COFFEE SCIENCE
ISSN: 1984-3909

eISSN: 1984-3909
Coffee Science, e172016, 2022

Doi: https://doi.org/10.25186/.v17i.2016

Effects of post-harvest process on volatile - sensory profile for coffee in Colombia

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Received in March 4, 2022 and approved September 22, 2022

ABSTRACT

The coffee fermentation process has been established as a determining stage regarding its quality and aroma. The objective of this study was to evaluate the differences that exist between five fermentation processes and within each process (at different fermentation times) based on their volatile and sensory profile. The processes evaluated were dry or natural (NA), semi-dry or honey (HO), and three variations of the wet process, called: conventional aerobic (CA), aerobic with previous fermentation in cherry (AFC) and anaerobic with previous fermentation in cherry (ANFC). The NA process obtained the highest score (86.63) in the sensory profile and statistically different from the other processes, followed by the process HO (84.79), ANFC (84.24), CA (84.21) and AFC (83.63). The volatile profile was determined by headspace solid phase micro-extraction (HS-SPME) and gas chromatography coupled to mass spectrometry (GC-MS). Fifty-one volatile organic compounds (VOCs) were tentatively identified. The main chemical families that allowed differentiating between groups of processes were ketones and pyrroles, and the VOCs that mainly contribute to differentiate between process or groups of processes are: ethanone, 1-(1H-pyrrol-2-yl)-, 2,5-dimethylpyrazine, 2-furanmethanol, 4-vinylguayacol, 2-methylfuran, 2- butanone, 2,3-dimethylpyrazine, acetylpyrazine, 1- (2-furanylmethyl) -1H-pyrrole and 2,2'-bifuran. Within each fermentation process (between treatments) no differences were found from the final score of the sensory analysis, but differences were found from volatile profile.

Key words: Volatile profile; time fermentation; GC-MS; SPME.

1 INTRODUCTION

In Colombia, the traditional wet-processing methods, use a method of fermentation to remove the cherry pulp surrounding the beans, this process has a great influence on the sensory quality and prestige of Colombian coffee in international markets (De Oliveira et al., 2019).

The last stages of coffee processing have been widely covered, so that in recent years there has been a change of focus in the modulation of coffee aroma towards the optimization of post-harvest processes and the treatment of coffee substrates (Lee et al., 2017). This change corresponds to the fact that, depending on the type of processing selected, beverages with particular tastes and aromas can be produced. Processing methods can be divided into three: dry, semi-dry or wet.

The dry process, also known as "natural", is the simplest, where the coffee is collected and can be washed or not, before being spread in a layer of 5 cm thick to be exposed to the sun until it reaches a humidity between 11-12%. In the semi-dry process, also known as "Honey" or "pulped natural" (Evangelista et al., 2014), the coffee cherrys are pulped, and the fermentation process takes place directly under the sun on a platform or concrete. In the wet process, the pulp and/or mucilage are mechanically removed, and the grains are fermented by submerging them in a large amount of water (Vilela et al., 2010).

From Colombia different studies have been carried out to identify how each type of process and its possible variations affect the chemical composition and/or quality of the coffee (Cortés-Macías et al., 2022); (Rodriguez; Guzman; Hernandez, 2020). Gonzalez-Rios et al. (2007) determined how three variants of the wet process and a mechanical process affect the volatile compounds in roasted coffee. Finding that the wet process in which the coffee beans were submerged in water and allowed spontaneous fermentation originated more pleasant notes and the treatment where the mechanical removal of the mucilage was used, provided the coffee with the lowest aroma quality. Which highlights the importance of the fermentation process to contribute to the quality of the beverage (Zhang et al., 2019). Puerta and Echeverry (2015) evaluated the wet process with other types of variations: fermentation without addition of water (solid) and submerged, and in turn with provision of air (Aerobic) and in closed processes (Anaerobic). Reporting that the solid and closed process can give more complex (untraditional) notes, but the submerged and open fermentations provide better control and less risk of cup defects.

The main function of coffee fermentation in any type of processing is to facilitate mucilage degradation by indigenous microorganisms consisting of pectinolytic yeasts (Masoud et al., 2004). However, the use of starter cultures that help control fermentation and have specific aroma profiles has been a topic of interest in recent times. This is how different

studies have evaluated the use of yeasts as starter strains in the dry process (Evangelista et al., 2014) (Da Silva et al., 2021), in the semi-dry process (Evangelista et al., 2014) (Ribeiro et al., 2017) or by inoculating the dry and semi-dry processes in parallel (Da Mota et al., 2020) (Bressani et al., 2021) concluding that certain yeasts can produce a drink with a distinctive flavor and good sensory quality.

(Bressani et al., 2021) compare the fermentation at different altitudes, finding that at altitudes below 900 m the use of yeasts could improve the cup quality of coffee and at altitudes above 900 m spontaneous fermentation is more promising. This highlights the potential of indigenous coffee microorganisms to produce good quality coffee.

Spontaneous fermentation has been studied comparing the three general types of processing (wet, semi-dry and dry) in parallel, focusing mainly on analyzing aroma precursor compounds such as sugars, caffeine, trigonelline and chlorogenic acids (Duarte; Pereira; Farah, 2010).

However, the results with different posthaverst process showed differences to quality aspects in sensory profile and linking to volatile profile, to what extent these changes in non-volatile profiles translate into volatile-aromatic profile differences between differentially processed coffees is less well known under Colombian conditions.

The purpose of this work was to evaluate the differences between five fermentation processes and within each process (at different fermentation times), based on their volatile and sensory profile. The processes evaluated were the dry (Natural-NA), semi-dry (Honey-HO) and three variations of the wet process named in this study as: Conventional Aerobic (CA), Aerobic with previous cherry fermentation (AFC) and Anaerobic with previous cherry fermentation (ANFC).

2 MATERIAL AND METHODS

2.1 Sampling site

The collection was carried out in October 2019, in the experimental farm of the Cooperativa de Cafetaleros del Norte del Valle (CAFENORTE) situated in the municipality of El Águila-Valle del Cauca, Colombia. The farm is located between 1450-1550 meters above sea level. The sampled lot had a size of 1 hectare with 2500 plants of the Castillo, Supremo and Caturra varieties. The age of the crop was 3 years old and it was in its second production cycle.

2.2 Fermentation experiments

The coffee cherries were manually harvested at the mature stage (red cherries) and were taken to the processing unit to be washed in 500-liter plastic boxes to separate the floating fruits. After washing, five fermentation processes were evaluated which correspond to three variations of the wet process (CA, AFC, ANFC), the semi-dry (HO) and dry (NA) process.

2.3 Treatments description

Aerobic conventional (CA): In this process, once the cherry was collected, it was immediately depulped and the coffee with mucilage were placed in open fermentation units (with air provision) without water addition (solid fermentation) and six different times (hours of fermentation were evaluated): 12, 16, 20, 24, 36 and 42 hours. For this purpose, 54 kg of coffee were pulped to obtain approximately 30 kg of coffee with mucilage and were distributed in six fermentation units (5 kg in each one), the containers used were made of polypropylene and had a capacity of 10 liters.

Aerobic with previous fermentation in cherry (AFC): This process was developed under the same conditions as the CA process, but a modification was included, this modification consisted of storing the coffee in plastic buckets (50 Kg capacity) for 15 hours after being harvested (previous cherry fermentation), this time simulates the practice that coffee growers can have of pulping the day after the coffee is harvested. Once this time was over, the coffee was pulped and finally processed as it was detailed in the CA process.

Anaerobic with previous fermentation in cherry (ANFC): This process was developed under the same conditions as the AFC process, but the fermentation units were closed (without provision of air). These units were adapted to allow the release of carbon dioxide produced by microorganisms and prevent the entry of air.

Semidry process: In this process, once the coffee was harvested, it was stored in clean tows for 15 hours (cherry fermentation), immediately this time ended, it was pulped and without removing the mucilage, it was subjected to immediate drying, exposing the coffee to different intensity of solar radiation to obtain three types of Honey: Yellow Honey (YH) (Direct sun exposure), Red Honey (RH) (Medium sun exposure) and Black Honey (BH) (Without sun exposure, covered with black plastic). 28 Kg of cherry coffee were pulped to obtain 15 Kg of coffee with mucilage and divided into 3 experimental units (5 Kg in each one).

Dry process: In this process, once the coffee was harvested, it was stored in clean tows for 24 and 36 hours (cherry fermentation). For each evaluated time 15 Kg of cherry coffee were placed.

For the wet processes and the fermentation in cherry for semidry and dry process were carried out at controlled temperature and relative humidity, in the agrochemical analysis laboratory of CAFENORTE. These variables were monitored with a HALTHEN brand digital thermohygrometer. The temperature presented an average of 21.2 °C ± 1.4 °C and the relative humidity presented an average of 40.4°C ± 2.8 °C.

2.4 Post-fermentation treatments

For the wet processes, once the respective fermentation time in each experimental unit had ended, the coffee was washed removing the remaining mucilage and was subjected to mechanical drying in a forced convection oven at 40 ° C. For the dry process, once the cherry fermentation time was completed for each experimental unit, the coffee cherry was subjected to drying under the same conditions as the wet processes. For the semi-dry process, once the coffee was pulped the fermentation units were placed in a parabolic dryer; parabolic drying presented a temperature that ranged between 29 and 35 °C, at a relative humidity between 45 and 75%, the drying time was eight days. The storage and stabilization process was carried out in the CAFENORTE storage center with temperature between 21 ± 2 °C and a moisture content of 65%.All the experimental units regardless of the process, were dried until reaching a moisture content between 10 and 12%, subsequently the coffee was packed in "Zipper GrainPro" bags and stored for 15 days to allow its stabilization. The samples of coffee was threshed in an INGSEC ING-C-250 laboratory thresher and finally, the green coffee was selected and roasted in PROBAT brand laboratory toaster according to the specifications of the (SCA, 2009).

2.5 Sensory analysis

The samples were prepared according to the cupping protocols of the (Specialty Coffee Association - SCA, 2009). For each sample, 120 g of coffee were roasted and subsequently ground in a BARATZA-MAESTRO brand electric mill. A panel of four trained "Q-Grader" certified tasters evaluated the study samples. The sensory attributes evaluated were: fragrance, flavor, aftertaste, acidity, body, uniformity, balance, sweetness, cleanliness and final score.

2.6 Analysis of volatile compounds

Roasted and ground coffee samples were extracted by HS-SPME-GC/MS (solid phase microextraction using the headspace mode, combined with gas chromatography coupled to mass spectrometry). Commercial 75-µm Carboxene/ Poldimethylsiloxane (CAR/PDMS) fiber (Supelco®) was used. Fiber was exposed for 30 min in a vial of 20 mL containing 1.5 g of sample. The system was immersed in controlled conditions at 60 °C in water bath.

Chromatographic analysis was performed in a gas chromatograph Shimadzu® GCMS-QP2010 plus with mass detector. Helium was used as the carrier gas; injection temperature was 230 °C, in splitless injection mode. ZB-1 ms capillary column (60 m × 0.25 mm I.D × 0.25 µm D.F; Phenomenex® brand) was used. A linear velocity mode was used with a total flow of 36.2 mL/min and a column flow of 2 mL/min. The oven temperature range began at 45 °C for 2 min, then increased 1 °C/min up to 58 °C staying for 2 min. Then, temperature increased 3 °C/min up to 100 °C for 2 min and finally, increased 10 °C/min up to 240 °C for 1 min. The total analysis time of 48 min. The ion source and interphase of mass spectrophotometer was at 300 °C. Ionization mode was electronic impact (EI) at 70 eV; the mass detection range was 40–350 Da in SCAN working mode at 0.93 kV and an acquisition time of 2 min after start. The identification of VOCs were performed using three methods: comparations of mass spectra experimentally obtained with the NIST14.0 library; determination of Kovats experimental retention index (RI Kovats) for each metabolite using an alkane standard (C7–C40) (reference 49,452 -U); and it was reviewed whether the compounds had already been previously reported as constituents of coffee in different investigations.

2.7 Statistical analysis

The final score of the sensory analysis were processed with the STATGRAPHICS Centurion XIX Version 19.0.17 software. The quantitative variable was compared by analysis of variance (ANOVA), followed by the Fisher's LSD multiple comparison test with a confidence level of 95%. The categorical data (attributes) described by the tasters were unified according to the taster's flavor wheel (Spencer et al., 2016) and analyzed through frequency tables.

The chemical data were organized in a matrix Xij, where i corresponds to the coffee samples and j the variables, represented by the relative area of the volatile components identified in all coffee samples. The analysis was carried out using the Metaboanalyst version 5.0 platform (Xia et al., 2009). In order to establish the main differences and similarities in the chemical composition between processes, a hierarchical clustering heatmaps was made and to identify potential biomarkers of the type of fermentation process used, a partial least squares discriminant analysis (PLS-DA) was performed and the VIP ((Variable Impact on Projections) coefficient was determined, which classifies the variables based on their explanatory power of separation between the processes. According to Alcantara, Dresch and Melchert (2021) the variables with a VIP>1 are the most relevant. Before the chemometric analysis, the data were subjected to logarithmic transformation in base 10 and autoscaling. Additionally, the normalized relative concentration was compared using ANOVA or if one of the assumptions was not met, the non-parametric Kruskal-Wallis test was performed, followed by the Bonferroni test with a confidence level of 95%.

In order to establish the main changes that occur with time and the relationships between the volatile profile and the sensory descriptors reported by the tasters, for the treatments of the wet processes, a grouping of the first three times was made. of fermentation 12, 16 and 20 hours and compared with the last three times 24, 36 and 42 hours.

3 RESULTS

3.1 Sensory analysis

When comparing the final score between the 5 fermentation processes (grouping the treatments within each process), it was identified that the NA process presents the highest final score and is statistically different from the other processes. Therefore, this is the process under which the best quality is obtained. On the other hand, when comparing the final score between treatments in each isolated process, it is evident that there are no statistically significant differences (Table 1). However, a criterion with commercial impact should be highlighted; according to the SCA a coffee with a final score greater than 85 points is classified as Specialty-Excellent. 5 treatments obtained a final score equal to or greater than 85 points: NA_24 h, NA_36 h, YH, RH and CA_36 h, for which these treatments should be considered for their potential to provide better quality coffee and obtain better economic compensation for coffee growers.

The relative frequency of the sensory descriptors reported by the tasters for the 5 processes evaluated are presented in Figure 1; as well as these graphs are presented for the treatments inside the processes, NA process (Figure 2), HO process (Figure 3) and wet processes (Figure 4).

3.2 Volatile profile

A total of 62 volatile compounds were detected for all the evaluated fermentation processes, where 51 of these were tentatively identified and 11 were reported as unknown (Table 2). The comparisons between processes were made from the chemical compounds without grouping and grouped in chemical families. Therefore, the detected compounds were grouped into the following 13 chemical families: Pyrazines (13), Furans (10), Ketones (7), Acids (3), Furanones (3), Pyrroles (3), Aldehydes (3) , Pyrones (2), Phenols (2), Esters (2), Pyridines (1), Lactones (1), Alcohols (1).

3.3 Comparison between fermentation processes

From the heatmap with hierarchical clustering it was possible to identify 3 main clusters, the first cluster made up of the wet aerobic processes CA and AFC, the second is made up of the ANFC and HO processes and finally the isolated NA process (Figure 5).

From inferential statistical analysis it was possible to determine the main chemical families that allow separating between fermentation groups, which are ketones and pyrroles (Table 3).

The pyrroles present statistically significant differences between two groups, the first group is the wet aerobic processes CA and AFC, the second; are the ANFC, HO and NA processes, the pyrroles content is higher in the first group. The ketones present statistically significant differences between the processes CA, AFC and a group consisting of ANFC, HO and NA, the concentration increases in the following direction CA→AFC→ (ANFC-HO-NA).

The discriminant analysis (PLS-DA) between process from the 62 compounds detected, provided 26 compounds with a VIP>1, to which an inferential statistical analysis was performed, finding that 10 of these compounds presented statistically significant differences from the mean or the median between two or more process groups (Table 3).

3.3.1 Dry process – Natural (NA):

Between the two evaluated treatments NA_24 h and NA_36 h, 10 compounds with statistically significant differences were found, 4 of these belong to the pyrazines family, with pyrazines being the chemical family that allows greater differentiation between the evaluated times and together with esters are the only two families that present statistically significant differences (Tabla 4).

Table 1: Results of the final score in the sensory analysis for all the treatments evaluated.

Process/treatment	12 h	16 h	20 h	24 h	36 h	42 h	Average	P value
CA	83.29^{a}	83.38^{ab}	84.75^{cd}	84.54^{bcd}	85.38^{d}	83.92^{abc}	84.21^{A}	0.0072
AFC	83.92^{ab}	83.96^{ab}	83.08^{ab}	83.96^{ab}	84.42^{b}	82.42^{a}	83.63^{A}	0.1830
ANFC	84.13^{a}	83.88^{a}	83.50^{a}	84.38^{a}	84.88^{a}	84.71^{a}	84.24^{A}	0.5550
	YH	RH	BH					
HO	85.00^{a}	85.22^{a}	84.16^{a}				84.79^{A}	0.3008
	24 h	36 h						
NA	86.44^{a}	86.81^{a}					86.63^{B}	*
					Comparison between processes			<0.0001

Mean values with different lowercase letters in the same row indicate statistical differences within each process (between treatments) at 5% significance level ($p < 0.05$) by Fisher's LSD test. Mean values in the column named "Average" with different capital letters indicate statistical differences between processes. (*) The treatments of the NA process did not meet the assumptions for the parametric analysis, for which the Kruskal Wallis test was carried out, concluding that there are no differences from the median (p-value = 0.3619).

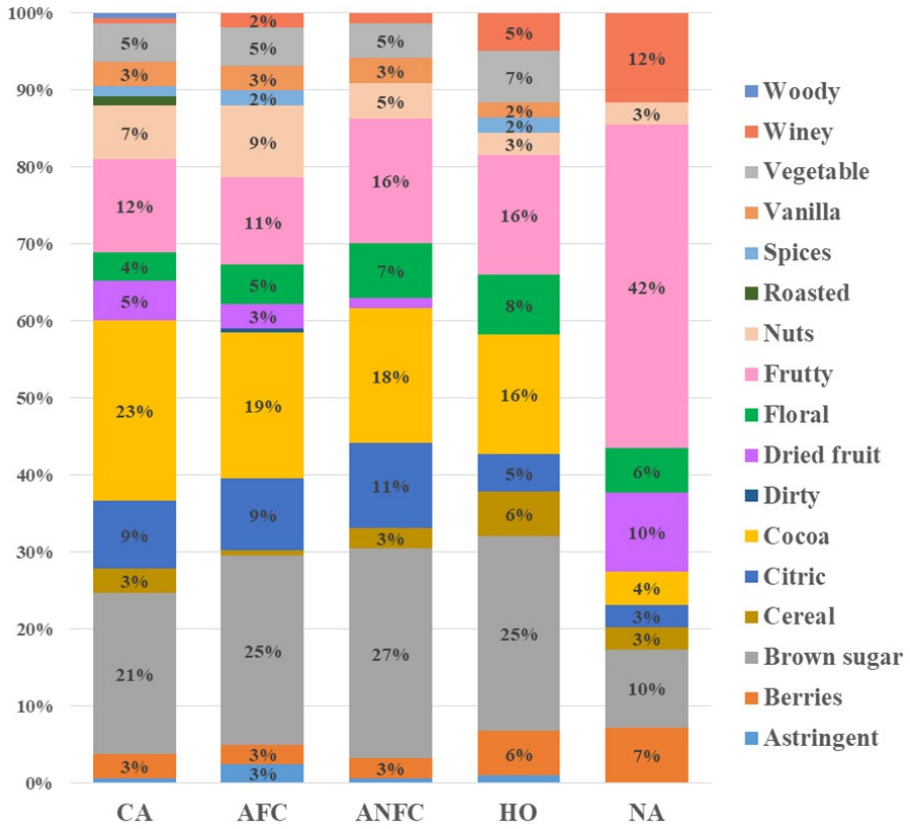

Figure 1: Relative frequencies for the descriptors reported in the fermentation processes.

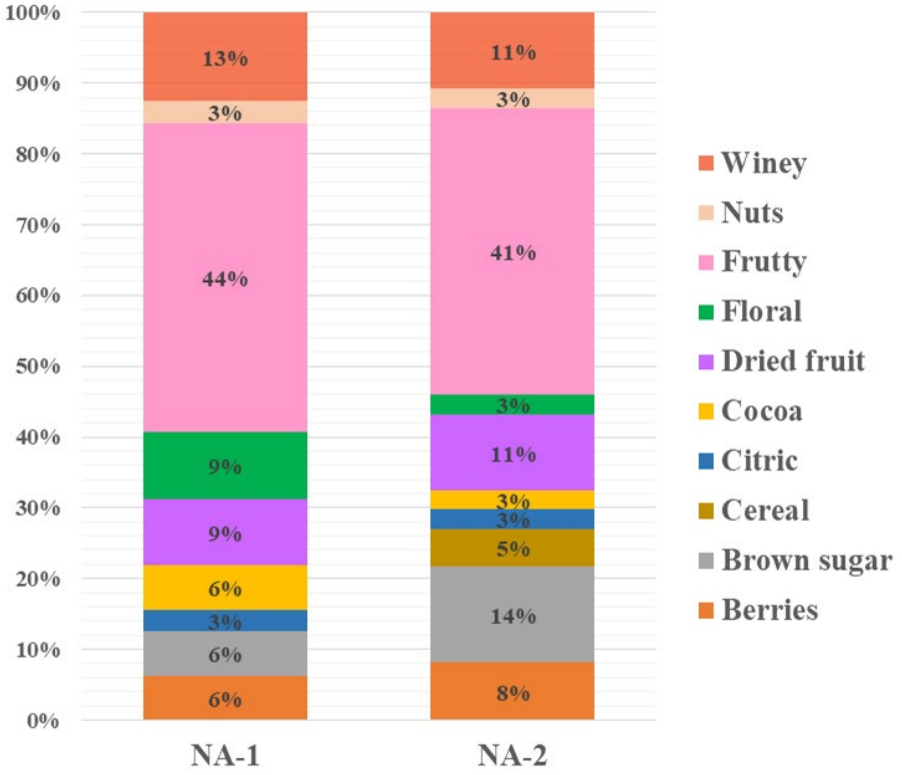

Figure 2: Relative frequencies for sensory descriptors reported in the NA process.

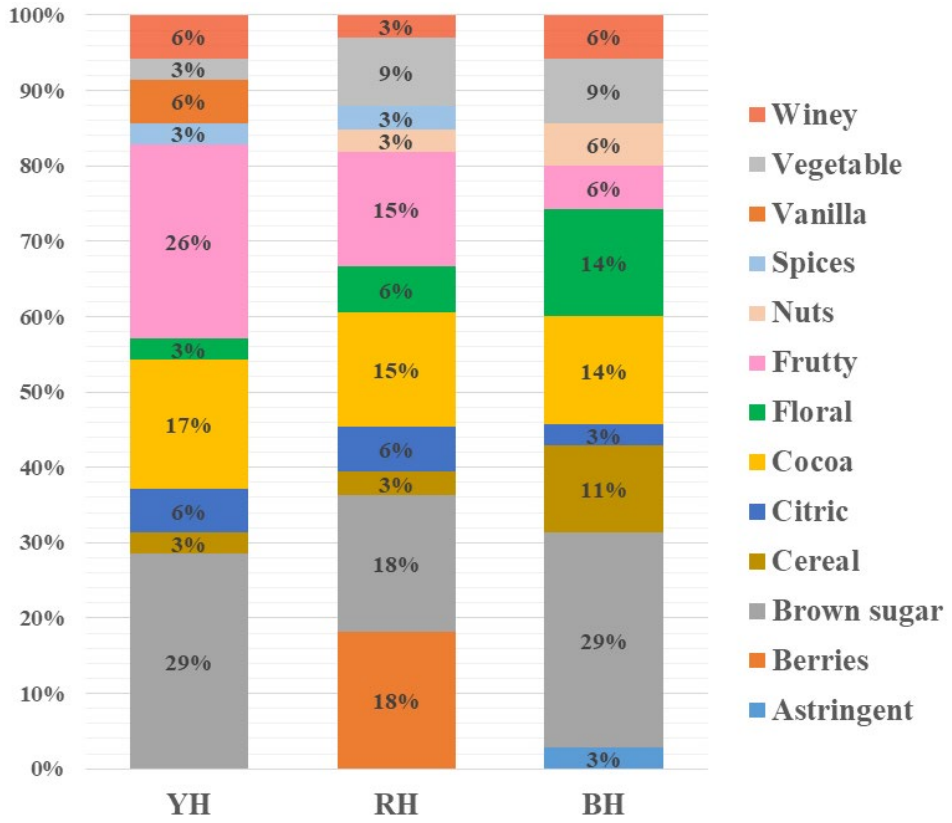

Figure 3: Relative frequencies for sensory descriptors reported in the HO process.

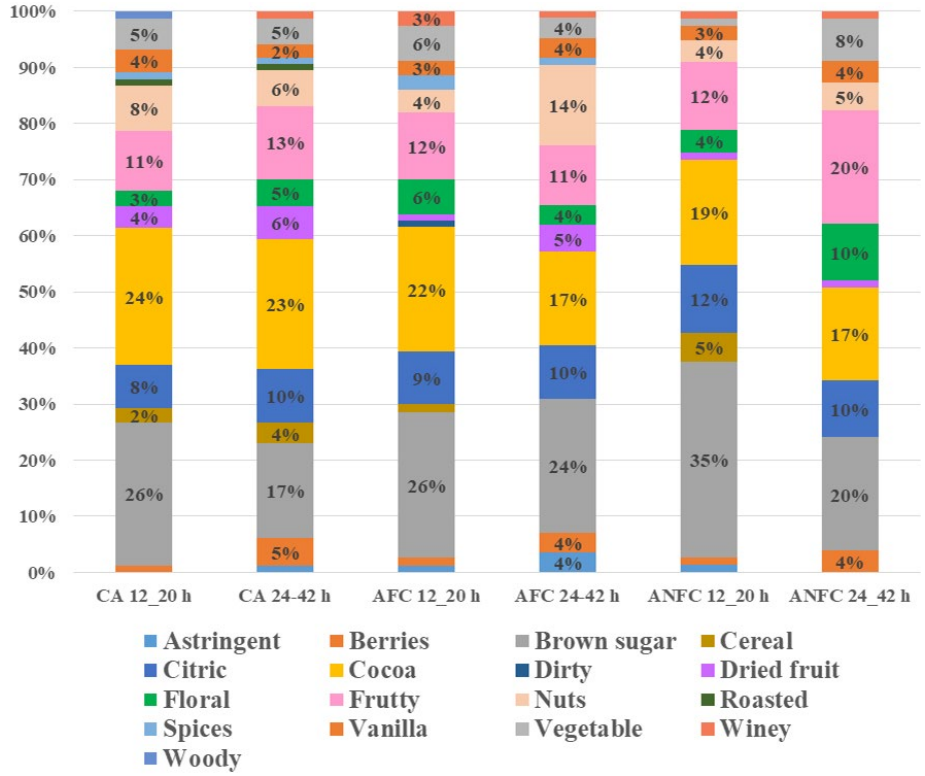

Figure 4: Relative frequencies for sensory descriptors - Wet processes.

Table 2: Volatile chemical composition of coffee samples subjected to different fermentation processes by HS-SPME/GC-MS.

No.	Compound	Similarity percentage	RI Kovats Cal.	RI Kovats Lit.	Sensory descriptors	Chemical group
1	Carbon dioxide	99%	<600	152	Odorless	Acids
2	Acetaldehyde	99%	<600	381,363,418	Pungent, Frutty	Aldehydes
3	Ethanol	98%	<600	444,440,649	Sweet, Alcohol	Alcohols
4	2-Propanone	98%	<600	475,503	Pungent, Sweetish	Ketones
5	Formic acid	96%	<600	512	Pungent, Sour	Acids
6	Methyl Acetate	97%	<600	517,531	Sweet, Frutty, Winey,	Esters
7	2,3-Butanedione	98%	<600	558,558	Buttery, Creamy, Caramellic	Ketones
8	2-Butanone	98%	<600	560,554	Ethereal, Frutty, Camphoreous	Ketones
9	Furan, 2-methyl-	97%	<600	595,604	Ethereal, Chocolate	Furans
10	Acetic acid	99%	624	617 - 710	Pungent, Sour, Vinegar-Like	Acids
11	2-Propanone 1-Hidroxy	98%	625	625,652	Pungent, Caramellic, Ethereal	Ketones
12	Butanal, 2-methyl-	96%	633	632,658,639	Cocoa , Frutty, Malty	Aldehydes
13	2,3-Pentanedione	98%	660	669,674,669	Fatty, Buttery	Ketones
14	Pyrazine	96%	702	702,704	Pungent, Sugar-Syrup, Nutty	Pyrazines
15	Pyridine	99%	713	717,717	Pungent, Nauseating, Sour, Fishy	Pyridines
16	3(2H)-Furanone, dihydro-2-methyl-	95%	759	760,776	Nutty, Astringent, Creamy	Furanones
17	Pyrazine, methyl-	98%	783	785,794,811	Popcorn, Nutty, Sweet, Pungent	Pyrazines
18	Furfural	99%	795	795,805,800	Bread, Sweet, Almond-Like, Woody	Furans
19	2-Furanmethanol	96%	828	827,827,834	Burnt, Caramellic, Brown	Furans
20	Cyclopent-4-ene-1,3-dione	90%	835	846	-	Ketones
21	Butyrolactone	97%	850	861,865,1282	Buttery, Oily, Caramel, Sweet	Lactones
22	2(5H)-Furanone	97%	852	863	Buttery	Furanones
23	2-Acetylfuran	97%	873	876,876,876	Balsamic, Tabaco, Cocoa	Furans
24	Pyrazine, 2,5-dimethyl-	97%	878	883,887,888	Nutty, Roasty, Cocoa-Like	Pyrazines
25	Pyrazine, ethyl-	96%	882	888,-,890	Nutty, Peanut Butter, Wood	Pyrazines
26	Pyrazine, 2,3-dimethyl-	97%	884	892,890,894	Nutty, Peanut, Green, Cocoa-Like	Pyrazines
27	5-Methylfurfural	97%	928	926,926,961	Almond, Break Like, Caramellic, Coffee-Like	Furans
28	Furfuryl Acetate	98%	962	966,967	Ethereal Floral, Spicy Herbal, Frutty, Banana	Furans
29	1H-Pyrrole-2-carboxaldehyde, 1-methyl-	96%	965	970	Cracker Popcorn	Pyrroles
30	Pyrazine, 2-ethyl-6-methyl-	95%	967	969,978,976	Frutty, Sweet, Roasted, Hazelnut-Like	Pyrazines
31	Pyrazine, 2-ethyl-5 methyl-	95%	971	973,978,976	Sweet, Sugar Syrup, Hazelnut	Pyrazines
32	Pyrazine, trimethyl-	95%	971	976,980,983	Roast, Potato, Nutty, Earthy, Cocoa	Pyrazines
33	Unknown 1	82%	974	984	Tarry, Mouldy, Earthy	NE
34	Acetylpyrazine	94%	982	987,987,1006	Roast, Sweet, Caramel, Nutty	Pyrazines
35	Corylon	95%	989	1000,1021	Caramel Strong	Ketones
36	Benzeneacetaldehyde	97%	1001	1002,1002	Pungent Green, Floral, Honey, Cocoa-Like	Aldehydes
37	2,2'-Bifuran	93%	1011	1047	Medicinal, Camphor	Furans
38	Unknown 2	87%	1020	1127	Nutty	NE

Continue...

Table 2: Continuation...

No.	Compound	Similarity percentage	RI Kovats Cal.	RI Kovats Lit.	Sensory descriptors	Chemical group
39	Ethanone, 1-(1H-pyrrol-2-yl)-	94%	1025	1026,1064,1028	Sweet, Pop Corn Like, Musty, Nutty, Coumarin-Like	Pyrroles
40	Furaneol	85%	1046	1046 - 1023	Caramel, Frutty	Furanones
41	Pyrazine, 3-ethyl-2,5-dimethyl-	95%	1055	1056 - 1062	Potato, Roast, Earthy	Pyrazines
42	Unknown 3	-	1059	-	-	NE
43	Maltol	95%	1075	1070 - 1070	Caramel, Frutty, Pineaple, Strawberry	Pyrone
44	2-Acetyl-6-methylpyrazine	89%	1080	1088	Roast, Cocoa, Pop corn	Pyrazines
45	2-Acetyl-3-methylpyrazine	91%	1084	1097	Nutty, Pop Corn	Pyrazines
46	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	85%	1107	1107	Caramel	Pyrone
47	Pyrazine, 2,3-diethyl-5-methyl-	80%	1129	1135	Nutty, Toasty, Potato	Pyrazines
48	Unknown 4	87%	1142	1106	Green, Nutty	NE
49	1H-Pyrrole, 1-(2-furanylmethyl)-	91%	1146	1148,1148	Hay, Pleasant Green, Cereal, Bready	Pyrroles
50	2-Furfuryl-5-methylfuran	80%	1153	1156,1156	Green - Cooked	Furans
51	Unknown 5	-	1156	-		NE
52	Methyl salicylate	87%	1165	1166,1169,1217	wintergreen, mint-Like	Esters
53	5-Hydroxymethylfurfural	87%	1177	1186,1267	Sweet, Herbaceous	Furans
54	Unknown 6	-	1185	-		NE
55	Unknown 7	-	1188	-		NE
56	Unknown 8	-	1210	-		NE
57	Unknown 9	-	1229	-		NE
58	Resorcinol, 2-acetyl-	91%	1240	-	Vanilla, Creamy	Phenols
59	5-Acetoxymethyl-2-furaldehyde	96%	1255	1304	Baked bread	Furans
60	Unknown 10	-	1265	-		NE
61	4-Vinylguaiacol	93%	1280	1282.1295	Spicy, Smoky, Clove-Like	Phenols
62	Unknown 11	-	1374	-		NE

RI kovats cal: Kovats retention index calculated in columnZB-1ms (60 m x 0.25 mm I.D. x 0.25 µm D.F.). IR Kovats lit: Kovats retention index of the literature (PubChem, NIST, FlavorNet). The sensory descriptors were extracted from the following references: (De Melo Pereira et al., 2019), (Caporaso et al., 2018), (Kıvançlı & Elmacı, 2016), (López-Galilea et al., 2006), (Akiyama et al., 2003). Compounds in bold have been listed as potent odorants.

Finding that the content of pyrazines increases and that of esters decreases when fermenting for 12 more hours (from 24 to 36 hours). Within the pyrazines family, methylpyrazine is the one with the greatest difference in concentration between the two times evaluated, establishing itself as the main marker compound of progress over time.

3.3.2 Semidry process – Honey (HO):

12 compounds and 3 chemical families were identified that presented statistically significant differences from the mean between the three types of HO evaluated (Table 5).

Within the 12 compounds, the one that presented the highest significant difference according to the ANOVA, being found in a different concentration in the three treatments, is 1-(2-furanilmethyl)-1H-Pyrrole and in turn this compound is the one that presented the highest positive linear fit with a correlation coefficient of 0.95. The linear fit was evaluated for the direction YH→RH→BH, (from the treatment with the highest exposure to solar radiation "YH" to the treatment with the lowest exposure "BH"). Therefore, this pyrrole is established as the main marker of the type of HO performed. Measuring the concentration of pyrroles as a group also makes it possible to differentiate between the three treatments. Because it is the chemical family with the highest significance and linear fit.

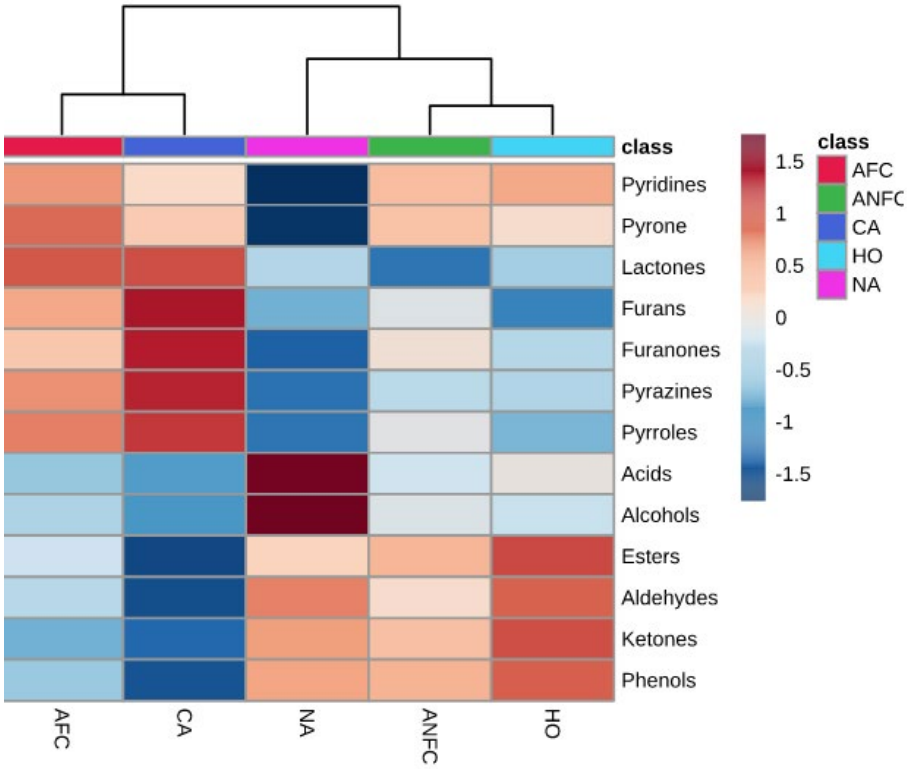

Figure 5: Comparison between fermentation processes based on chemical families using a heatmap with hierarchical clustering.

Table 3: Main compounds and chemical groups that allow differentiating between groups of processes

	VIP	p-Value	CA	AFC	ANFC	HO	NA
Compound							
Ethanone,_1-(1H-pyrrol-2-yl)-	1.69	6.41E-20	0.99^{a}	0.49^{b}	-0.38^{c}	-1.08^{d}	-1.71^{e}
4-Vinylguaiacol*	1.63	1.41E-11	-1.20^{a}	-0.32^{a}	0.52^{b}	1.18^{b}	1.03^{b}
Pyrazine,_2,3-dimethyl-*	1.50	2.63E-08	0.57^{a}	0.63^{a}	-0.33^{b}	-0.81^{b}	-1.66^{b}
Furan,_2-methyl-*	1.49	7.78E-10	-0.90^{a}	-0.41^{a}	0.61^{b}	0.96^{b}	1.10^{b}
2-Butanone*	1.48	2.49E-10	-0.75^{a}	-0.23^{a}	0.68^{b}	0.72^{b}	1.16^{b}
Acetylpyrazine*	1.47	1.00E-10	0.83^{a}	0.23^{a}	-0.55^{b}	-0.89^{b}	-1.13^{b}
2,2'-Bifuran*	1.35	8.02E-11	0.44^{a}	0.28^{a}	-0.36^{b}	-1.10^{b}	-0.86^{b}
2-Furanmethanol	1.30	1.41E-08	0.72^{a}	0.45^{a}	-0.40^{b}	-0.51^{b}	-1.56^{c}
Pyrazine,_2,5-dimethyl-	1.24	5.28E-08	0.66^{a}	0.47^{a}	-0.39^{b}	-0.43^{b}	-1.57^{c}
1H-Pyrrole,_1-(2-furanylmethyl)-	1.20	1.74E-07	0.59^{a}	0.61^{a}	-0.41^{b}	-0.84^{b}	-1.11^{b}
Chemical group							
Ketones	-	1.08E-17	-1.06^{a}	-0.47^{b}	0.62^{c}	1.27^{c}	0.83^{c}
Pyrroles*	-	3.82E-10	0.81^{a}	0.59^{a}	-0.36^{b}	-1.00^{b}	-1.52^{b}

Values of normalized relative concentration with different lowercase letters in the same row indicate statistical differences between processes. Variables without asterisk (*) present statistically significant differences from the mean and with asterisk, from the median.

Table 4: Volatile compounds with statistically significant differences in their normalized relative concentration between the treatments of the NA process.

	p-Value	NA_24 h	NA_36 h
Compound			
Pyrazine, methyl-	0.010552	-0.72	1.09
Pyrazine	0.016403	-0.82	0.10
Pyrazine 2.5-dimethyl-	0.018389	-2.26	-0.88
Acetylpyrazine	0.019863	-1.69	-0.71
Carbon dioxide	0.01999	1.13	-0.28
2-Propanone	0.022628	1.28	0.32
Methyl Acetate	0.023156	1.10	-0.64
Unknown 6	0.023406	0.15	1.36
Maltol	0.037656	-1.15	-2.02
Acetaldehyde	0.046012	1.14	0.55
Chemical group			
Pyrazines	0.0170	-1.88	-0.41
Esters	0.0248	1.06	-0.67

Fisher's LSD Multiple Comparison Test with a confidence level of 95%.

Table 5: Volatile compounds with statistically significant differences in their normalized relative concentration between the treatments of the HO process.

Compound	Pearson r (YH-RH-BH)	p-Value	YH	RH	BH
1H-Pyrrole, 1-(2-furanylmethyl)-	0.9507	0.0003817	-1.65^{a}	-0.70^{b}	-0.16^{c}
3(2H)-Furanone, dihydro-2-methyl-	-0.69293	0.00063475	0.18^{a}	0.49^{a}	-0.78^{b}
Unknown _8	-0.94975	0.00089636	0.51^{a}	0.09^{b}	-0.27^{c}
Unknown_11	0.66528	0.0012919	-0.10^{a}	-0.51^{a}	0.98^{b}
Cyclopent-4-ene-1,3-dione	-0.89299	0.0039399	0.13^{a}	-0.43^{b}	-0.67^{b}
Pyrazine, 2-ethyl-6-methyl-	0.86456	0.0041743	-0.89^{a}	-0.01^{b}	0.20^{b}
5-Hydroxymethylfurfural	-0.79913	0.0045504	1.33^{a}	-0.68^{b}	-0.72^{b}
Furfuryl Acetate	0.89056	0.0048525	-1.73^{a}	-0.83^{b}	-0.42^{b}
Pyrazine, ethyl-	-0.0048806	0.0048749	-1.52^{a}	0.18^{b}	-1.53^{a}
Ethanone, 1-(1H-pyrrol-2-yl)-	0.89892	0.0069474	-1.38^{a}	-1.09^{a}	-0.76^{b}
Furaneol	-0.80432	0.0072349	-0.18^{a}	-0.30^{a}	-1.89^{b}
Resorcinol, 2-acetyl-	0.53962	0.0098325	0.54^{a}	-0.08^{a}	1.53^{b}
Chemical group					
Pyrroles	0.93208	0.0023	-1.54^{a}	-1.05^{b}	-0.55^{c}
Pyrazines	0.69661	0.0154	-1.26^{a}	-0.04^{b}	-0.19^{b}
Phenols	0.44721	0.0254	1.11^{a}	0.83^{ab}	1.43^{b}

Mean values with different lowercase letters in the same row indicate statistical differences between treatments. (Fisher's LSD Multiple Comparison Test with a confidence level of 95%).

3.4 Wet processes

In the CA process, 11 compounds and 3 chemical families presented statistically significant differences between the two time blocks (Table 6). Lactones and pyridines are made up of a single compound, γ-butyrolactone and pyridine, respectively. Therefore, it is more precise to comment on the changes of the individual compounds, being then the ketones the only group that presented differences between the two time blocks.

Table 6: Volatile compounds with statistically significant differences in their normalized relative concentration between the pooled treatments of the CA process.

Compound	p-Value	CA_12-20 h	CA_24-42 h
5-Methylfurfural	0.0017	-0.39	0.86
y-Butyrolactone	0.0059	-0.07	1.03
Furaneol	0.0145	1.21	0.57
Unknown_8	0.0225	0.47	-1.00
Maltol	0.0399	-0.67	0.21
2-Propanone 1-Hidroxy	0.0403	-0.91	-1.45
Pyridine*	0.0017	-0.22	0.62
2-Furanmethanol*	0.0041	0.56	1.14
Corylon*	0.0152	0.09	0.80
Unkown_1*	0.0152	0.27	-0.27
Pyrazine, ethyl-*	0.0469	0.33	-0.15
Chemical group			
Lactones	0.0073	-0.05	1.03
Ketones*	0.0007	-0.67	-1.41
Pyridines*	0.0023	-0.22	0.62

Variables without asterisk (*) present statistically significant differences from the mean and with asterisk, from the median.

In the AFC process, 9 compounds and 2 chemical families presented statistically significant differences between the two time blocks (Table 7). The greatest regularity found was the decrease in pyrazines content with advancing fermentation time, where of the 9 compounds that presented statistically significant differences, 5 compounds are pyrazines.

In the ANFC process, 14 compounds presented statistically significant differences between the two time blocks (Table 8).

4 DISCUSSION

In the NA process, the high concentration of alcohols, specifically Ethanol (the only compound that constitutes this group) was related with the higher frequency of the descriptor “Winey” reported by the tasters in the sensory analysis. This process also has the lowest concentration of pyrones, furanones, pyrazines and the second lowest of furans (after the HO process). The low concentration of these families and especially of furans and pyrazines which are the two most abundant families in coffee (Angeloni et al., 2021), providing sweet/caramel/malt and nuts/roasted/earthy/cocoa notes respectively Sunarharum; Williams; Smyth,(2014), Caporaso et al. (2018), Akiyama et al. (2003), was reflected in the low frequency with which the tasters reported notes of brown sugar, nuts and cocoa in the sensory analysis. The low relative concentration of the most abundant families contributes to the non-masking of notes and the above average concentration for the esters and aldehydes families, which are related to fruity notes (Ludwig et al., 2014), it was reflected in the high frequency with which the tasters reported fruity notes, dried fruit and berries for this process.

Table 7: Volatile compounds with statistically significant differences in their normalized relative concentration between the pooled treatments of the AFC process.

Compound	p-Value	AFC_12-20 h	AFC_24-42 h
Pyrazine, methyl-	0.00016514	0.50	-0.54
Pyrazine, 2,5-dimethyl-	0.0010791	0.99	-0.04
Unknown_1	0.012904	0.37	-0.16
Formic acid	0.015433	-0.83	0.00
Pyrazine, trimethyl-*	0.0152	0.35	-0.20
2-Acetylfuran*	0.0193	0.27	-0.20
2-Acetyl-3-methylpyrazine	0.021326	0.66	0.20
Pyrazine, 2-ethyl-6-methyl-	0.021645	0.36	-0.24
Ethanol*	0.0379	-0.29	0.02
Chemical group			
Pyrazines	0.0011	0.70	-0.07
Alcohols*	0.0379	-0.28	0.02

Variables without asterisk (*) present statistically significant differences from the mean and with asterisk, from the median.

The higher frequency of the winey and fruity sensory descriptors, and the lower frequency of the brown sugar and cocoa notes of the NA process compared to the wet and semi-dry processes were consistent as one way of establishing coffees with consistent flavor profiles (Seninde; Chambers, 2020).

The HO and ANFC processes form a cluster, indicating that they are similar in chemical composition. For these processes, the frequency of brown sugar and cocoa notes increased with respect to the NA process, which is mainly related with the increase in the concentration of ketones, which have been described with creamy and caramel notes (López-Galilea et al., 2006) and from other families such as

pyrones, furanones and pyrazines. On the other hand, they are the processes with the highest concentration of esters, which was reflected in the report of floral notes and their relative concentration above the average for aldehydes was related with the fact that after the NA process they are the processes for which the fruity note was most frequently reported (De Melo Pereira et al., 2019).

Table 8: Volatile compounds with statistically significant differences in their normalized relative concentration between the pooled treatments of the ANFC process.

Compound	p-Value	ANFC_12-20 h	ANFC_24-42 h
2-Furanmethanol*	0.0005	0.16	-0.67
Cyclopent-4-ene-1,3-dione	0.0010527	-0.73	-0.01
Pyrazine, 2,5-dimethyl-	0.0057421	-0.73	-0.06
Furfural*	0.0118	-0.48	0.08
Formic acid	0.011987	0.12	0.78
Unknown_2	0.016386	-0.50	-0.12
Pyrazine, trimethyl-	0.018478	-0.81	-0.16
Maltol*	0.0305	0.77	0.14
Pyridine*	0.0469	0.66	-0.09
Unknown_10	0.029975	0.53	-0.25
Benzeneacetaldehyde*	0.0469	-0.71	-0.46
Ethanol	0.040671	0.00	0.40
Unknown_8	0.047664	-0.25	0.69
Furfuryl Acetate	0.049042	-0.05	-0.67

Variables without asterisk (*) present statistically significant differences from the mean and with asterisk, from the median.

The wet aerobic processes (CA-AFC) formed a cluster which distanced itself from a second, broader cluster formed by the NA, HO and ANFC processes. Indicating that under these two general clusters different aroma profiles are obtained in a coffee beverage. The wet aerobic processes were characterized by the highest concentration of pyrroles, pyrazines and furans, which is consistent with what was reported by Arruda et al. (2012), however in the study in question they report that the wet process has a higher concentration of phenols and aldehydes than the dry and semi-dry processes, which is not consistent with our results, this could be due to the fact that in this study they are minority families and their concentration may present greater variation.

Ethanone, 1-(1H-pyrrol-2-yl)- is the predictive variable with the greatest weight to differentiate between the different fermentation processes (VIP=1.69), which is confirmed by performing the ANOVA and the subsequent multiple range test, where it is evident that its concentration presents statistically significant differences among all processes. However, the 10 compounds mentioned must be considered in future studies to establish calibration models that allow differentiating between fermentation processes.

In the natural process treatments, there was a relationship between the change in the volatile profile and the frequencies of the sensory descriptors reported by the tasters, where for the fermentation time of 36 hours, a decrease in the frequency of the report of the floral and fruity notes was evidenced, which can be attributed to the decrease in esters as a group and to the compounds maltol and acetaldehyde.

On the other hand, for this fermentation time there was an increase in the frequency with which the brown sugar descriptor was reported, which is attributed to the increase in the content of pyrazines and mainly of pyrazine and acetylpyrazine, which have been recognized for providing sweetness (among other attributes). In such a way that when fermenting for 12 more hours (from 24 to 36 hours) there were statistically significant differences in the volatile profile, but not in the final score of the beverage.

For the honey process treatments, a relationship was found between the volatile profile and the descriptors reported by the tasters. The treatment with the highest concentration of pyrroles was the BH, which was well related to the higher frequency of the descriptor "Cereal" characteristic of this family and which is not desired in the beverage (De Melo Pereira et al., 2019) (Pereira et al., 2020). The fruit descriptor was in higher concentration in the YH and RH treatments compared to the BH. Which is related to the concentration of furaneol, one of the most relevant furanones due to its fruit descriptors to pineapple and strawberry (Dionísio et al., 2012). The higher frequency of the floral and vegetal descriptors reported by the tasters for BH and RH with respect to YH, was well related to the powerful odorants that present these attributes: furfuryl acetate and 2-ethyl-6-methyl-pyrazine (this pyrazine has been associated with floral notes, being an exception within its family as it does not share the traditional notes (López-Galilea et al., 2006), the two compounds are found in low concentration in the YH treatment and in different concentration and higher in RH and BH.

In the CA process, the highest content of ketones between 12 to 20 hours, which contribute to the brown sugar descriptor (López-Galilea et al., 2006) and especially 1-hydroxy-2-propanone that has been described as "Potent caramel". It was related with the greater frequency with which the tasters reported notes of brown sugar between 12 and 20 hours.

In the AFC process, the highest concentration on average for the first three times of 2-acetylfuran which has been described as balsamic/tobacco/cocoa, added to the highest content of pyrazines: 2,5-dimethylpyrazine and trimethylpyrazine, for which the cocoa descriptor has been reported, was reflected in the greater frequency with which the tasters reported the note to Cacao between 12 and 20

hours (Pereira et al., 2020). Formic acid has been described as "powerful bitter" and was found to tend to increase over time, being at a higher concentration between 24 to 42 hours and at the same time for this interval there was a tendency to increase the report of the astringent note by the tasters. Therefore, the effect that this volatile could have on the astringency of the beverage should be considered and studied in more detail.

For ANFC process, among the main findings, it was evidenced that contrary to the trend presented in the AFC process, the pyrazines: trimethylpyrazine and 2,5-dimethylpyrazine, are found in higher content between 24 and 42 hours. Being therefore the main compounds that indicate the change in aroma precursors when fermenting with or without air disposition. additionally, the greater frequency of the report of the vegetal and floral notes by the tasters in the interval of 24 to 42 hours, was well related with the higher content of Benzeneacetaldehyde which has been described with a green-powerful, floral and sweet smell (De Melo Pereira et al., 2019).

5 CONCLUSIONS

The NA process was the one that obtained the highest score, so it is under which better quality coffees are obtained and 5 treatments obtained a score higher than 85 points. It is considered "special-excellent coffee": CA_36h, YH, RH, NA_24h, NA_36h.

Within each fermentation process (between treatments) no differences were found from the final score of the sensory analysis, but differences were found from volatile profile. Ketones and pyrroles were the chemical families that allowed to differentiate to a greater extent between two groups of processes; the first the wet aerobic processes CA and AFC, and the second; ANFC, HO and NA processes.

Under the wet aerobic processes (CA-AFC) a different aroma profile is obtained from that obtained for the wet anaerobic process (ANFC) which is more similar to the HO process. Therefore, with the anaerobic process, a washed coffee could be obtained with an aromatic profile that weighs the profile of the wet processes with that of the processes semi-dry and dry.

In the NA proccess changes were identified between the treatments, finding that when fermenting for 12 more hours (from 24 hours to 36 hours), the floral and fruity character of the drink is reduced, which is related to the decrease of esters, maltol and acetaldehyde; and the descriptor to brown sugar increases, which was related to the increase in the content of pyrazines. In the HO process the family and the chemical compound that allow a statistically significant distinction between the three treatments are the pyrroles and 1-(2-furanylmethyl) -1H-Pyrrole, respectively, increasing their concentration in the direction YH→RH→BH. Among the main findings found in the evolution of the aromatic profile with the advance of time within the CA process, was the reduction in the content of pyrazines and ketones, which was related to the decrease in the description of brown sugar. Within the AFC process the main finding was the decrease in pyrazine content and in the ANFC process there were no significant changes in the volatile profile with time.

6 ACKNOWLEDGEMENTS

To the Cooperativa de Cafetaleros del Norte del Valle and its manager Julian Alberto Gonzalez Espinal, for supplying the coffee and allowing the development of the sensory analysis and to Martha Viviana Zuluaga Rojas (Caldas University) for the methodological support.

7 AUTHOR CONTRIBUTIONS

AFA wrote the manuscript, performed the experiment, collected and interpreted the results, AMVH supported the establishment of the experimental design, the statistical analysis and reviewed the manuscript, and GTO supervised the experiment and approved the final version of the work.

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