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Variability of volatile compound profiles during two coffee fermentation times in northern Peru using SPME-GC/MS

Variabilidade dos perfis de compostos voláteis durante dois períodos de fermentação do café no norte do Peru usando SPME-GC/MS

Jhordy Perez[1], Martha Steffany Calderon[1,2*], Danilo Edson Bustamante[1,2], Aline Camila Caetano[1,3], Jani Elisabet Mendoza[1], Samia Littly Jahavely Fernandez-Güimac[1]

[1]Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas, Instituto de Investigación para el Desarrollo Sustentable de Ceja de Selva (INDES-CES), Chachapoyas - Perú
[2]Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas, Instituto de Investigación en Ingeniería Ambiental (IIIA), Facultad de Ingeniería Civil y Ambiental (FICIAM), Chachapoyas - Perú
[3]Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas, Facultad de Ingeniería y Ciencias Agrarias (FICA), Chachapoyas - Perú

***Corresponding Author:** Martha Steffany Calderon, Universidad Nacional Toribio Rodriguez de Mendoza de Amazonas, Instituto de Investigación en Ingeniería Ambiental (IIIA), Facultad de Ingeniería Civil y Ambiental, Calle Higos Urco n° 342-350-356, 10101, Chachapoyas - Perú, e-mail: martha.calderon@untrm.edu.pe

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Abstract

The time of the fermentation process of coffee from northern Peru is variable (9 to 48 hours) since coffee farmers do not use standardized processes, causing a variety of coffee qualities. This study aimed to identify volatile compounds in both short (9 hours) and long (32 hours) coffee fermentation processes from coffee farms in northern Peru using Solid-Phase Microextraction-Gas Chromatography/Mass Spectrometry (SPME-GC/MS) to associate the coffee quality and diversity of volatile compounds. Sensory analyses showed that the short fermentation process (SFP) scored 77.8 ± 0.39 and had chocolate, wood, cardboard, dry, fatty and rough notes, while the long fermentation process (LFP) showed higher punctuations 85.5 ± 3.16 and citrus, fruity, floral, caramel and chocolate sensory attributes. A total of 90 compounds were found in the SFP, whereas 141 compounds were identified in the LFP. Significant differences in the relative abundance of 14 chemical compounds were reported in the SFP and LFP ($p < 0.05$). From these results, the presence of benzaldehyde, methional, hexanal, 2-heptanone, pentadecane, 1-butanol-3-methyl-acetate, and benzene-acetic acid ethyl ester seems to impact the quality of coffee. The analysis of similarities showed that coffee samples (5 h and 9 h) during the SFP were very variable, whereas coffee samples from LFP showed some tendency to group, which may be related to the difference in altitude and temperature in coffee farms making comparison between them difficult. In addition, this study highlights the complex relationship between coffee fermentation and flavour and the influence of several factors and variables that may affect the composition of flavour and aroma precursors in green coffee beans obtained from wet fermentation.

Keywords: Coffee; Fermentation time; Peru; Sensory; SPME-GC/MS; Volatile compound; Wet fermentation.

Resumo

O tempo de fermentação do café do norte do Peru é variável (9 a 48 horas), pois os cafeicultores não utilizam processos padronizados, causando uma variação na qualidade dos cafés. O objetivo deste estudo foi identificar compostos voláteis tanto em processos de fermentação de café curtos (9 horas) quanto longos (32 horas) de fazendas de café no norte do Peru, utilizando SPME-GC/MS para associar a qualidade do café à diversidade dos compostos voláteis formados. As análises sensoriais mostraram que o processo de fermentação curto (SFP) resultou em pontuação 77,8 ± 0,39 com notas de chocolate, madeira, papelão, secas, gordurosas e ásperas, enquanto a fermentação longa (LFP) mostrou pontuações de 85,5 ± 3,16 e atributos cítricos, frutados, florais, caramelo e chocolate. Um total de 90 compostos foram encontrados no SFP, enquanto 141 compostos foram identificados no LFP. Diferenças significativas na abundância relativa de 14 compostos químicos foram verificadas entre os processos SFP e LFP (p < 0,05). A partir destes resultados, a presença de benzaldeído, metional, hexanal, 2-heptanona, pentadecano, 1-butanol-3-metil-acetato e éster etílico do ácido benzenoacético parece ter impacto na qualidade do café. A análise das semelhanças mostrou que as amostras de café após 5 h e 9 h de fermentação no SFP foram muito variáveis, enquanto as amostras de café do LFP mostraram alguma tendência ao agrupamento, o que pode estar relacionado com as diferenças de altitude e temperatura nas fazendas de café, tornando difícil a comparação entre elas. Além disso, este estudo destaca a complexa relação entre a fermentação e o sabor do café, e a influência de vários fatores e variáveis que podem afetar a composição de precursores de sabor e aroma em grãos de café verde, obtidos a partir da fermentação úmida.

Palavras-chave: Café; Tempo de fermentação; Peru; Sensorial; SPME-GC/MS; Composto volátil; Fermentação úmida.

Highlights

- A total of 157 volatile compounds was identified in both short (9 h) and long (32 h) coffee fermentation processes from Peru.
- Ninety volatile compounds were found in the short fermentation process, whereas 141 were found in the long process.
- The volatile compounds in coffee samples collected at 5 and 9 hours during the short fermentation process were very different from each other.
- According to sensory attribute, coffee produced using a long fermentation process scored higher than that produced using a short process (85.5 ± 3.16 *versus* 77.8 ± 0.39 points).
- This study highlighted the complexity of coffee fermentation and the influence of several factors in the attributes and final quality of coffee.

1 Introduction

Coffee is one of the most popular beverages in the world and is one of the most valuable agricultural products produced by approximately 170 countries employing over 25 million people worldwide (Rodrigues Vegro & Florencio Almeida, 2020). Coffee is attributed to many health benefits, such as antibiotic, anti-inflammatory, hepatoprotective, antioxidant, and stimulant properties (Janissen & Huynh, 2018). Additionally, the pleasant taste sensation, comprised of a balanced combination of bitterness, acidity, nuttiness and astringency, makes coffee a unique beverage (Cheng et al., 2016). In the last decade, the international coffee market has undergone significant transformations regarding the increasing demand for “special coffees” (Bertrand et al., 2012). Accordingly, coffee quality is one of the key criteria when purchasing this product, as it is associated with desirable flavours specific to a particular geographical region (Guo et al., 2014). To date, several factors have been debated as determinants of coffee quality and chemistry, including microclimate, processing, and fermentation parameters as well as geographic location, soil, and coffee microbiota (Evangelista et al., 2014b; Pereira et al., 2020).

The mucilaginous layer of coffee cherries is composed of 84.2% water, 8.9% protein, 4.1% sugar, 0.91% pectic substances, and 0.7% ash (Schwan & Fleet, 2014). This mucilage is removed by the fermentation process through enzymes naturally found in the coffee fruit and microbiota acquired from the soil environment (Haile & Kang, 2019; Elhalis et al., 2020). It has been demonstrated that these microorganisms (i.e., yeasts, bacteria, and fungi) play an important role in the degradation of mucilage by producing various enzymes, alcohols, and acids during the fermentation process (Elhalis et al., 2020). In this fermentation process, coffee grains are exposed to a diversity of aromas and flavour compounds, and they are associated with more than 700 volatile and non-volatile compounds that contribute to the final coffee flavour (Farah et al., 2006). These compounds are divided into different classes depending on their chemical structure as follows: furans, pyrazines, ketones, pyrroles, phenols, hydrocarbons, acids, anhydrides, aldehydes, esters, alcohols, and sulfurs (Flament, 2002). Nevertheless, the desirable aroma of coffee is produced by a balance in the composition of some volatile compounds, including the predominant pyrazines followed by furans, aldehydes, ketones, phenols, and sulfur compounds (Maeztu et al., 2001; Akiyama et al., 2005). Characterization of volatile compounds has been mainly focused on green and roasted coffee from semidry fermentation methods (Evangelista et al., 2014a; Martinez et al., 2017), whereas there is a lack of information regarding the volatile compounds involved in wet fermentation.

For the specific characterization of volatile compounds in coffee beans, different techniques are employed. For instance, headspace extraction, Solid-Phase Microextraction (SPME), Needle-Trap Device (NTD), and Headspace Sorptive Extraction (HSSE) are widely used along with Gas Chromatography/Mass Spectrometry (GC/MS) since these methodologies are single sample, robust and reproducible when concentrating analytes on fibers with different types of sorbents (Guo et al., 2014). In South America, few studies have associated results from volatile compound characterization and coffee quality. For instance, 53 different volatile compounds were identified in raw coffee samples from Brazil using SPME-GC/MS (Toci & Farah, 2008). Additionally, Evangelista et al. (2014b) identified 48 volatile compounds in fermented and roasted beans as well as more than 800 volatile compounds in roasted and green coffees from Brazil by HS-SPME/GC. Although coffee from northern Peru has been characterized as a specialty coffee based on its peculiar characteristics in terms of its sensory quality (aroma and flavour), the volatile compounds involved in these peculiarities are unknown (Russell, 2022).

The time of the fermentation process of coffee from northern Peru is variable among coffee farms (9-48 hours) since coffee farmers do not use standardized techniques and follow specific criteria according to particular conditions (i.e. altitude, temperature, coffee variety, ripeness of the fruit, etc.), resulting in a variety of coffee qualities (Puerta et al., 2015). This scenario might involve a diversity of volatile compounds during the fermentation process of coffee in northern Peru. Accordingly, this study aimed to identify volatile compounds in both short (9 hours) and long (32 hours) coffee fermentation processes from coffee farms in northern Peru using the headspace SPME method in conjunction with GC/MS to associate the coffee quality and diversity of volatile compounds.

2 Materials and methods

2.1 Sample collection and handling

Fruits of coffee (*Coffea arabica* L.) were harvested on farms from the Amazonas and Cajamarca Regions located at 950 and 1800 m above sea level, respectively. Approximately 500 g of pulp-bean mass of spontaneous fermentation was collected at 5 and 9 hours (short fermentation process commonly performed in Amazonas) and at 6, 12, 18, 22, 27, and 32 hours (long fermentation process commonly performed in Cajamarca) (Elhalis et al., 2020). The temperature of ferments at each time

was measured using a multiparameter instrument (Clarkson HI9126). The samples were stored in a tank with liquid nitrogen (MVE XC 47/11, USA) (-190 °C) and transferred to the Laboratory of Molecular Biology and Genomics at the Universidad Nacional Toribio Rodriguez de Mendoza de Amazonas, Peru.

2.2 Sensory analysis

200 g of green coffee samples were roasted and prepared following the sensory analyses of the Specialty Coffee Association of America (2012) to be cupped by a panel of three trained and certified tasters (Q-Graders) to evaluate the following 10 sensory attributes: aroma, flavour, aftertaste, acidity, body, uniformity, balance, sweetness, cleanliness and score on a total 100 points scale. According to the Specialty Coffee Association of America (2018), a coffee attribute is defined as an aromatic aspect when coffee is brewed with hot water and it includes valuable ones such as i) flavour (impression of all taste sensations that go from the mouth to the nose), ii) aftertaste (duration of positive taste attributes emanating from the back of the palate), iii) uniformity (consistency of flavours in various cups of the tasted sample), iv) balance (how the different flavour attributes interact), v) cleanliness (absence of negative impressions), and vi) score (adding of individual scores of primary qualities).

2.3 Chromatographic analysis

Volatile compounds were extracted using the SPME technique and analyzed by GC–MS. Fermented coffee beans (2 g) were crushed using liquid nitrogen and a mortar and a pestle. Divinylbenzene/carboxen/polydimethyl-siloxane (DVB/CAR/PDMS) 50/30 mm fiber (Supelco Co., USA) was used to extract the volatile compounds from the sample. An equilibration time of 15 min at 50 °C was used, and then the fiber was exposed for 45 min at the same temperature (Lee et al., 2017). The compounds were analyzed using a GC (Agilent Technologies, 7890B GC System, USA) equipped with a mass detector (Agilent Technologies 5977B MSD) and a DB-5MS UI capillary column (60 m x 0.25 µm diameter x 1 µm packing thickness). Temperature programming started at 50 °C, ramped up to 170 °C at 3 °C/min and ramped up to 250 °C at 8 °C/min (Lee et al., 2017). The injector and detector temperatures were maintained at 250 °C. Helium (He) was used as the carrier gas at a flow rate of 1.5 mL/min. Injections were performed by exposing the fiber to the volatile compounds for 4 min. Volatile compounds were identified by comparison of the mass spectra of each sample compound and the NIST 2017 library database. The identity of the compounds was confirmed by injection of the n-alkane standard (C_{10} to C_{40}) and comparison of their Linear Retention Index (LRI) (Costa et al., 2019). All samples were analyzed in triplicate.

2.4 Statistical analysis

Analysis of Variance (ANOVA, $p < 0.05$) and Tukey's test of the relative abundance of volatile compounds among fermentation times were performed in RStudio v4.1.2 software. The data of volatile compounds involved in organoleptic characteristics (Table 1) were analyzed by a heatmap and Principal Component Analysis (PCA) using the *Stats*, *FactoMineR*, *factoextra* and *ggplot2* libraries in RStudio. An m × n matrix was constructed with the relative percentage of the relative areas of the n chromatographic peaks identified for the different samples (Evangelista et al., 2014b). The data were automatically scaled.

Table 1. Volatile compounds showed significant differences among collection times during the SFP and LFP.

Chemical Group	Compound and CAS Registry Number	Relative area (%)							
		LFP (h)						SFP (h)	
		6	12	18	22	27	32	5	9
Alcohols	Phenylethyl alcohol (60-12-8) **	4.29	6.4	3.26	8.43	5.42	13.1	11.32	5.63
	1-Butanol, 3-methyl-, acetate (123-92-2) ***	9.70	7.60	7.49	19.43	19.81	6.01	0.67	1.82
Aldehydes	Benzaldehyde (100-52-7) ***	7.71	6.94	5.91	3.34	8.54	6.19	21.28	0.00
	Methional (3268-49-3) ***	0.62	0.78	0.61	0.84	0.99	0.55	6.84	0.74
	Hexanal (66-25-1) ***	2.04	1.78	1.79	2.19	2.24	2.08	3.71	0.66
Alkanes	Tetradecane (629-59-4) ***	5.79	4.76	3.22	6.46	11.33	7.45	3.30	3.69
	Pentadecane (629-62-9) *	2.80	2.40	1.64	1.08	1.05	1.37	0.00	0.37
Aromatic hydrocarbons	Styrene (100-42-5) **	9.64	5.99	5.60	1.69	2.07	2.95	3.09	5.47
Benzenes	Ethylbenzene (100-41-4) ***	1.14	0.56	0.28	0.13	0.09	0.12	0.28	0.57
Esters	Benzeneacetic acid, ethyl ester (101-97-3) *	3.66	3.27	7.71	4.68	8.05	8.55	0.00	2.45
Ketones	2-Heptanone (110-43-0) ***	1.62	1.50	1.66	0.18	0.05	0.09	1.27	1.85
Pyrazines	Pyrazine, 2-methoxy-3-(2-methylpropyl)-(24683-00-9) **	1.63	2.21	1.76	0.55	0.68	0.62	2.67	5.57
Terpenes	o-Cymene (527-84-4) **	2.21	0.87	0.99	0.13	0.00	0.06	0.36	0.83
Others	1-Butanol, 2-methyl-, acetate (624-41-9) *	0.96	1.00	1.08	10.02	3.51	0.00	0.00	0.25

Asterisks in the volatile compounds indicate that they were significantly different among fermentation times at (*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$. Grey colour shows significant differences.

3 Results and discussion

3.1 Sensory analysis

The coffee from the long fermentation process (henceforth LFP) scored 85.5 ± 3.16 and showed citrus, fruity, floral, caramel, and chocolate attributes. On the other hand, the coffee from the short fermentation process (henceforth SFP) scored 77.8 ± 0.39 and showed sensory attributes such as chocolate, wood, paperboard, dry, fatty, and rough. These coffee scores were significantly different ($p < 0.01$) (Figure S1, in Supplementary Materials). Chocolate was the only attribute reported in both fermentation processes. Based on these sensory attributes, coffees with fruity, floral, citrus, and caramel notes (those of LFP) are considered specialty coffees, whereas those with earthy, sour, harsh and woody notes are classified as medium- to low-quality coffees (Puerta et al., 2015).

3.2 Volatile compounds in green coffee from SFP and LFP

A total of 157 volatile compounds were detected by SPME-GC/MS in short and long fermentation processes (3 acids, 13 alcohols, 16 aldehydes, 13 alkanes, 2 aromatic hydrocarbons, 5 benzenes, 24 esters, 1 ether, 2 furans, 10 ketones, 2 pyrazines, 9 terpenes, and 57 compounds belonging to other chemical groups; Table S1, in Supplementary Materials). All of these compounds showed peaks with similarities higher than 80% and matched the linear retention index (LRI).

A total of 90 compounds were found in the SFP (70 at 5 h and 73 at 9 h), whereas 141 compounds were identified in the LFP (83 at 6 h, 81 at 12 h, 86 at 18 h, 69 at 22 h, and 75 at 32 h). The highest relative abundances at each coffee fermentation time were reported for alcohols (28.7% ± 3.8%), esters (15.4% ± 8.5%), and alkanes (11.2% ± 2.8%) during the LFP and for aldehydes (14.6% ± 9.9%) in the SFP (Table S1 in Supplementary Materials), Figure 1). The relative abundance of these groups varied slightly during the LFP, whereas the aldehydes (37.0% to 2.8%) and esters (1.2% to 8.4%) strongly fluctuated during the SFP (Figure 1, Table S2, in Supplementary Materials).

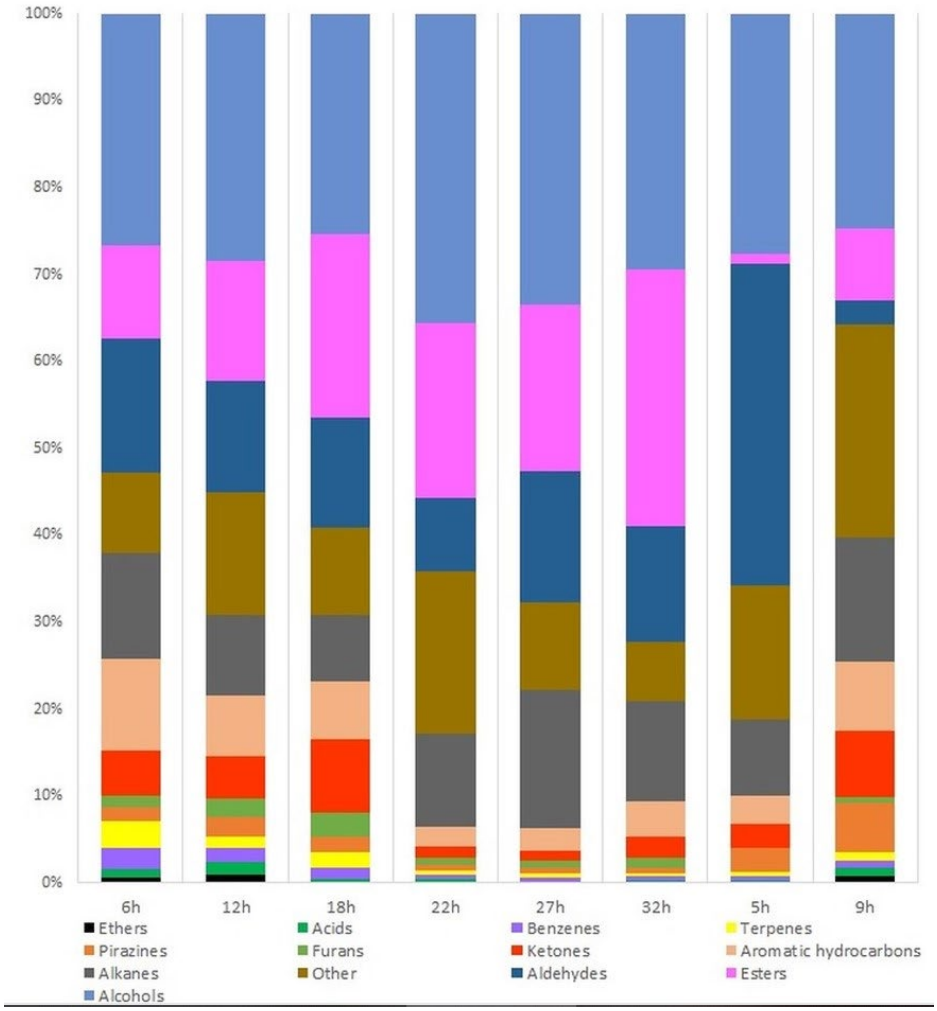

Figure 1. Relative abundances of volatile chemical groups detected in fermented coffee beans during short (5 h, 9 h) and long fermentation (6 h, 12 h, 18 h, 22 h, 27 h, 32 h) processes.

More than 900 volatile compounds have been reported in coffee beans (Haile & Kang, 2019), and the majority of these compounds have been recorded from semidry fermentation processes (Evangelista et al., 2014a; Martinez et al., 2017). Conversely, only 10-37 volatile compounds were reported from green coffee in the wet fermentation process, with alcohols (phenylethyl alcohol) and aldehydes (4-hydroxy-3-methylacetophenone) being the most abundant compounds (Figueroa-Hurtado, 2013; Martinez et al., 2017). Additionally, 13-86 (mainly alcohols, acids, and aldehydes) and 32-42 (mainly furans and pyridines) volatile compounds were identified from green and roast coffee, respectively (Toci & Farah, 2008; Martinez et al., 2017; Tsegay et al., 2019). Our study registered at least three-fold the number of volatile compounds reported in previous studies using wet fermentation processes in green coffee beans (Bertrand et al., 2012; Evangelista et al., 2014a; Martinez et al., 2017).

Conversely, the chemical profile of coffee obtained by wet fermentation from Loja, southern Ecuador (Figueroa-Hurtado, 2013) and Lonya Grande, Bagua, Peru (Marek et al., 2020) using GC/MS identified only 37 and 19 volatile compounds, respectively. These studies performed a single analysis, mainly at the end of the coffee fermentation process, instead of characterizing the entire process (Figueroa-Hurtado, 2013; Marek et al., 2020). Our study analyzed eight different times (two in SFT and six in LFT) throughout the fermentation process of coffee from northern Peru, with alcohols, aldehydes, and esters being the dominant chemical groups (Tsegay et al., 2019).

3.3 Comparison of volatile compounds and organoleptic attributes in SFP and LFP

Significant differences in the relative abundance of 14 chemical compounds were reported in the SFP and LFP ($p < 0.05$, Table 1, Figure 2). The aldehydes (i.e., benzaldehyde, methional, and hexanal) were abundant at the beginning of the SFP (Figure 3a-c). Additionally, 2-heptanone (ketone) was predominant at 5 h and 9 h of the SFP and at 6 h, 12 h, and 18 h of the LFP (Figure 3d). Pentadecane (alkane) was higher in the LFP (6 h and 12 h) than in the SFP (5 h and 9 h) (Figure 4a), and its highest values were at 32 h in the LFP. Also in the LFP, two compounds, 1-butanol-2-methyl-acetate (alcohol) and benzeneacetic acid, ethyl ester (ester) were significantly higher at 22 h and 27 h in the former (Figure 4b) and at 18 h, 27 h, and 32 h (Figure 4c) in the later. The remaining seven compounds were significantly abundant at specific times in the SFP and LFP [i.e., 2-methoxy-3-2-methylpropyl (9 h of the SFP); styrene, ethylbenzene, and o-cymene (6 h of the LFP); 1-butanol-2-methyl-acetate (22 h of the LFP); tetradecane (27 h of the LFP); and phenylethyl alcohol (32 h of the LFP) (Table 1, Figure 4d, Figure S2 in Supplementary Materials)].

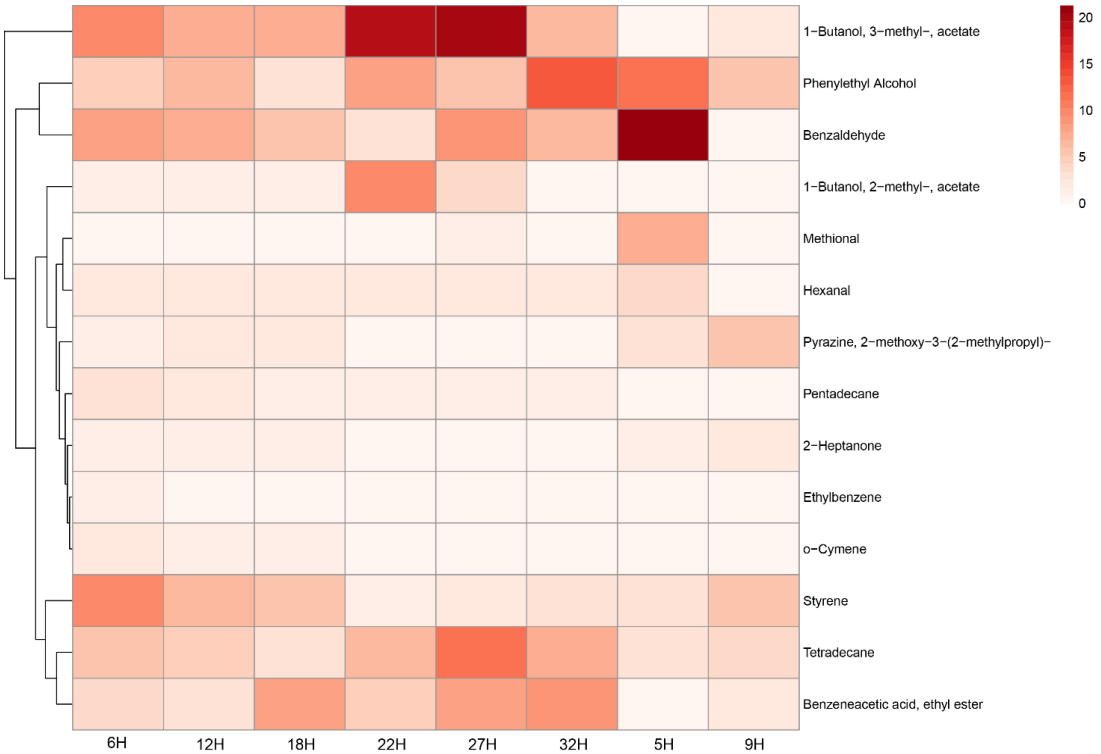

Figure 2. Heatmap showing the differences in the relative abundances of 14 volatile compounds that showed significant differences (x-axis) produced throughout the SFP (5 and 9 h) and LFP (6, 12, 18, 22, 27, and 32 h) (y-axis).

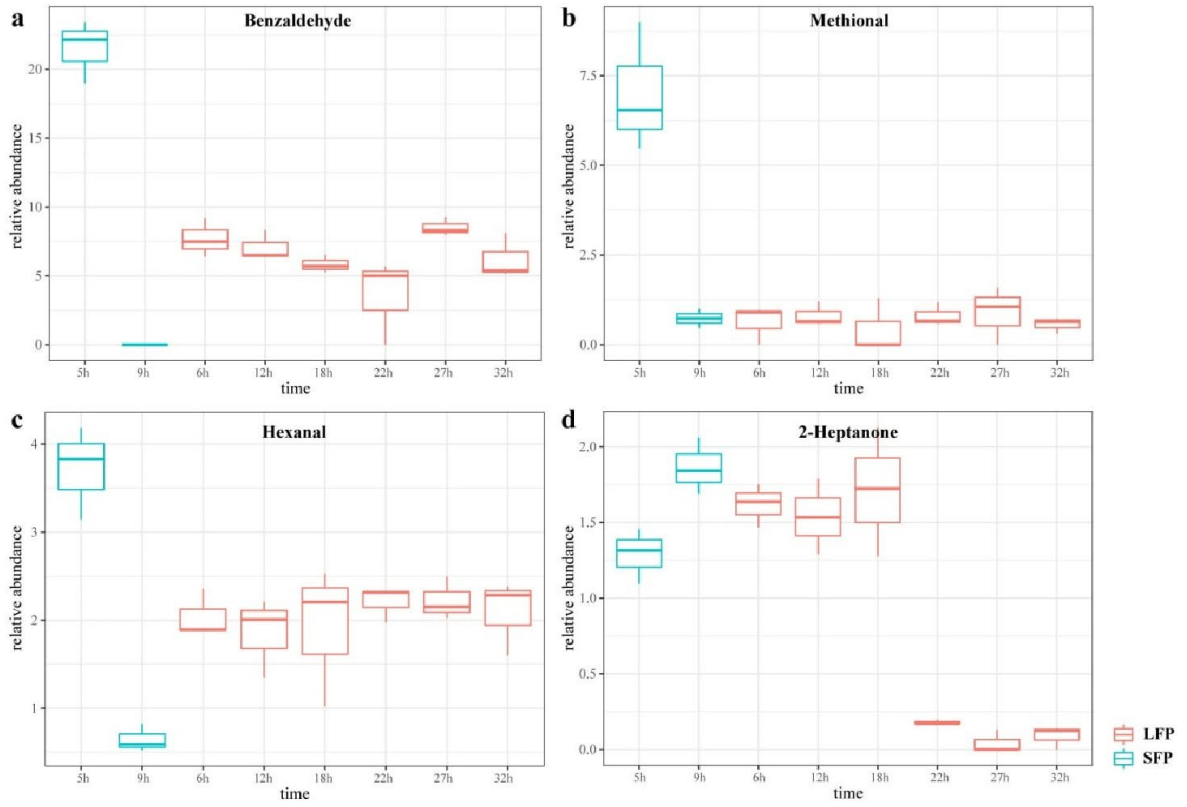

Figure 3. Relative abundances of the volatile compounds benzaldehyde (a), methional (b), hexanal (c), and 2-heptanone (d) produced throughout the SFP (5 and 9 h) and LFP (6, 12, 18, 22, 27 and 32 h).

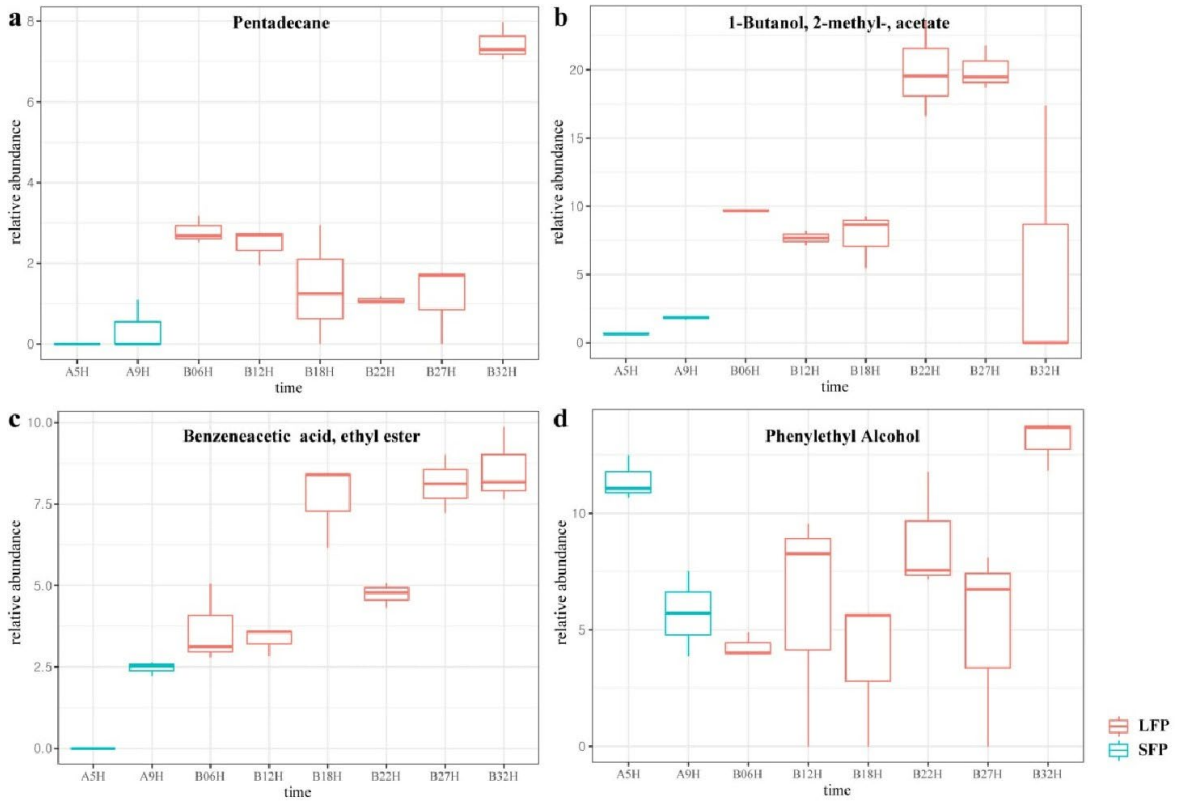

Figure 4. Relative abundances of the volatile compounds pentadecane (a), 1-butanol-3-methyl-acetate (b), benzeneacetic acid, ethyl ester (c), and phenylethyl alcohol (d) produced throughout the SFP (5 and 9 h) and LFP (6, 12, 18, 22, 27 and 32 h).

Only ~5% of compounds (out of 900 reported) are capable of impacting coffee aroma (Flament, 2002; Toledo et al., 2016). The chemical groups pyrazines, furans, aldehydes, ketones, phenols, and sulfurs have been highlighted as the main odoriferous compounds in coffee (Akiyama et al., 2005). The presence of aldehydes is associated with almond, bitter, cherry, chocolate, and malt odours (Maeztu et al., 2001; Ramirez-Gaona et al., 2017; Hamdouche et al., 2019). In this study, aldehydes (i.e., benzaldehyde, methional, and hexanal) were observed throughout the LFP but were limited in the SFP (Figure 3a-c), confirming the features proposed by the certified tasters in the sensory analyses. These aldehydes have been reported as secondary metabolites of Lactic Acid Bacteria (LAB) and yeast, and they carry to the process fruity, apple and almond aromas (Rosca et al., 2016; Mukisa et al., 2016). Additional analyses should be included to confirm the effect of hexanal during and after the fermentation of coffee since the reduction of hexanal has been observed after overtime roasting (Yang et al., 2016).

2-Heptanone (ketone) is associated with coconut and woody flavours, butter notes, and caramel odour (Maeztu et al., 2001; Akiyama et al., 2005; Ramirez-Gaona et al., 2017), and it is one of the compounds emitted by fresh coffee berries (Flament, 2002). 2-Heptanone almost disappeared at the late stages of the LFP, but it was present in the SFP (Figure 3d). Additionally, the relative abundances of pentadecane (waxy flavour), 1-butanol-3-methyl-acetate (fruity and sweet flavours), and benzeneacetic acid, ethyl ester (anise and chocolate flavours) (Ramirez-Gaona et al., 2017) were significantly higher in the LFP than in the SFP (Figure 4).

Postharvest processing has a substantial impact on the aroma quality of coffee (Toledo et al., 2016). Analyses of raw coffee seeds from wet fermentation demonstrate that they have better aroma quality and resulted in high concentrations of alcohols (i.e., ethanol and 2-phenylethanol) that provide sweet and floral aromatic notes (Flament, 2002). Phenylethyl alcohol has been mentioned to be a dominant compound in green coffee (Tsegay et al., 2019), giving a pleasant floral-woody, honey-like character. Although this compound was abundant only at the final stages of the LFP (32 h), its attributes were detected in the sensory analyses. Additionally, pyrazine has a characteristic pungent, sweet, and slightly ammoniacal odour (Toci & Farah, 2008). Pyrazine, 2-methoxy-3-(2-methylpropyl) is considered a special class of earthy-musty odourant (Wang et al., 2020); therefore, its significant abundance in the SFP might be linked to the paperboard notes found in the sensory analysis.

The combination of the 14 predominant volatile compounds in the LFP and SFP might give specific attributes to the coffee in the sensory analyses. Nevertheless, further analyses sampling coffees exposed to different climatic conditions, agricultural management, and postharvest processes might provide insights into the chemical profile involved in the characteristics of the coffee from northern Peru.

3.4 Other volatile compounds in SFP and LFP

Three other volatile compounds (i.e., 2-butenal, 3-methyl; 3-pentanone; and furan, 2-pentyl; Table S2, Supplementary Materials) that are extensively associated with organoleptic attributes in coffee (such as fruity and mellow; sweet and fruity; green beans/fruity aromas, respectively) were found in the SFP and LFP of coffee from northern Peru (Hamdouche et al., 2019; Elhalis et al., 2020; Ramos et al., 2020; Zou et al., 2022). Low levels of 2-butenal, 3-methyl (1.06%) and 3-pentanone (0.08%) at 5 h and furan, 2-pentyl (0.59%) at 9 h were reported in the SFP (Table S3, Supplementary Materials). During the LFP, the abundances of 2-butenal, 3-methyl (0.0-0.36%); 3-pentanone (0-0.78%); and furan, 2-pentyl (0.7-1.91%) were barely detected (Table S3, in Supplementary Materials). Among these compounds, furans are crucial compounds and abundant in roasted coffee, and their formation is linked to sucrose hydrolytic products in Maillard reactions during fermentation, providing herbal or fruity notes (Evangelista et al., 2014a). In this study, only low levels of furans (i.e., furan-2,3-dihydro-4-methyl, furan-2-pentyl) were identified at every fermentation time. It was stated that low concentrations (0.7 ppm) of furans impart bitter, wintergreen notes and mouthfeel sensations (i.e. sensations arising from the interactions of ingested food with saliva) (Flament, 2002; Frances, 2022). Accordingly, furans might have slightly impacted the aroma of coffee from northern Peru.

3.5 Analysis of similarities of volatile compounds from the SFP and LFP

The PCA was applied to the percentages of the relative abundance of the 14 volatile compounds for each time of the SFP and LFP to represent the dataset variance. The analysis of compounds was performed by means of eigenvalue confirmation with a data variability that exceeded 80% after compound confirmation. The plane of the first two main axes of the PCA of these 14 volatile compounds accounted for 31.9% of the first component and 25.6% of the second component of the total variation (Figure 5). The PCA showed that coffee samples at 5 h and 9 h from the SFP were very different from each other, suggesting that the presence of the 14 compounds was variable. Conversely, coffee samples from the LFP showed some tendency to group. Samples from the initial (6 h, 12 h, and 18 h) and late (22 h, 27 h, and 32 h) stages in the LFP clustered in the PCA, suggesting that those volatile compounds occurred in a stable manner throughout each stage. Additionally, the coffee samples at 9 h from the SFP clustered with those of the late stage of the LFP, suggesting similarities in their chemical profiles. The vectors representing pyrazine, 2-methoxy-3-(2-methylpropyl) and 2-heptanone are positively correlated, however, negatively correlated to 1-butanol-2-methyl-acetate and strongly influenced PC1 (Figure 5). Additionally, the vectors representing aldehydes (benzaldehyde, methional, and hexanal) and phenylethyl alcohol are positively correlated and mainly influence PC2. The compounds influencing PC1 and PC2 showed a strong impact on the final sensory properties of coffee from the LFP and SFP (Figure 5).

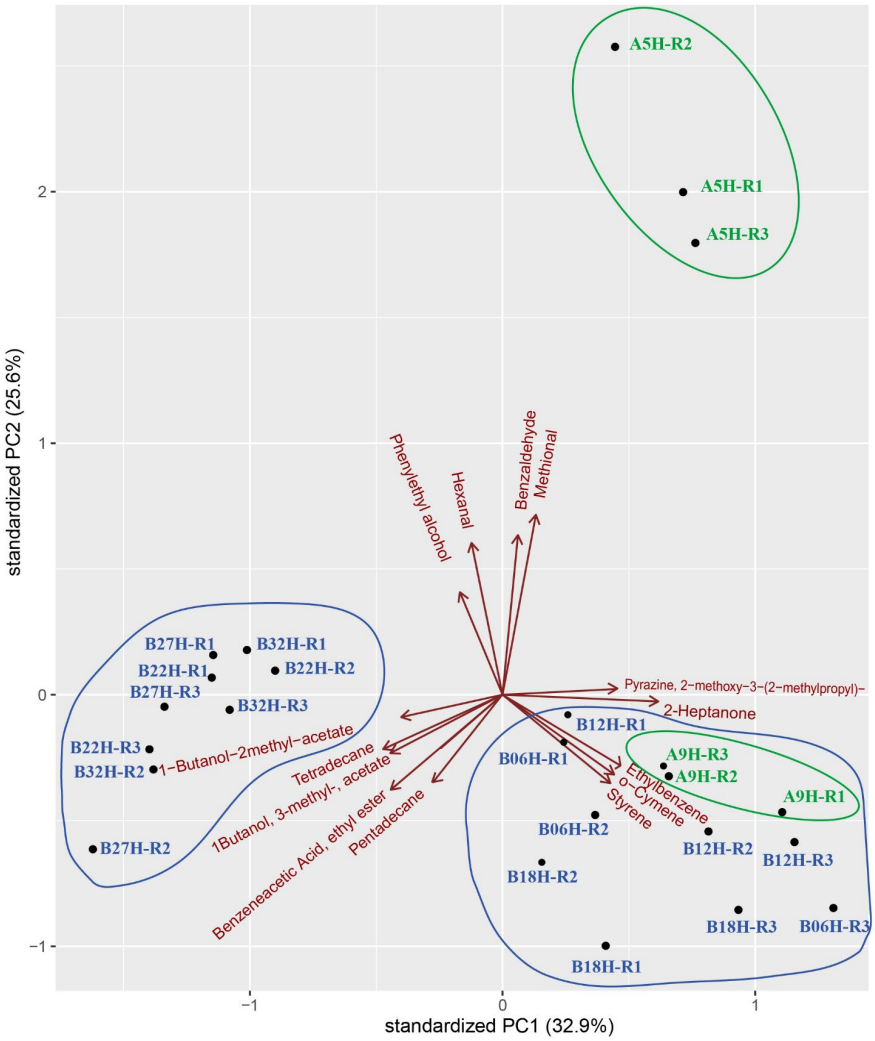

Figure 5. Plot of principal component analysis (PCA) of 14 volatile compounds that showed significant differences throughout the SFP (5 and 9 h) and LFP (6, 12, 18, 22, 27 and 32 h).

The lack of uniformity in the relative abundances of volatile compounds throughout the fermentation process might be attributed to the rapid volatilization of compounds due to temperature. The temperature ranged from 22.5 to 23.0 °C during the SFP, whereas it fluctuated from 17.3 °C to 18.5 °C during the initial stage (6 to 18 h) and from 19.9 to 20.4 °C during the late stage (27-32 h) of the LFP. As the temperature increases, the volatility of the aroma compounds generally rises (Schneiderbanger et al., 2011). The aldehydes (i.e., benzaldehyde, methional, and hexanal) disappeared, and pyrazine was produced as time progressed at high temperatures in the SFP (Table 1). Accordingly, reducing the fermentation temperature has a possible application for improving flavour and aromatic profiles (Shi et al., 2022).

In addition, differences in the volatile profile of various coffee fermentation processes might be associated with the altitudes since the location of coffee farms for the SFP (950 m.a.s.l) and LFP (1800 m.a.s.l) varied by 850 m. Although differences in the profile of volatile compounds of green coffee from Ethiopia in a range of 1500 to 2300 m.a.s.l. were not observed (Tsegay et al., 2019), altitude has been considered a key factor in explaining differences in coffee quality (Bertrand et al., 2012). Certainly, other intrinsic factors would also influence the differences observed between the SFP and LFP, such as native microorganisms, soil composition, shade level, and harvest periods (Cheng et al., 2016).

4 Conclusion

This study highlights the complex relationship between coffee fermentation and flavour and the influence of several factors and variables that may affect the composition of flavour and aroma precursors in green coffee beans obtained from wet fermentation. Fermentation time (short *versus* long fermentation process) might have a key role not only in the attributes and final quality of beverages but also in the differences in the presence of volatile compounds in both types of processes. Since fermentation relies on microorganisms inhabiting coffee cherries and surrounding areas, additional studies including this biological component are needed in northern Peru to understand the mechanisms underlying the coffee quality variation exhibited by geographically similar coffee farms. In addition, complementary studies are required to evaluate the influence of temperature and fermentation time on coffee final attributes The optimization of this important process to generate desirable traits, such as floral and fruity attributes with decreased bitterness and acidity, should be further explored, especially with the use of native microorganisms and the standardization of the processes, including the time of fermentation.

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SUPPLEMENTARY MATERIAL

Supplementary material accompanies this paper.

Table S1. List of volatile compounds identified in fermented coffee beans during long (L) and short (S) fermentation processes. The values are the mean of three replications. Data are expressed as the relative areas in percentage. Asterisks by the chemical compounds indicate that they were significantly different among fermentation times at (*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$.

Table S2. Relative abundances (in percentage) of volatile chemical groups identified in fermented coffee beans during the LFP and SFP. The values are the mean of three replications.

Table S3. Relative abundances (in percentage) of volatile chemical groups identified in fermented coffee beans during the LFP and SFP. The values are the mean of three replications.

Fig. S1: Flavour attributes and scores of coffee beverages obtained by two fermentation processes (short and long) performed by Q-Graders through cupping.

Fig. S2: Relative abundances of the volatile compounds 2-methoxy-3-2-methylpropyl (a), styrene (b), ethylbenzene (c), o-cymene (d), 1-butanol-2-methyl-acetate (e), and tetradecane (f) produced throughout the SFP (5 and 9 h) and LFP (6, 12, 18, 22, 27 and 32 h).

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