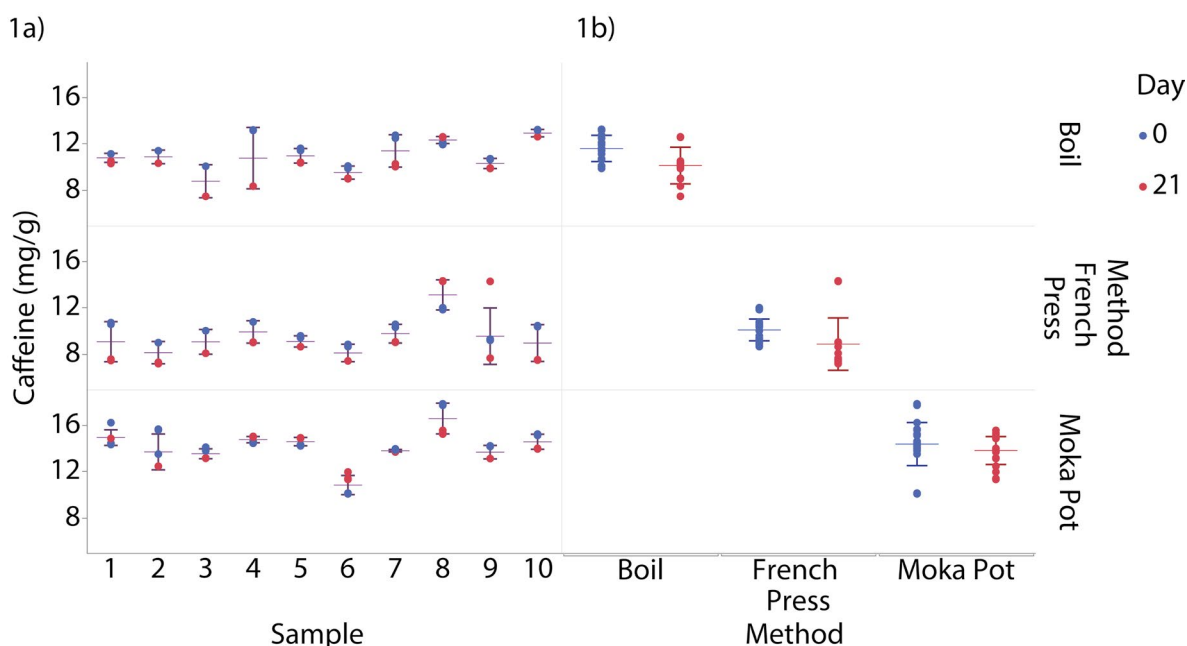


Figure 1: Amount of caffeine in coffee powder. 1a) Each horizontal bar represents the mean ($\pm$ SD) caffeine amount (mg/g) in ten different samples extracted using boiling, French press, and Moka pot methods. 2b) Each horizontal bar represents the mean ($\pm$ SD) caffeine amount (mg/g) in all the samples extracted using boiling, French press, and Moka pot methods. Dots depict jittered raw data, with blue dots indicating day 0 and red dots indicating day 21

4 DISCUSSION

4.1 Caffeine content

Caffeine plays a crucial role in the flavor profile of coffee, with its bitter taste adding complexity and balance to the beverage, complementing other flavor compounds extracted during the brewing process. Coffee enthusiasts appreciate the interplay between caffeine and other flavor components, such as acids and sugars, contributing to the overall taste experience.

The significant differences observed in caffeine content among various extraction methods shed light on the complex

caffeine extraction process from coffee beans. Each method, whether the Moka pot, boiling, or French press, offers a unique approach to extracting caffeine, showcasing the multifaceted nature of this process. The Moka pot method, known for its high-pressure brewing, likely enhances caffeine solubility and extraction efficiency compared to boiling and French press methods. The increased pressure exerted during Moka pot brewing enables thorough extraction of caffeine molecules from coffee grounds, resulting in the highest observed caffeine concentration among the three methods. Conversely, the French press method's relatively gentler extraction process may lead to lower caffeine concentrations. While this method

Table1: Summary of Caffeine content (mg/g) and pH of the 10 samples using Boiling, French Press and Moka pot brewing methods.

Brewing Method	Sample	Day 0 Caffeine (mg/g)	Day 21 Caffeine (mg/g)	Day 0 pH	Day 21 pH
Boiling	1	11.17±0.01	10.48±0.13	5.76±0.29	5.77±0.06
	2	11.44±0.01	10.37±0.01	5.45±0.14	5.47±0.2
	3	10.11±0.01	7.52±0	5.65±0.12	5.69±0.2
	4	13.2±0.02	8.39±0	5.5±0.19	5.54±0.08
	5	11.56±0.1	10.43±0.03	5.34±0.18	5.37±0.16
	6	10.06±0.13	9.04±0.03	5.6±0.08	5.63±0.21
	7	12.68±0.15	10.15±0.13	5.46±0.22	5.48±0.2
	8	12.08±0.11	12.62±0.03	5.83±0.17	5.88±0.29
	9	10.73±0.05	9.94±0.03	5.2±0.23	5.13±0.07
	10	13.24±0.04	12.65±0.01	5.2±0.15	5.2±0.2
French Press	1	10.68±0.07	7.54±0.06	5.53±0.07	5.7±0.17
	2	9.04±0.01	7.3±0.07	5.27±0.21	5.4±0.1
	3	10.06±0.02	8.13±0.02	5.48±0.26	5.65±0.12
	4	10.82±0.02	9.07±0.03	5.33±0.26	5.47±0.21
	5	9.56±0.1	8.69±0.02	5.18±0.11	5.3±0.2
	6	8.82±0.11	7.46±0.01	5.43±0.14	5.53±0.25
	7	10.52±0.15	9.07±0.02	5.31±0.06	5.41±0.23
	8	11.95±0.09	14.3±0.02	5.62±0.1	5.81±0.23
	9	9.26±0.06	9.9±3.8	4.97±0.22	5.12±0.18
	10	10.43±0.04	7.55±0.02	5.02±0.1	5.17±0.1
Moka Pot	1	15.01±1.05	14.88±0.01	5.53±0.24	5.49±0.21
	2	14.93±1.21	12.5±0.01	5.36±0.11	5.33±0.19
	3	13.93±0.17	13.18±0.02	5.46±0.25	5.51±0.08
	4	14.53±0.09	15.01±0.04	5.37±0.2	5.48±0.17
	5	14.27±0.05	14.92±0.04	5.32±0.2	5.32±0.14
	6	10.14±0.03	11.59±0.35	5.53±0.23	5.5±0.27
	7	13.91±0.06	13.72±0.02	5.44±0.22	5.34±0.07
	8	17.78±0.06	15.38±0.16	5.62±0.2	5.65±0.07
	9	14.22±0.04	13.16±0.01	5.2±0.2	5.18±0.05
	10	15.17±0.06	13.99±0.03	5.19±0.11	5.12±0.06

allows for prolonged water-to-coffee contact, facilitating comprehensive flavor extraction, it may not maximize caffeine extraction compared to methods involving higher pressures or temperatures. Additionally, variables such as grind size, water-to-coffee ratio, and brewing duration can influence caffeine extraction efficiency in the French press method.

Shrestha *et al.* reported the caffeine content of coffee samples from Kathmandu and Kaski of Nepal on a dry weight basis to be 11 to 15.2 mg/g for roasted coffee beans and 11 to 13.5 mg/g for coffee powder. They used the simple infusion (boiling) method of brewing with 30 minutes of extraction time, which is rather long for home brewing. In this study, the highest average concentration of caffeine (Day 0) was 17.8 mg/g for sample 8 brewed using a Moka pot. In contrast, the lowest average concentration of caffeine was 8.8 mg/g for sample 6 using a French press. Caporaso *et al.* (2014) reported the extraction efficiency of the Moka pot method to be 9.2 mg/g using ground-roasted Arabica coffee samples from Naples, Italy (Caporaso *et al.*, 2014). Another study by Fox *et al.* (2013) reported the caffeine concentration of 28 coffee samples worldwide (Fox *et al.*, 2013). They used the simple infusion extraction method but heated the solution for 10 min after adding the coffee samples. The reported concentration of caffeine for blended coffee samples is 10.6–19.9 mg/g, whereas for Arabica coffee samples it is 10.5–12.8 mg/g. The coffee samples with the lowest and highest caffeine concentrations originated from Brazil (10.5 mg/g) and Colombia (12.8 mg/g). We have reported

a similar average concentration of caffeine (Day 0) using the boiling method (10–13.2 mg/g), although the extraction time was less (3 min). Our result suggests Nepali coffees are quite potent in terms of caffeine concentration in coffee beans. The average caffeine content in fresh Nepalese coffee (Day 0) using French press, boiling, and Moka brewing methods is 10.1, 11.6, and 14.4 mg/g. Assuming 10 g of coffee beans per serving and 400 mg/day of safe daily caffeine dosage, consumers should limit their intake to less than four servings of coffee using a French press and boiling method and less than three servings of coffee using a Moka pot (Wikoff *et al.*, 2017).

The observed decrease in caffeine content over the 21 days highlights its temporal dynamics. Since the unsealed coffee samples were stored under standard laboratory conditions, humidity may play a significant role. The unsealed samples may absorb moisture, affecting the coffee samples' effective weight during the brewing process. Processes such as degradation and oxidation can also lead to a decline in caffeine concentration over time. While we observed this pattern empirically, we did not measure degradation products or environmental factors such as humidity. Therefore, while factors like oxidative degradation and moisture uptake have been proposed in the literature as contributors to caffeine loss, we present these mechanisms as plausible hypotheses rather than confirmed explanations (Lin *et al.*, 2022; Pazmiño-Arteaga *et al.*, 2022). Future studies, including water activity measurements and analysis of degradation markers, would

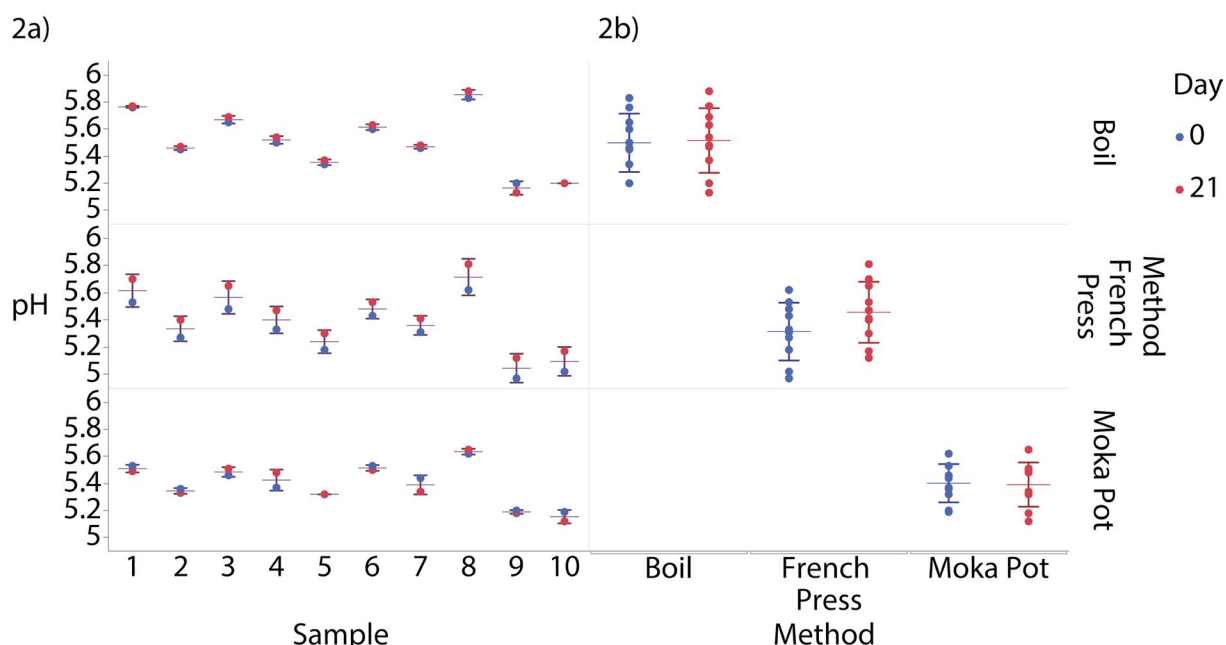


Figure 2: pH value of coffee. 1a) Each horizontal bar represents the mean ($\pm$ SD) pH in ten different samples extracted using boiling, French press, and Moka pot methods. 2b) Each horizontal bar represents the mean ($\pm$ SD) pH in all the samples extracted using boiling, French press, and Moka pot methods. Dots depict jittered raw data, with blue dots indicating day 0 and red dots indicating day 21.

be necessary to verify these mechanisms. These observations underscore the importance of consuming fresh coffee for those seeking higher caffeine intake or desiring the full sensory experience and quality of coffee.

Understanding the intricacies of caffeine extraction from coffee beans can help coffee enthusiasts and professionals optimize their brewing methods to achieve desired caffeine levels and flavor profiles in their coffee beverages. Caffeine, a natural alkaloid, contributes significantly to coffee consumption's sensory experience and physiological effects.

Additionally, studying caffeine extraction using different methods provides insights into the dynamics of solubility, diffusion, and chemical interactions occurring during brewing. Investigating temperature, pressure, particle size, and extraction time is vital to optimizing caffeine extraction efficiency and quality in coffee production. This ongoing research aims to enhance the understanding and appreciation of coffee's complex chemistry while improving the overall coffee experience for consumers.

4.2 pH of brewed coffee

Understanding the variations in pH levels among extraction methods is essential for discerning the sensory attributes of brewed coffee (Gloess et al., 2013). Acidity, a crucial component in coffee, contributes to its perceived brightness, liveliness, and flavor complexity. The acidity profile of coffee can vary widely depending on factors such as bean origin, roast level, and brewing method. Coffee enthusiasts and professionals often consider pH levels when evaluating brewed coffee's sensory characteristics and overall quality (Gloess et al., 2013; Rao; Fuller, 2018).

The pH of a brewed coffee reflects the concentration of hydrogen ions, which directly impacts its perceived acidity. In this context, acidity is a desirable characteristic that enhances the coffee's flavor complexity and brightness. The observed variations in pH levels among different extraction methods provide intriguing insights into the acidity profiles of the extracted coffee solutions, significantly influencing the sensory experience of coffee drinkers.

The literature shows a significant variation in the reported pH of coffee samples. Although the species, geographical location, roasting method, and brewing techniques are also very diverse. Gloess et al. analyzed coffee samples using nine different brewing methods and reported the pH in the range of 5.51 (filtered coffee) to 5.92 (French press) (Gloess et al., 2013). A previous study reported that the pH of hot (French press method of extraction) and cold (simple infusion for 7 hours at room temperature) brew coffee was 4.92 to 5.10 and 4.96 to 5.13, respectively (Rao; Fuller, 2018). Moon et al. (simple infusion in hot water for 3 hours) reported the pH of green

and roasted coffee beans in the range of 5.68 to 5.85 and 5.41 to 5.79, respectively. In our study, we found the pH on Day 0 and Day 21 to be in the range 4.97 (French press) to 5.83 (boiling) and 5.12 (French press and moka) to 5.88 (boiling), respectively.

The higher pH observed in the boiling method compared to the Moka pot and French press methods suggests that boiling may extract more acidic compounds from the coffee powder. Prolonged exposure to high temperatures during boiling facilitates the extraction of various organic acids in coffee beans, such as acetic, malic, formic, lactic, chlorogenic, and quinic acids. These acids collectively contribute to the perceived acidity of the coffee.

Moreover, the observed pH variations underscore the importance of selecting appropriate brewing methods to achieve desired acidity profiles in coffee beverages. Different techniques extract different proportions of organic acids, leading to distinct acidity profiles in the final cup. For example, the gentle immersion brewing of the French press method may yield a smoother and less acidic coffee than prolonged exposure to high temperatures in the boiling method. Our study did not include compound-specific profiling. Although previous research has identified variations in acid content across brewing methods, we refrain from asserting definitive biochemical mechanisms based on our data alone (Batali et al., 2021; Rune et al., 2023; Yeager et al., 2023; Santanatoglia et al., 2024). Instead, we propose this as a potential explanation warranting further targeted investigation, such as organic acid profiling contributing to coffee acidity and their impacts on flavor perception and consumer preference. Understanding the intricacies of acidity in coffee enables producers and enthusiasts to refine brewing techniques and optimize flavor profiles to meet diverse consumer preferences and elevate the coffee experience.

5 CONCLUSIONS

In conclusion, our study reveals the relationship between caffeine content, pH levels, and sensory attributes in brewed coffee. Caffeine significantly influences flavor, with high-pressure methods like the Moka pot yielding higher concentrations. Freshness is key to experiencing coffee's full sensory potential. pH variations reveal insights into the organic acids extracted; boiling extracts more acids than immersion methods like French press. This study will refine brewing methods, optimize flavors, and meet diverse preferences.

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7 AUTHOR CONTRIBUTIONS

Conceptual Idea: Koirala, S.; Pokharel, P.; Bhdanari D. R., Methodology design: Koirala, S.; Pokharel, P.; Bhdanari D. R., Data collection: Koirala, S.; Pokharel, P.; Devkota S., Data analysis and interpretation: Koirala, S.; Pokharel, P., and Writing and editing: Koirala, S.; Pokharel, P.; Bhdanari D. R..

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