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Variation in the Alcohol Components of *Coffea arabica* L. Wastewater Distillate Fermented Under Different Conditions

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ABSTRACT

Coffee is the second-most consumed beverage in the world, and its high demand is covered by countries such as Peru, where the waste generated in production causes environmental pollution. We sought to determine the concentration of alcohol components and other volatiles compounds present in the distillate, after fermenting wastewater from the first wash in the wet processing of *Coffea arabica* var. catimor under five conditions: C1 (pasteurized + 0.325 g sucrose + 8.000 g *S. cerevisiae*), C2 = (pasteurized + 8.000 g *S. cerevisiae*), C3 (0.325 g sucrose + 8.000 g *S. cerevisiae*), C4 = (8.000 g *S. cerevisiae*), and C5 = (natural state). The solid-phase microextraction technique was used to determine the composition of the distillates by gas chromatography (GC). 35 components were detected, 11 of them under all conditions. Ethanol was the most abundant element in all five fermentation conditions. Condition 1 shows the highest value at 97.29 $\mu g.mL^{-1}$, though all five concentrations can be considered high. This study shows that wastewater from the wet processing of coffee can have agro-industrial use as a value-added product. Postharvest Peruvian coffee is amenable to strategies aligned with the sustainable development goal of reducing food losses along production and supply chains.

INTRODUCTION

Coffee is the second-most traded commodity in the world after oil (Girotto et al. 2018) and the second-most consumed beverage (Perrone et al. 2008). Peru is one of the main coffee-exporting countries, whose production is mainly driven by five regions, one being the Amazon region (Salas-López et al. 2020). Although coffee represents an important commercial asset at the international level (Acosta-Alba et al. 2020), its production has environmental impacts (Garde et al. 2017). The residues generated during fermentation in coffee processing plants are potential environmental problems and cause water pollution due to their high organic component and acidic nature (Woldesenbet & Chandravanshi 2016). When released directly into the environment, they are harmful to water bodies, flora, and aquatic fauna (Pérez-Sariñana et al. 2015).

The processing of coffee begins with the manual harvesting of ripe cherries (Rattan et al. 2015). Then the cherries can be processed by two methods, wet and dry (Gathuo et al. 1991). In the wet process, large volumes of high-strength wastewater are generated and they require treatment before disposal (Gathuo et al. 1991), in Peru there are no records of farms that perform the recycling process on these waters. The waste from this process contains large amounts of organic substrates that are suitable for bioconversion into value-added bioproducts (Zhang et al. 2019).

The coffee cherries are pulped and fermented between 12 and 18 h and then are usually dried (Davydenko et al. 2020, Sanz-Uribe et al. 2017). Waste from wet coffee processing, due to its high sugar content, can be used in the production of bioethanol (Zhang & Ordway 2003), which provides an alternative energy source from waste biomass and solves the problem of its elimination in the environment, as well as the problems it can cause to human health (Woldesenbet et al. 2016). However, the fermentation parameters are not precisely known. Besides the microorganisms present in the residue, it is also possible to use *Saccharomyces cerevisiae* inoculum (Einfalt et al. 2020). *S. cerevisiae* is capable of metabolizing C6 sugars (glucose, fructose, mannose, and galactose) (Kim et al. 2016). This can happen due to the presence of oxygen, which shows favorable conditions for yeast growth, and during anaerobiosis fermentation (utilization of sugar for energy production) occurs (Zhang & Ordway 2003). Approximately 95% of the industrial ethanol in the world is obtained through the fermentation of sugars, which must be separated as efficiently as possible from water-ethanol, but this is difficult due to its chemical affinities (Acosta & Zumalacárregui 2016).

Several microorganisms take part in the fermentation of coffee, and it is expected that these microorganisms participate in the fermentation of the must; however, these microorganisms could affect the quality of the distillates.

Pasteurization can be an alternative to eliminate these microorganisms and use only inoculum (Chagua & Malpartida 2020).

Between 2011 and 2015, up to 389,733 ha of coffee were replaced in Peru by the catimor variety, as it is resistant to coffee leaf rust (*Hemileia vastatrix*) (Díaz & Carmen 2017). Therefore, the waste is useful for the food, pharmaceutical, and cosmetic industries (Ferrell & Cockerill 2012), coffee wastewater contains reducing sugars that could become substrates for fermentation (De Bruyn et al. 2017).

The wet coffee process requires large amounts of water; some authors report a range between 1 and 10 m^3 of water per ton of coffee cherries and up to 15 m^3 of water per ton of clean beans (Hans-Dieter et al. 2009). Considering that Peru produced 228,000 tons of coffee in 2020 (Sanz-Uribe et al. 2017), 34,200,000 m^3 of wastewater was underutilized. Therefore, this work determined the abundance of alcohol components present in the distillate obtained after fermenting wastewater from the first washing in the wet processing of *C. arabica* under natural conditions, after pasteurization, and after being subjected to the activity of *S. cerevisiae* to determine their potential agro-industrial use as value-added products. In this way, coffee production is aligned with the sustainable development goals, namely, goal 12.3, which is to reduce per capita food waste by half and reduce food losses along with production and supply (Ferrell & Cockerill 2012).

MATERIALS AND METHODS

Obtaining Coffee Wastewater

C. arabica var. catimor was collected in May 2019 at the experimental station of the Universidad Nacional Toribio Rodríguez de Mendoza, district of Huambo, at coordinates 6° 20′ 10″ S, 77° 27′ 58″ W and an altitude of 1630 masl. The coffee wastewater was collected from the first wash of the wet process with 7 °Brix and transferred to the Agro-industrial Engineering laboratory in 5-liter containers.

Execution of the Experiment for Fermentation, Distillation and net Yield

Five conditions were selected after the standardization procedure. For sucrose, pre-tests were carried out at 0.300 $kg.L^{-1}$; 0.325 $kg.L^{-1}$; 0.350 $kg.L^{-1}$; 0.375 $kg.L^{-1}$ and 0.400 $kg.L^{-1}$. And for *S. cerevisiae*, pre-tests were performed at 8 $g.L^{-1}$ and 9 $g.L^{-1}$. A 2-liter fermenter was set up for each treatment. Then, according to treatment Table 1, determined the best condition was pasteurized, and sugar was added to a continuous stream (0.325 g; samples C1 and C3).

For the activation of *S. cerevisiae*, water was heated in an electric laboratory cooker until the temperature reached 80°C. Then the mixture was cooled to 40°C, at which time 8 $g.L^{-1}$ *S. cerevisiae* was added. Commercial brown sugar was also added. All treatments were fermented at room temperature (16-19°C) for 7 days, and the fermentation was stopped by refrigeration until further distillation (Wang et al. 2020). Then, 180 mL of each condition (C1, C2, C3, C4, and C5) was added to the distillation system. The distillation was cut off when the first 25 ml was obtained.

Obtaining the Distillates

The distillates were made in a laboratory distiller with a capacity of 1 L. We kept the temperature of the balloon around 75°C without letting it exceed 80°C. The distillates were packed in amber glass flasks until further analysis.

Volatile Compounds Analysis by SPME-GC-MS and net Yield Obtaining

The analyses of volatile compounds of the alcoholic distillates were performed using the solid-phase microextraction technique (SPME) and were analyzed by gas chromatography coupled to mass spectrometry (GC-MS), based on the method developed by Wang (2020) with some modifications. The distillate was transferred to a 20 mL vial with 1-butanol solution (1-butanol, 5 µL of 1.25 $µg.µL^{-1}$ water) as the internal standard. The amounts were chosen as representative of a high concentration of volatile substances. The vial was hermetically sealed and taken to a dry bath at 55°C for 15 min. After the equilibration period, the divinylbenzene/carboxen/polydimethylsiloxane fiber was exposed to the headspace of the vial for 30 min. After that time, the fiber was placed in the injection port at 250°C in split mode (1:1) and exposed for 6 min for the desorption of the compounds.

The presence and relative abundance of alcohol components present in the distillates were determined with a gas chromatograph (Agilent Technologies 7890B GC system) coupled to a mass detector (Agilent Technologies model 5977B MSD) equipped with a DB-5 MS IU capillary column (60 m length × 0.25 µm inner diameter × 1 µm film thickness). The total run time was 52 min. The oven program was as

Table 1: Characteristics of the different conditions of fermentation in *C. arabica* var. catimor wastewater distillate.

Fermentation condition	Pasteurized	Added sucrose [$kg.L^{-1}$]	*S. cerevisiae* ($g.L^{-1}$)
C1	YES	0.325	8
C2	YES	NO	8
C3	NO	0.325	8
C4	NO	NO	8
C5	NO	NO	NO

follows: initial temperature of 40°C maintained for 2 min, rising by 3°C/min to 50°C and maintained for 1 min, rising to 110°C at a rate of 5°C/min and maintained for 1 min, rising to 200°C at 6°C/min, and rising to 250°C at 8°C/min, where it remained for approximately 11.5 min. The mass spectrometer recorded the total ionic current (70 eV) in a mass range of m/z 30 to 550 (scan mode). The compounds were identified by comparison with the NIST library and the criteria for the identification of each compound required a mass spectrum matching score of ≥70. For every sample, extraction, injection, detection, and identification were performed in quintuplicate on all samples. Of these, the execution in duplicate was without internal standard, and in triplicate with internal standard (with 1-butanol). A threshold of at least two detections was established to express the results of the presence and absence of each identified compound in the samples. Semi-quantitative concentrations of each compound were obtained and were multiplied by a constant for better visualization of the data. This is because the aliquots of the analyzed samples were quite small compared to the 20 mL vial used. These quantities were chosen to take into account that this sample constituted a distillate and, therefore, it was estimated that it contained a high concentration of volatile substances. However, it is important to note that this did not prevent the detection of the different compounds and the estimation of their relative concentration. Finally, after quantification of the compounds, the net yield for the condition was obtained for each ml of distillate. Only the values for the alcoholic components that were repeated in all conditions are shown.

RESULTS

The GC analysis revealed the presence of 35 alcohol components in *C. arabica* var. catimor fermented under different conditions Table 2. We found 11 bioalcohol components under all conditions (ethanol; 1-propanol; 1-butanol, 2-methyl-; 1-butanol, 3-methyl-; hexanoic acid; 2-furan methanol, 5-ethenyltetrahydro-α,α,5-trimethyl-, cis-; trans-linalool oxide (furanoid); linalool; phenylethyl alcohol; octanoic acid; benzaldehyde, 2,4-dimethyl-; and acetic acid, 2-phenylethyl ester). All are included in industrial use lists, and six of them have been cataloged for human consumption (Nguyen et al. 2021). Ethanol was the most abundant element. It was present in all five fermentation conditions, and condition 1 (unpasteurized + sucrose + 8 g *S. cerevisiae*) yielded the highest value, with 972.9 $\mu g.\mu L^{-1}$ concentration. We also consider the ethanol values for the other conditions substantial (926.0 $\mu g.\mu L^{-1}$ in C2 and 874.2 $\mu g.\mu L^{-1}$ in C3). The 1-Dodecanol ranked second in conditions 1, 2, and 3, with values of 368.6, 362.4, and 271.8 $\mu g.\mu L^{-1}$, respectively, while in condition 4 its value decreased to 9.7 $\mu g.\mu L^{-1}$, and in condition 5 it disappeared. The compound trans-Linalool oxide (furanoid) showed its highest concentration in condition 3.

The Yield of Alcohol Produced in the Distillates

Fig. 1 compares the abundance of the main compound identified in the 5 conditions assayed, including ethanol. Of the 11 compounds identified in the distillates of the five conditions, ethanol was the most abundant, followed by 2-benzaldehyde, 2,4-dimethyl- in all cases. Conditions C1, C2, and C3 yielded distillates with higher ethanolic contents of 97.29, 92.6, and 87.42 $\mu g.mL^{-1}$, respectively. Conditions C4 and C5, without the addition of sucrose or yeast (fermented with native flora present in the collected wastewater), yielded distillates with lower ethanolic content (20.50 to 34.42 $\mu g.mL^{-1}$). The content of the other compounds present was proportional in both groups Fig. 1.

DISCUSSION

The 35 components in *C. arabica* var. catimor distillates found here correspond to quantitative GC measurements that have been performed frequently with a remarkable degree of reproducibility in agreement with Novotny (2003) and Lopes et al. (2020), and the search for the sustainable use of these components by adding value is an ideal transition to an economy of biological resources (Woldesenbet et al. 2016).

The alcohol components that were found in all the distillates of this study have important properties for industrial use (Brugnera et al. 2006). Their main use is agro-industrial because of the high amounts of ethanol (Xiao & Lu 2014). Other uses for its components are, for example, rocket fuel, the manufacture of plastics, agricultural pesticides, for medications to treat cancer patients (Nguyen et al. 2021), contact lenses, and textile cleaning (Wang et al. 2020). Some other potential uses are the manufacture of other chemical products, etching metals, solvent, and chemical laboratory analysis, fabric dyes, nylon production, and tanning of leathers (Sampaio et al. 2013). Another six components identified here have agro-industrial uses: 1-butanol, 2-methyl- (Kim et al. 2019); 1-butanol, 3-methyl- (Kim et al. 2019); linalool and benzaldehyde, 2,4-dimethyl- (Kim et al. 2019), especially in the production of alcoholic beverages, to improve the taste of their food products (Kim et al. 2016), as an aroma and essence of artificial fruits (Kim et al. 2016), and others.

Ethanol was the most abundant compound under all five conditions of this work. It is classified as one of the most important products of primary metabolism and one of the most produced in the world (Chin et al. 2011). It can reduce pollution from agricultural waste (Clarke & Bakker 2004). Work has been done to optimize these processes to achieve

Table 2: Semi-quantitative concentrations (mean ± standard deviation) of alcohol components of the wastewater distillate of *Coffea arabica* L. fermented under five different conditions.

Name	CAS-Number	Retention Time	Conditions (mean × 10000) a				
			C1	C2	C3	C4	C5
Hydrazine **, ***	302-01-2	4.494	ND	ND	31.1 ± 2.4	ND	ND
Hydrazinecarboxamide ****	57-56-7	4.491	ND	38.3 ± 4.3	ND	ND	ND
Ethanol *,**, ***	64-17-5	5.349	972.9 ± 55.5	926.0 ± 27.4	874.2 ± 40.9	344.2 ± 14.2	299.0 ± 20.5
Acetic acid**	64-19-7	8.764	92.3 ± 9.7	114.3 ± 10.6	97.3 ± 7.0	42.5 ± 10.1	ND
Ethyl Acetate*,**	141-78-6	9.816	23.7 ± 1.7	19.8 ± 1.3	15.2 ± 0.7	ND	22.0 ± 3.4
1-Propanol, 2-methyl-*,**	78-83-1	10.415	19.1 ± 0.9	19.3 ± 1.6	17.7 ± 0.5	5.5 ± 2.0	6.2 ± 1.4
Acetoin*	513-86-0	13.9	ND	80.3 ± 15.1	72.6 ±10.4	ND	ND
1-Butanol, 3-methyl-*,**	123-51-3	15.088	55.0 ± 2.7	49.4 ± 5.3	50.4 ± 3.1	31.2 ± 10.9	43.0 ± 6.7
1-Butanol, 2-methyl-*,**	137-32-6	15.285	ND	ND	ND	8.2 ± 3.0	11.6 ± 2.0
1-Cyclopentyl-2,2-dimethyl-1-propanol ***	337966-85-5	15.279	30.2 ± 4.0	ND	ND	ND	ND
Butanoic acid**	107-92-6	16.507	ND	ND	ND	6.4 ± 1.5	ND
Furfural**	98-01-1	19.544	ND	ND	ND	9.7 ± 3.2	9.0 ± 1.1
3-Furaldehyde	498-60-2	19.546	ND	ND	196.7 ± 7.9	ND	ND
Propanedioic acid, propyl	616-62-6	20.804	ND	14.1 ± 2.6	ND	ND	ND
Pentanoic acid*,**	109-52-4	20.805	14.2 ± 2.0	ND	10.4 ± 1.3	9.6 ± 3.5	9.6 ± 3.3
1-Hexanol	111-27-3	20.971	ND	ND	ND	ND	4.2 ± 0.6
CH3C(O)OCH(CH3)C(O)CH3**	4906-24-5	21.644	12.7 ± 1.3	ND	13.0 ± 1.4	ND	ND
1-Heptanol	543-49-7	22.366	ND	ND	ND	ND	4.0 ± 0.3
Hexanoic acid*,**	142-62-1	24.767	47.8 ± 7.1	47.6 ± 3.2	36.8 ± 3.8	17.0 ± 5.9	18.7 ± 5.5
Benzyl alcohol	100-51-6	27.678	ND	ND	9..3 ± 0.5	ND	ND
2-Furanmethanol, 5-ethenyltetrahydro-.alpha.,.alpha.,5-trimethyl-, cis-***	5989-33-3	29.025	89.5 ± 1.5	90.4 ± 1.7	96.4 ± 2.3	10.8 ± 2.0	13.0 ± 1.9
trans-Linalool oxide (furanoid)**	34995-77-2	29.513	65.0 ± 1.5	68.4 ± 2.3	69.1 ± 3.6	8.2 ± 1.8	9.5 ± 1.8
1,6-Octadien-3-ol, 3,7-dimethyl-, formate	115-99-1	29.648	ND	ND	26.2 ± 0.4	ND	ND
Linalool*,**	78-70-6	29.654	26.4 ± 1.5	25.1 ± 1.1	ND	ND	15.0 ± 0.4
Phenylethyl Alcohol**	60-12-8	30.384	48.5 ± 8.7	56.2 ± 4.7	57.3 ± 6.4	23.9 ± 5.3	ND
Octanoic acid**	124-07-2	31.168	42.2 ± 10.3	48.7 ± 7.8	27.4 ± 8.1	ND	ND
alpha.-Terpineol**	98-55-5	32.912	18.3 ± 2.1	ND	17.9 ± 0.6	ND	ND
Benzaldehyde, 2,4-dimethyl-*	15764-16-6	33.643	109.3 ± 14.7	104.7 ± 5.0	105.5 ± 9.3	17.9 ± 4.8	4.4 ± 0.7
Acetic acid, 2-phenylethyl ester**	103-45-7	34.268	18.5 ± 2.1	17.0 ± 1.0	13.1 ± 1.1	ND	18.7 ± 1.6
2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-, (E)-*****	23726-93-4	37.562	14.9 ± 0.7	ND	9.0 ± 0.6	ND	ND
1-Dodecanol**	112-53-8	39.083	368.6 ± 35.7	362.4 ±18.6	271.8 ± 15.7	ND	ND
2,4-Di-tert-butylphenol*****	96-76-4	39.843	82.3 ± 6.8	80.1 ± 6.9	71.0 ± 3.1	ND	ND

ND: Compound not determined, *use in human consumption, ** industrial use, *** medicinal use, **** no use/unknown use. a Concentration relative (μg. μL^{-1}) of the components was multiplied by 10000.

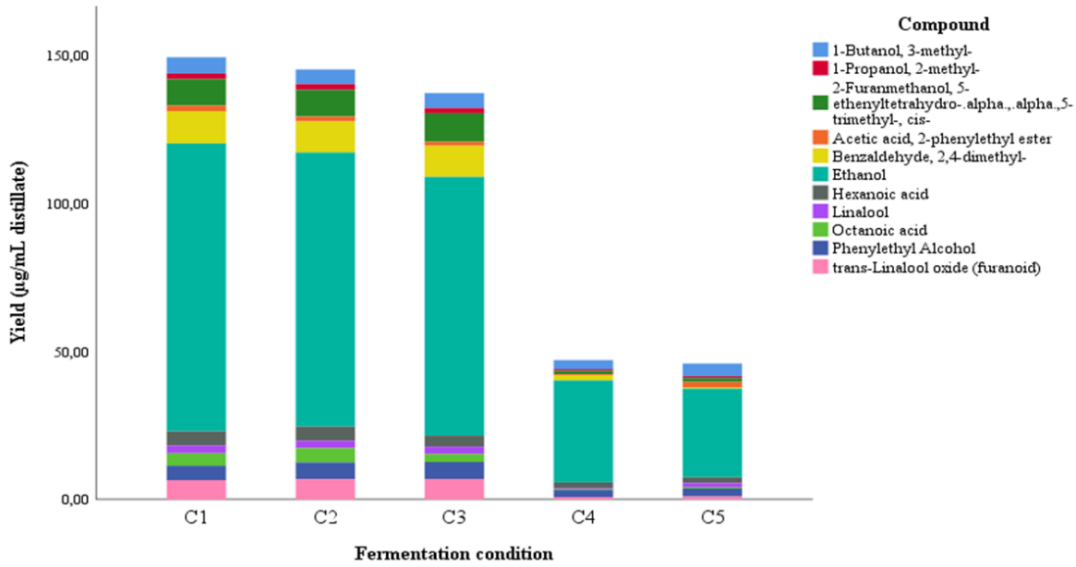

Fig. 1: The concentration of the 11 compounds present in the distillates obtained from fermented coffee wastewater under five conditions.

higher levels of sugar consumption and ethanol production due to effective exposure to the substrates (Ryan et al. 2004). Studies have shown when other residual coffee wastes, such as whole husks and ground coffee, have been used directly as substrates for the production of fermentative ethanol, final yields (g of ethanol/g of the substrate) of 0.008 and 0.007, respectively, have been achieved (Novotny 2003), 0.33 of coffee mucilage (Vidra & Németh 2018) and, this study reported 0.097 $g.mL^{-1}$ of wastewater. Despite the variation in its value between the different types of coffee waste, we think that the results reflect the presence of high ethanol values in this study.

The addition of sugar and *S. cerevisiae* to fermentation influences the ethanol yield (Roehr et al. 2001), as well as the concentration relative to the rest of the components that we measured. A lower amount of ethanol could be due to the consumption of sugar by other microorganisms that interfere with the fermentation process (Woldesenbet et al. 2016). In this sense, the initial concentration of sugars has positive effects on the fermentation, and the yield and productivity depend on the initial concentration of sugars (Phisalaphong et al. 2007). Here, the single application of 8,000 g of *S. cerevisiae* led to the greatest abundance of ethanol in the distillate (972.9 $\mu g.\mu L^{-1}$). Coffee wastewater subjected to saccharification produced yields (based on sugar content) of ethanol of 15.3 $g.L^{-1}$ (Choi et al. 2012), 0.55 $g.L^{-1}$ (Bonilla-Hermosa & Schwan 2014), and 0.33 g ethanol/g sugar (Silva et al. 2010).

Thus, the present results support *S. cerevisiae* as the most common microorganism to produce ethanol due to its high consumption of sugars (Vučurović 2011). The results of the relative concentrations of the components are presented in Fig. 1.

The ethanol content and the presence of furfural compound in Condition 1 could have resulted from the application of *S. cerevisiae*. However, furfural is undesirable, because of the affectation of specific cell growth, and cell mass yield in ATP and ethanol, depending on its concentration in the fermentation medium (Palmqvist & Hahn-Hägerdal 2000). On the other hand, the presence of octanoic acid is associated with odors such as rancid and fatty, in this study, the lowest values of this compound (0.37-0.45 $\mu g.mL^{-1}$) were evidenced for the C4 and C5 conditions. *S. cerevisiae* synthesizes a wide variety of alcohols during fermentation (Franca et al. 2008), and 1-propanol was found in all the assessed conditions.

Propanol, isobutane, and isoamyl alcohol are part of the so-called fusel oil and are formed by alcohols with more than two carbon atoms; they are formed by the fermentation of sugars. These compounds were also present in the distillates of this study. *S. cerevisiae* has a natural ability to adapt skillfully to inhibitory environments (Kim et al. 2020), and ethanol is the main byproduct of yeast fermentation, as its generation is notably associated with cell growth, viability, and metabolic activity (Mussatto et al. 2012).

In fermentation condition 1, the highest concentration of furfural was found, at 226.7 $\mu g.\mu L^{-1}$. Other studies have detected butyric, propionic, and acetic acids, which represent a possibility for the use of other sources as substrates (de Melo Pereira et al. 2014).

Linalool can be produced by *S. cerevisiae* and *Pichia guilliermondii* in association with coffee (Ezeji et al. 2007). It was most abundant in condition C1, with a value of 26.4

$\mu g.\mu L^{-1}$. It has been identified as a fresh, citrus, and woody aroma in food products (Lee et al. 2009).

Acetic acid, was highest in condition C1, with a value of 114.3 µg/L. 2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-, (E)- was not detected under condition C4 or C5. 1-Dodecanol was most abundant under condition C1, with a value of 368.6 µg/µL. Similarly, the compound 2,4-di-tert-butylphenol yielded the highest value of 82.3 $\mu g.\mu L^{-1}$, characteristic compounds of the distillates of coffee wastewater that are aromatic (Vázquez & Dacosta 2007). Acetic acid, present in conditions 1-4, is an unpleasant taste in coffee distillates and could damage the final quality of the distillates by giving them an onion flavor (Ezeji et al. 2007). However, we consider these values unimportant because they had less than 92.3 $\mu g.\mu L^{-1}$, and the condition C5 did not yield acetic acid.

CONCLUSION

SPME and GC-MS allowed us to detect 35 alcohol components at different relative abundance values in five different distillates obtained from the wastewater fermentation of the first wash in the wet processing of *Coffea arabica* var. catimor. All distillates had ethanol as the most abundant alcohol, suggesting the potential agro-industrial use of Peruvian coffee as a postharvest value-added product.

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