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Aroma profiles and cupping characteristics of coffee beans processed by semi-carbonic maceration process

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ABSTRACT

The goal is to improve the flavors of Arabica coffee by combining semi-carbonic maceration (SCM) process, with enzymatic process at various lengths and temperature. Five different variations of SCM processes were investigated: pulped natural process (control); at 15 °C for 10 days (SCM-15); at 20 °C for 10 days (SCM-20); with 0.01 g.L^{-1} pectinases A (polygalacturonase, pectin esterase and pectin lyase); at 20 °C for 5 days (SCM-PA); and with 0.03 g.L^{-1} pectinases B (polygalacturonase, pectin esterase, pectin lyase and ß-glucanase) at 20 °C for 5 days (SCM-PB). The chemical composition of intact mucilage and green bean coffee were analyzed. The environments created by using SCM fermentation process at 20 °C combined with pectinase A and pectinase B produced green bean coffee with higher content of acidity and reducing sugar. The volatile compounds of roasted coffee were examined by Gas Chromatography-Mass Spectrometry (GC-MS). Fifty-one volatile compounds belonging to 12 chemical classes were identified. The different total peak area, indicates that most volatile compounds, increases during SCM, and SCM process combined with enzymatic method. The Specialty Coffee Association of America; SCAA sensory analysis was performed by ten trained specialty coffee judges. The final product of coffee beans, which has been treated with SCM process at 15 and 20 °C have obtained the highest score in fragrance, flavor, aftertaste, body, balance and overall impression. While the enzyme treatment has attained the highest scores in acidity. This study demonstrates that the proposed process may be useful for the improvement of the cup quality.

Key words: Coffee; semi-carbonic maceration process; pectinase; gas chromatography-mass spectrometry; cup quality.

1 INTRODUCTION

The flavors of coffee are a direct result from the green beans' production process, from harvest to storage (Agresti et al., 2008). The coffee bean production has been divided into three categories: dry natural, pulped natural, and wet hulled or fully washed. These processes are applied to remove the mucilage and to reduce the moisture content of the beans (Poltronieri; Rossi, 2016). It may involve controlled fermentation, to obtain the desired characteristics, in terms of flavors, proteolysis and lipolysis, synthesis of volatiles such as aldehydes and ketones, free fatty acids and acidity in the end product. The different forms of coffee processing are rapidly evolving towards specialty coffee. A new trend is to study assisted fermentation by using microorganisms as starter strains, enzymes treatment (Poltronieri; Rossi, 2016), and recently carbonic process (Gudi, 2017).

Carbonic maceration is a winemaking technique, exploiting the adaptability of intact grape berries to an oxygen-deprived medium, enriched with carbon dioxide. This adaptation is reflected almost instantly inside each berry by the transition from a respiratory to fermentative anaerobic metabolism. The fermentation involves whole coffee cherries in a vacuum sealed environment filled with carbon dioxide. The Carbonic maceration promotes different biochemical interactions, as well as changes in sensorial, chemical and microbiological profile of coffee (Brioschi et al., 2021). The coffee cherries absorbed carbon dioxide gases and begin oxygen free and yeast free fermentation that breaks down the sugars and increase the acidity in the berries. A number of reactions take place inside the coffee cherries to create unique flavors in the cup. There are a number of ways to remove coffee cherries from its beans, such as the washed process or the natural process. The carbonic process produces more fruity flavors, such as strawberry, raspberry, cherry, banana and bubblegum which is the hallmark for this process. This method has been adopted in many countries around the globe and has highly increased the value of coffee beans on the international market (Gudi, 2017).

SCM is similar to carbonic maceration. It is a technique, which creates a carbon dioxide-concentrated atmosphere for a short amount of time inside the fermentation tank. There are two ways to create carbon dioxide gas. When the coffee cherries, at the bottom of the fermentation tank, are being squished or torn, causing nutrients to leak. Natural microorganisms from coffee cherries will feed on those leaking nutrients, creating alcohol and carbon dioxide. These gases will combine with the added carbon dioxide, creating a carbon dioxide-rich atmosphere inside the fermentation tank, leading to a complex biochemical reaction (Robinson, 2006).

Commercial enzymes widely used in different food industries are proteases, glucosidases, glucanases and pectinases (Hüfner; Haßelbeck, 2017). Pectinases are used in various types of fermentation. During coffee fermentation process pectinases removes mucilage layers from coffee beans and enhance flavors, creating the finest of coffee (Kubra, 2018). Traditionally,

fermentation of coffee is made with pectolytic microorganisms to remove the layer of mucilage from the coffee beans. With the same purpose in mind, commercial enzyme containing pectinase is sprayed onto the coffee beans. A cheaper alternative to the enzymatic treatment is to use, the filtrate of inoculated fermentations. The enzyme treatment significantly reduces fermentation time (Amorim; Amorim, 1977; Kashyap et al., 2001; Serrat et al., 2002; Silv et al., 2000).

The complete degradation of pectin requires assistance of several enzymatic activities such as: polygalacturonase (PG), pectinlyase, pectinesterase and acetylesterase, in order to break the complex molecules into smaller fragments. Polygalacturonase (homogalacturonan-hydrolase) is the hydrolytic depolymerization of the polygalacturonic acid chain which cleave single galacturonic acid units from either the middle or the end of the chain (exo-activity, exoPG, EC 3.2.1.67). Pectinlyase (EC 4.2.2.2 and 4.2.2.9) is the nonhydrolytic cleavage of the polygalacturonic acid chain. Pectinesterase (EC 3.1.1.11) is the hydrolytic cleavage of methanol from the D-galacturonic acid chain, causing drastic viscosity reduction in the liquid portion of the mash (Mojsov, 2013; Mojsov et al., 2015).

The experiment also included ß-glucanases-treated coffee beans, which was used to degrade polysaccharides (Kubra, 2018). They are primarily used to filter and maturewines, especially wines made from botrytised grapes, to hydrolyse the high molecular weight glucan produced by *Botrytis cinerea*. The ß-1,3-glucanase activity is essential because it cleaves glucan and releases glucose and gentobiose (Humbert-Goffard et al., 2004). The process of adding enzymesgreatly reduces the time required for the release of polysaccharides when aging wines over lees. Commercial products are frequently a mixed of different enzymes that considerably increases the polysaccharide content of wines (Palomero et al., 2007).

The coffee cherries/ The Final product of SCM processes had greater potential in terms of active-aroma attributes than the ones treated by dry process (Jitjaroen; Chaisri; Panjai, 2023). However, there are only a few scientific studies on coffee semi-carbonic maceration process. Therefore, the aim of this article was to study the effect of temperature and enzymatic treatment of the semi-carbonic maceration techniques on coffee. This would help to understand whether volatile compounds and sensorial aspects can be effectively used to tailored fermentation processes. This would help to develop a new technique for those who desire to step the world of specialty coffee.

2 MATERIAL AND METHODS

2.1 Fermentation conditions

In 2019 Arabica coffee (*Coffea arabica* L.) cherries has been harvested by hand from Chae-sorn district, Lampang province, (1,053 meters above sea level (18.54°N, 99.24°E)) in the northern part of Thailand. The experiments were carried out in sealed tanks, each one with 30 kg of pulped coffee beans (40% tank capacity). Carbon dioxide (99.9% purity) (PubChem CID:280) was injected to the tanks with 1 bar pressure, until its volume capacity reached 60% (Novatech; 1637, Australia). Five different SCM processes were studied: the control sample, using pulped natural process; at 15 °C for 10 days (SCM-15); at 20 °C for 10 days (SCM-20); mixed with 0.01 $g.L^{-1}$ pectinases A (containing polygalacturonase, pectin esterase and pectin lyase; Lallzyme c-max, Lallemand, Australia) at 20 °C for 5 days (SCM-PA); lastly mixed with 0.03 $g.L^{-1}$ pectinases B (containing polygalacturonase, pectin esterase, pectin lyase and ß-glucanase; Lallzyme mmx, Lallemand, Australia) at 20 °C for 5 days (SCM-PB). The coffee beans were then sun-dried until the moisture content reaches approximately 12% (Pereira, 2021).

2.2 Determination of chemical compounds in coffee

The mucilage of pulped coffee beans during fermentation has been controlled on a daily basis by examining the following parameters: pH by pH meter (Sartorius; Docu-pH, Germany), titratable acidity by titration (compared to citric acid)(modified from Reta et al., 2017), and reducing sugar by Lane and Enon method, and the dried green bean was monitored via moisture content until the desire moisture content approximately 12% of the coffee beans were achieved (Pereira, 2021), by using hot air oven method (Association of official analytical chemists, 2000). It was stored at 25 °C (Makri et al., 2011) for three months before being chemically analyzed. The cherries are then hulled and sorted to obtain the perfect green beans.

The volatile aroma compounds (VACs) were examined by using Solid-phase microextraction–gas chromatography mass spectrometry (SPME/GC-MS). One hundred grams of green bean coffee were roasted using a coffee roaster (Probat; Werke, Germany) at medium roasting profile, and at a constant temperature of 230 °C for 7 min to a level corresponding to Lightness (L) 30-31 (Jitjaroen; Chaisri; Panjai, 2023). Ground coffee (0.5 g), NaCl (0.5 g) (PubChem CID: 5234), and distilled water (5 ml) were put into a 20 ml vial and stirred at 300 ppm, 40 °C. The SPME fiber (50/30 µm DVB/Carboxen/ PDMS, Supelco, Inc. USA) was exposed to the headspace for 30 min. A GC-MS was performed on an Agilent gas chromatograph, model 7890A, coupled with an Agilent 5975C mass selective detector (Agilent technologies, USA). Analytes were separated on an HP-5MS capillary column (30 m x 0.25 mm, 0.25 µm) (J&W, USA), then increased to 230 °C for 5 min. The analysis was conducted with a carrier gas (Helium) (PubChem CID: 19357400) flow rate of 1.0 $ml.min^{-1}$ and the MS was operated in the electron-ionization (EI) mode at an ionization voltage of 70 eV. The detector temperature was set at 250 °C. The identification of volatile components was done by measuring and comparing the mass spectra with the Wiley and NBS data system library (Min; Kwon;

Park, 2015). The relative abundance of each volatile compound presented as peak area distribution found in the samples.

2.3 Sensory evaluation

The sensory analysis method of Specialty Coffee Association of America was performed by ten trained specialty coffee judges, for coffee brewing and roasting (Lingle, 2011; Specialty Coffee Association of America - SCA, 2015). The fermented green coffee was roasted to a level corresponding to Lightness (L) 30-31 by a roaster (Ikawa V3, England). Then fifty grams of green beans from each sample were roasted. For the sensory evaluation, three cups of each sample were tasted, with one session of sensory analysis for each repetition.

The evaluated sensory attributes were grouped into subjective and objective categories. The subjective attributes were fragrance/aroma, flavor, acidity, body, balance, aftertaste and overall impression. They were scored according to their quality on a scale of 6 to 10 points with 0.25 points increments. The objective category included uniformity, sweetness, and clean cup. The objective attributes were scored on a scale from 0 to 10 points, with 2 points awarded for each cup that presented satisfactory levels of each attribute. The sum of all these evaluated attributes is the total score (SCA, 2015). In addition to these evaluations, the panelists were also asked to describe the characteristic fragrance, aroma and flavor of each coffee.

2.4 Statistical analyses

The assay was carried out in a Completely Randomized Design for Physio-chemical composition, Balance Incomplete Block Design for sensory evaluation, with three replications and five conditions of fermentation. The results are expressed as means±standard deviation. Significant differences between treatment samples for all parameters were determined using a one-way ANOVA. A Duncan's Multiple Range Test was conducted to establish the differences among mean values. Statistical analyses were carried out using SPSS v.17 software package. The threshold p-value chosen for statistical significance was $p \leq 0.05$ (Ritthiruangdej, 2018).

3 RESULTS

3.1 Mucilage of pulped coffee characteristic

The experiments have discovered that the mucilage of coffee cherries contain the following properties, 3.59 pH, 6.83 $g.L^{-1}$ acidity, 11.0 °Brix total soluble solids, and 3.22% reducing sugar (Table 1).

Utilizing SCM fermentation process under different circumstances will affect the properties of post-fermentation coffee mucilage differently. Most acidity values tend to increase and reach its peak, with the highest acidity (10.29 $g.L^{-1}$) and found among the samples which were fermented at 20 °C (SCM-20). On the other hand, reducing sugar will mostly decrease, with the least amount of reducing sugar (2.72%) found in the sample fermented at 20 °C combined with pectinase B (SCM-PB).

3.2 Green bean characteristic

The different conditions, in which the SCM process was applied to, significantly influenced chemical composition of green bean coffee (Table 2). By comparing all samples with the control sample, it was apparent that every sample has seen an increase in pH value (5.66-5.83), a decrease in reducing sugar (4.55-4.90%), with the highest acidity found in samples SCM-PA (1.56 $g.L^{-1}$), and SCM-PB (1.82 $g.L^{-1}$). Results indicates that, by raising the fermentation temperature from 15 °C (SCM-15) to 20 °C, combined with the use of enzymes (SCM-PA and SCM-PB), the acidity value tends to increase as well. Furthermore, by increasing fermentation time, from 5 days (SCM-PA and SCM-PB) to 10 days (SCM-15 and SCM-20), the production of acidity reduces significantly.

Table 1: Chemical composition of the intact mucilage of coffee cherries after different conditions of semi-carbonic maceration process.

Treatment	Pulped natural process (Control)	SCM-15	SCM-20	SCM-PA	SCM-PB
pH	3.59 ± 0.01^{c}	3.93 ± 0.03^{a}	3.77 ± 0.02^{b}	3.82 ± 0.05^{b}	3.79 ± 0.04^{b}
Titratable acidity as citric acid, ($g.L^{-1}$)	6.83 ± 0.10^{b}	7.77 ± 0.76^{b}	10.29 ± 0.28^{a}	7.30 ± 0.52^{b}	6.45 ± 0.98^{b}
Total soluble solids (°Brix)	11.00 ± 0.04^{a}	8.50 ± 0.62^{b}	6.32 ± 0.51^{d}	7.45 ± 1.11^{c}	7.05 ± 0.68^{d}
Reducing sugar (%)	$3.22\pm0.08^{a,b}$	$3.35\pm0.28^{a,b}$	3.02 ± 0.85^{b}	3.81 ± 0.46^{a}	2.72 ± 0.37^{b}

mean±sd (n=3) ($p\leq0.05$), Different superscript letters with in the same row represent significant difference among cultivars.

Table 2: Chemical properties comparison of green bean coffee with different conditions of semi-carbonic maceration process

Treatment	Pulped natural process (Control)	SCM-15	SCM-20	SCM-PA	SCM-PB
pH	5.50 ± 0.02^{d}	5.83 ± 0.02^{a}	5.75 ± 0.06^{b}	5.66 ± 0.04^{c}	5.74 ± 0.02^{b}
Titratable acidity ($g.L^{-1}$, dm, as citric acid)	$1.68\pm0.04^{a,b}$	$1.34\pm0.10^{b,c}$	1.19 ± 0.36^{c}	$1.56\pm0.26^{a,b}$	1.82 ± 0.07^{a}
Reducing sugar (%, dm)	5.00 ± 0.02^{a}	4.55 ± 0.09^{b}	4.55 ± 0.29^{b}	5.00 ± 0.24^{a}	4.90 ± 0.01^{a}

mean±sd (n=3) ($p\leq0.05$), Different superscript letters with in the same row represent significant difference among cultivars.

3.3 Volatile compounds

There are five different types of SCM processes representing in the study. The peak area distribution of volatile compounds in roasted coffee samples were identified (Table 3, Figure 1 and 2). Fifty-one of which are described in the literatures as volatile aroma compounds in coffee. All of which have previously been identified in coffee. The compounds have been classified into 12 groups based on the chemical properties of the compounds (1 alcohols, 2 esters, 4 aldehydes, 3 ketones, 1 acid, 12 pyrazines, 6 pyrroles, 1 pyridine, 1 sulphide-containing compound, 12 furans, 5 phenols and 3 miscellaneous). Literature descriptions of their aroma profile varied from flowery, fruity, nutty, grassy, sour, herb, chocolate, medicinal smoky. It should be observed that the peak area distribution alone does not dictate which compound is the most important compounds present in coffee, but rather suggests compounds that are likely to have a large impact on the coffee aroma.

3.4 Sensory evaluation

This study shows that the sensory attributes of the semi-carbonic maceration samples displayed no significant difference to the control samples ($p>0.05$), with the exception of acidity, which varied from the control samples at every step of the process (Figure 1).

Individual analysis showed that the SCM processes at 15 and 20 °C have obtained the highest scores for fragrance, balance and overall impression (7.62-7.75, 7.37-7.56 and 7.56-7.62, respectively). The addition of enzymes attained the highest scores for acidity (7.06-7.25). The final score of all samples were within the same range (81.12-81.93). The panelists qualitatively described the Control treatment as lemon, nutty, earth, honey, vanilla, while the SCM-15 process as honey, lemon, caramel, and clove, the SCM-20 process has been described as orange, nutty, pepper, and jackfruit. At the same time, the SCM-PA has been characterized as pepper, cinnamon, and ripe fruit, the SCM-PB process has been given the attributes of lemon, tea, banana, honey and herb (Data not shown).

4 DISCUSSION

4.1 Mucilage of pulped coffee characteristic

The mucilage of coffee bean is a clear layer of gel in the middle of the coffee cherry (mesocarp), which consists of glucose, protein, starch, fiber, carbohydrates, oils and pectin (Arya; Rao, 2007; Haile; Kang, 2019). The coffee parchment is being wrapped by a layer of mucilage, naturally created once the coffee cherry has ripened (Belitz; Grosch; Schieberle, 2009). The compounds of coffee cherry mucilage vary depending on a number of factors, such as variety, and coffee cherry ripeness. The more mucilage, the better the resulting coffee tastes, and aromas will be (Meenakshi; Jagan, 2007).

Due to the dense phase CO_2, created during the SCM fermentation processes, the resulting chemical changes involve a decrease in cytoplasm pH, explosive cell rupture, modification of a cell's membrane, inactivation of key enzymes and extraction of intracellular substances (Gunes; Blum; Hotchkiss, 2005; Liu et al., 2014). The pH value is key in determining the end point of the fermentation process, in order to prevent coffee from over fermenting to the point in which the microorganisms involved during the fermentation create undesirable aroma substances, causing a drop in coffee qualities (Haile; Kang, 2019).

Nevertheless, samples which used SCM fermentation have all seen a decrease in reducing sugar. A previous research supports this set of data, explaining that complex changes occurred during the SCM fermentation process including converting a small amount of sugar into alcohol. For example, during the wine production, the SCM process would be able to produce 1.5–2% (v/v) alcohol, generally the wine would be fermented at 30-32 °C for 5-8 days, or at 15 °C for 20 days (Tesniere; Flanzy 2011). The process metabolites sugar into carbon dioxide gas as well as creates more acidic, alcoholic and volatile compounds (Haile; Kang, 2019). This will positively affect the cup quality of coffee.

4.2 Green bean characteristic

Depending on the environment, green bean coffee will be able to differently absorb acidic solutions from the fermentation liquid. The level of acidity also depends on temperature, time and process, which is consistent with the report of Reta (2017), who has discovered that the fermentation of coffee by using the Ohmic reactor method at 30 °C (increase in acidity) and 40 °C (decrease in acidity). However, with the increase of fermentation time, acidity decreases. By analyzing the acidity-time trend, it can be surmised that, microbial growth is happening inside this fermentation process. Through the use of available substrates combined with longer fermentation time, various compounds will be digested. Including acidic solutions inside coffee beans, which will be merged with the fermentation liquid resulting to a decrease in coffee acidity (Reta et al., 2017).

The acidic solutions decrease the total soluble solids of the mucilage from 11 to 6.32-8.5 °Brix as well as decrease reducing sugar in green bean coffee. The long fermentation time causes liquid to seep from the coffee pulp and pooled together at the bottom of the fermentation tank. Thus, parts of the fermented coffee beans are submerged under the liquid for longer period of time leading to a decrease in reducing sugar (4.50-4.55%) and other compounds of coffee beans.

Table 3: Peak area distribution related to volatile compounds in roasted coffee samples from different semi-carbonic maceration processes.

RT	Tentative compounds	CAS no.	Area[1] (Ab*S) x 10^6					Literature descriptions[2]
			Control	SCM-15	SCM-20	SCM-PA	SCM-PB	
Alcohol								
23.69	Furfuryl alcohol	98000	183.21±25.2	174.08±19.25	211.07±20.54	222.23±22.13	188.64±17.54	burnt
Ester								
18.65	2-Furfuryl formate	13493975	31.19±4.21	43.52±4.52	43.29±4.25	1.29±0.13	32.87±2.47	ethereal
19.91	Furfuryl acetate	623176	99.90±15.21	115.85±17.21	71.72±12.35	64.28±9.45	58.46±6.98	fruity
Aldehyde								
17.92	Furfural	98011	471.19±26.09	367.79±23.18	422.81±35	514.82±32.16	485.88±29.91	sweet, woody, almond, grass fragrant, baked, bread hay
19.72	Benzadehyde	100527	nd	9.44±0.56	nd	8.73±0.43	nd	sweet, oily, almond, cherry
21.24	5-Methylfurfural	620020	231.09±25.67	228.67±19.18	268.69±25.54	329.18±31.54	271.04±24.09	sweet, spicy, sweet, caramel, caramel, maple
24.78	2-Thiophenecarboxaldehyde	98033	nd	nd	12.28±0.82	2.26±0.09	11.67±0.1.14	not found
Ketone								
6.92	Isobutyl methyl ketone	108101	5.90±0.12	3.70±0.19	nd	nd	3.70±0.24	sharp, solvent, green, herbal, fruity, dairy, spice
19.47	2-Furyl acetone	6975606	7.83±0.92	8.76±0.87	7.72±0.64	4.70±0.36	8.79±0.95	caramel, fruity, spicy, radish
21.58	4-Cyclopentene-1,3-dione	930609	10.46±0.15	nd	3.67±0.13	2.30±0.09	6.43±0.16	not found
Acid								
27.43	3-Methyl crotonic acid	541479	7.33±0.54	8.70±0.69	10.84±1.28	15.79±1.67	12.38±1.33	green, phenolic, dairy
Pyrazine								
12.44	2-Methylpyrazine	109080	nd	49.78±6.80	4.83±0.93	0.29±0.05	119.64±16.5	nutty
13.89	2.5-Dimethylpyrazine	123320	39.11±7.95	30.22±6.43	44.80±8.47	42.69±8.16	40.25±6.52	grassy, fruity, chocolate, roasted nuts, earth, hazelnut, corn, roasted coffee, almonds,
14.02	2.6-Dimethylparazine	108509	50.57±7.02	26.44±4.23	35.21±5.26	17.19±2.48	37.27±6.21	chocolate, cocoa, roasted nuts, fried, cooked, hazelnut, corn, alcohol, potatoes, earthy
14.13	2-Ethylpyrazine	13925003	76.51±1.82	60.41±1.35	75.50±1.69	74.66±1.58	65.14±0.98	nutty, peanut, butter
14.59	2.3-Dimethypyrazine	5910894	10.54±1.12	6.13±0.77	9.84±0.89	8.53±0.73	8.83±0.96	nutty, roasted, popcorn, corn, moldy, alcohol, fruity
15.67	2-Ethyl-6-methylpyrazine	13925036	38.68±1.05	33.98±1.68	42.40±1.45	44.73±1.67	41.37±1.56	flowery, fruity, hazelnut
15.86	2-Ethyl-5-methyl pyrazine	13360640	27.67±0.89	23.68±0.73	29.12±1.17	30.14±0.95	28.42±0.83	coffee, nutty, roasted, grassy
16.23	2-Ethyl-3-methyl Pyrazine	15707230	9.83±1.03	8.32±1.12	10.12±1.17	15.13±1.56	12.68±1.30	nutty, peanut, corn, sweet, alcohol, passion fruit, papaya
16.32	2.3.5-Trimethypyrazine	14667551	11.39±1.51	11.45±1.67	11.98±1.35	12.14±1.41	11.24±1.37	toast, potato, beans, popcorn
17.03	2.6-Diethylparazine	13067271	nd	nd	4.47±0.13	5.33±0.25	nd	nutty, maize
17.42	2.5-Dimethyl-3-ethylpyrazine	13360651	12.45±0.70	13.77±0.98	16.50±1.06	8.87±1.20	13.53±0.21	earthy, roasted

Continue.

Table 3: Continuation.

RT	Tentative compounds	CAS no.	Area[1] (Ab*S) x 10^6					Literature descriptions[2]
			Control	SCM-15	SCM-20	SCM-PA	SCM-PB	
25.31	Pyrazinecarboxamide	98964	nd	4.91±0.57	7.18±0.84	5.47±0.63	6.91±0.74	not found
Pyrrole								
8.52	1-Methyl-1H-pyrrole	96548	6.65±1.05	7.89±1.31	9.65±1.86	0.35±0.06	5.56±0.29	smoky, woody, herbal
19.33	1H-pyrrole	109977	nd	7.80±0.98	10.93±1.43	0.34±0.046	25.51±2.95	musty, beefy, coffee, sweet, ethereal
22.71	1-Methyl-2-formylpyrrole	1192581	15.81±1.89	22.25±2.67	22.61±2.78	28.78±2.97	17.96±1.91	roasted
28.35	1-Furfurylpyrrole	1438944	5.34±0.23	11.50±0.58	12.37±0.96	15.79±1.82	12.65±1.08	vegetable
32.24	2-Acetylpyrrole	1072839	5.49±0.45	9.41±0.56	8.36±0.86	10.41±1.25	7.42±0.84	musty
33.62	2-Formylpyrrole	1003298	7.98±1.51	11.33±1.79	14.51±1.95	17.43±2.1	12.55±1.47	musty, beefy, coffee
Pyridine								
10.40	Pyridine	110861	nd	5.59±0.41	4.04±0.26	nd	5.41±0.59	sour, putrid, fishy, amine, bitter, roasted, dry peas, sweet, burnt
Sulphur								
18.42	Furfurylmethylsulphide	1438911	nd	5.66±0.36	8.73±0.49	0.19±0.02	nd	onion, garlic, sulfuraceous, vegetable, meaty
Furan								
4.79	2-Methylfuran	534225	8.71±0.09	10.15±0.12	nd	nd	nd	pungent, fruity, chocolate, burnt, ethereal acetate
5.51	2,5-Dimethylfuran	625865	nd	6.04±0.41	5.19±0.52	0.22±0.08	nd	ethereal
7.20	2-Vinylfuran	1487189	3.96±0.72	5.37±0.13	8.69±0.18	3.62±0.09	3.16±0.08	ethereal, rum, cocoa
8.61	2-Vinyl-5-methylfuran	10504139	7.04±0.98	5.96±0.85	11.18±1.64	5.48±0.82	nd	not find
10.97	Furfuryl methyl ether	13679464	5.06±0.12	5.46±0.63	6.54±0.78	0.23±0.03	6.84±0.45	coffee
17.92	Furfural	98011	471.19±35.67	367.79±29.37	422.81±39.65	514.82±46.89	485.88±49.56	sweet, woody, almond, fragrant, baked, bread
18.65	2-Furfuryl formate	13493975	31.19±4.08	43.52±4.89	43.29±3.94	1.29±0.04	32.87±2.12	ethereal
19.17	2-Acetyl furan	1192627	81.29±5.34	67.59±8.01	77.59±7.95	93.07±10.17	80.48±9.58	smoky, spicy
19.48	2-Butylfuran	4466244	7.83±0.35	8.76±0.49	8.55±0.62	9.14±0.85	6.96±0.34	fruity, winey, sweet, spicy
19.91	Furfuryl acetate	623176	99.90±7.34	115.85±12.80	71.72±6.80	64.28±7.60	58.46±6.70	fruity
21.24	5-Methylfurfural	620020	231.09±25.67	228.67±21.63	268.69±27.36	329.18±34.60	271.04±31.04	Sweet, spicy, sweet, caramel, caramel, maple
22.02	2-Furfurylfuran	1197406	nd	5.58±0.25	7.63±0.36	3.76±0.47	5.35±0.38	roasted
Phenol								
8.58	3-Methylphenol (m-Cresol)	108394	nd	nd	nd	0.28±0.13	8.11±0.46	dry, tarry, medicinal-leather
14.14	2-Methylphenol (o-Cresol)	95487	nd	51.89±5.80	81.41±8.71	1.26±0.11	113.06±9.54	band-aid, medicinal, smoky
14.14	4-methylphenol (p-Cresol)	106445	85.22±7.01	nd	nd	nd	nd	band-aid, phenol

Continue.

Table 3: Continuation.

RT	Tentative compounds	CAS no.	Area[1] (Ab*S) x 10^6					Literature descriptions[2]
			Control	SCM-15	SCM-20	SCM-PA	SCM-PB	
29.27	2-Methoxyphenol (Guaiacol)	90051	nd	7.76±0.68	7.32±0.79	3.90±0.48	nd	phenolic, burnt, smoky, corn, medicinal, camphor, peppery
37.59	2-Methoxy-4-vinylphenol(4-vinylguaiacol)	7786610	6.69±0.72	8.67±0.94	7.28±0.87	6.11±0.65	9.40±0.38	phenolic, clove
Miscellaneous								
4.79	4(5)-Methylimidazole	822366	nd	nd	1.86	nd	3.13±	burnt sugar
12.34	1-Vinylimidazole	1072635	84.46±4.80	5.75±0.38	68.24±3.91	53.35±1.98	29.68±1.67	slight amine
10.54	1,3-Cyclohexadiene	592574	nd	nd	nd	6.22±0.13	nd	not found

[1] mean value, n=3;

[2] are taken from the literature (Coste, 1992; Akiyama, 2005; Akiyama, 2007; Bassoli, 2019; Bressanello, 2017; Czerny, 2000; Guowan; Zheng; Zhao, 2011; Grosch, 2001; Kumazawa, 2006; Maeztu, 2001; Thammarat, 2018; The Good Sense Company, 2019; Weschenfelder, 2015; Yang, 2016)

nd= not detected.

Furthermore, results of the study show that, by combining SCM fermentation process with pectinase at 20 °C will result in green bean coffee with higher acidity and reducing sugar than any other samples. According to the results, pectinases A and B (pectin esterases, polygalacturonases, pectin lyases) tend to hydrolyzes pectic substances. Depending on its mode of action and substrate preference, the enzymes may increase pectic acid, and oligosaccharide (Whitaker, 1990). The advantage of adding enzymes into coffee fermentation process, is that they degrade the mucilage into free sugar molecules (Poltronieri; Rossi, 2016).

Accoding to (Dubourdieu et al., 1981; Palomero et al., 2007), a fermentation with ß-glucanases, similar to the SCM-PB sample, allows for a quicker breaking of the cell walls by hydrolyzing the ß-*O*-glycosidic linkages of the ß-glucan chains, leading to the release of glucose and oligosaccharides inside of wine, (Dubourdieu et al., 1981; Palomero et al., 2007), resulting in sweeter taste and more intense flavors (Rodriguez-Nogales et al., 2012). ß-Glucan is a widespread molecule- in the cell wall of many organisms, such as yeast, bacteria, fungi, algae and plants, acting as major structural elements of yeast cell wall. They form the deposits at the bottom of the wine fermentation tanks (Varelas et al., 2016; Cecchini et al., 2016). However, currently there are insufficient studies on the relationships between ß-glucanase and ß-glucan during the fermentation process of coffee beans. Future in-depth studies are needed.

According to the results of the study, pre-treatment of the coffee cherries using the SCM process, will positively impact the fermentation process. The process helps release mucus and soluble into the fermentation media. The longer the fermentation, the lower the pectin content of coffee. As fermentation progress, organisms will degrade coffee pectin into acid compounds. Fermented coffee is relatively easy to wash, and dries faster (Pimenta et al., 2009; Reta et al., 2017).

One of the most important aspects of SCM isthe different levels of impact, it has on the flavor profiles of brewed coffee. Acidity and sweetness in brewed coffee are major cupping characteristics aiming for emotional responses. The acidity of the final product shows an increase in sour notes, which correlated with the present of hydrogen ion concentration inside the coffee brews (Manzocco; Lagazio, 2009). Acidity impacted brewed coffee the most. At the same time, sweetness and fragrances are characteristics which has gain the most appeal among consumers (Cusielo et al., 2019).

4.3 Volatile compounds

The coffee production directly affects in the presence of volatile compounds in the coffee brew (Sunarharum; Williams; Smyth, 2014). In order to control the quality of coffee samples, they were roasted with medium roasting level (Franca, 2009), which has been described as suitable to develop the highest content of coffee aroma compounds (Schenker, 2002). Studies have shown variations in temperature and

enzyme within the semi-carbonic maceration process directly impacted the generated precursors in green bean. This leads to chemical reactions such as caramelization, Maillard chemistry, oxidation and pyrolysis, resulting in the development of colour and flavor in the final roasted coffee (Sunarharum; Williams; Smyth, 2014).

The furan typically has caramel-like odors since they are the result of sugar pyrolysis (Coffee Research Institute, 2001). The concentration of total furans and general compounds originated from chlorogenic acid degradation (Moon; Shibamoto, 2009). They are found to be the predominant group among the coffee aromatic compounds in this study. The different SCM processes increase the amount of furan as compared with the control. The SCM process influenced coffee samples with the largest concentration of 2-methylfuran, 2,5-dimethylfuran, 2-furfuryl formate and furfuryl acetate in the SCM-15 process, 2-vinylfuran, 2-vinyl-5-methyfuran, 2-furfuryl formate and 2-furfurylfuran in the SCM-20 process, furfural, 2-acetyl furan, 2-butylfuran and 5-methylfural in the SCM-PA process, and furfuryl methyl ether in the SCM-PB process.

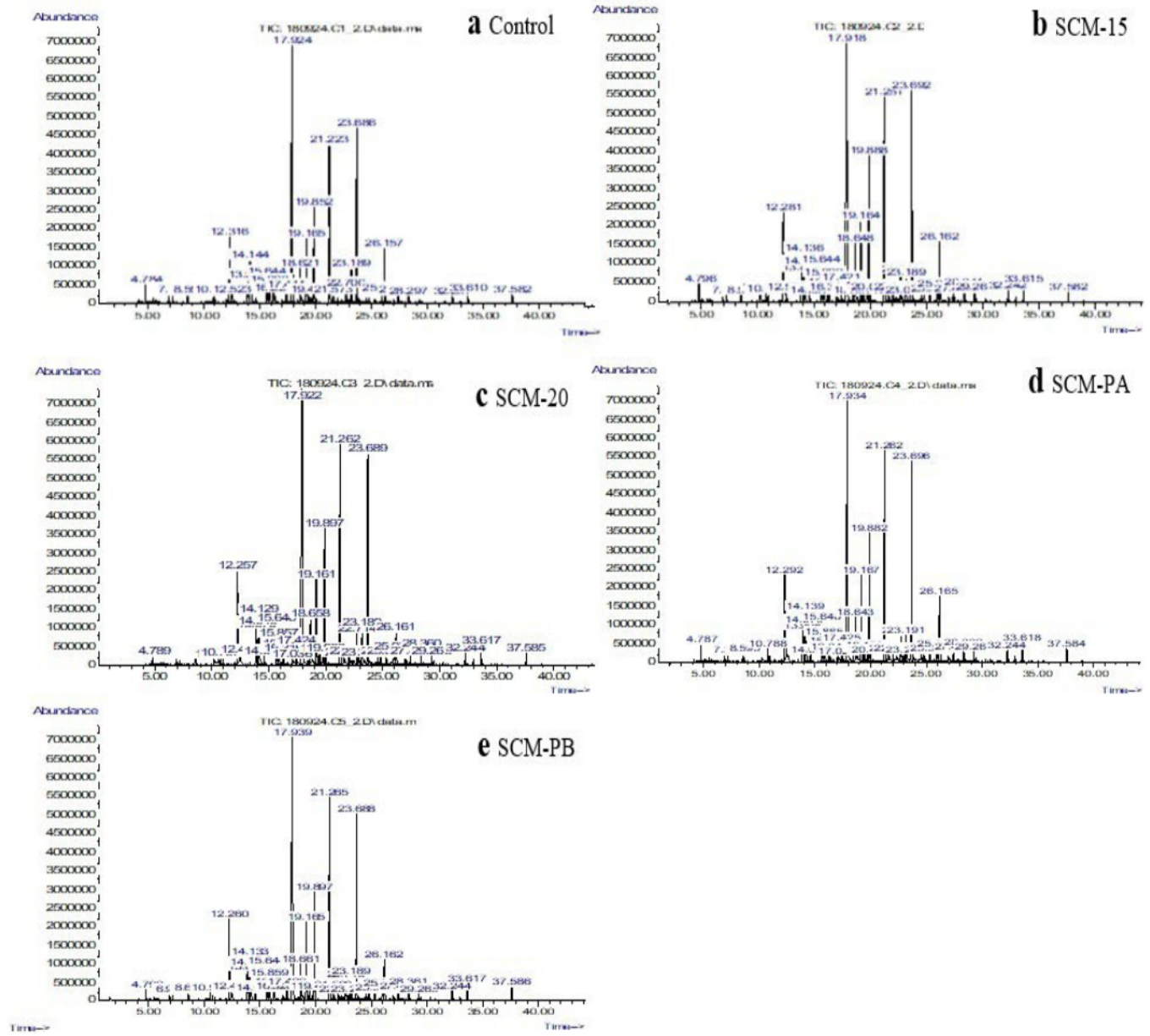

Figure 1: The representative of peak chromatogram analyzed by GC-MSD of the roasted coffee from the five different SCM processes: a) pulped natural process (control); b) at 15 °C for 10 days (SCM-15); c) at 20 °C for 10 days (SCM-20); d) with 0.01 g.L^{-1} pectinases A (polygalacturonase, pectin esterase and pectin lyase) at 20 °C for 5 days (SCM-PA); and e) with 0.03 g.L^{-1} pectinases B (polygalacturonase, pectin esterase, pectin lyase and ß-glucanase) at 20 °C for 5 days (SCM-PB).

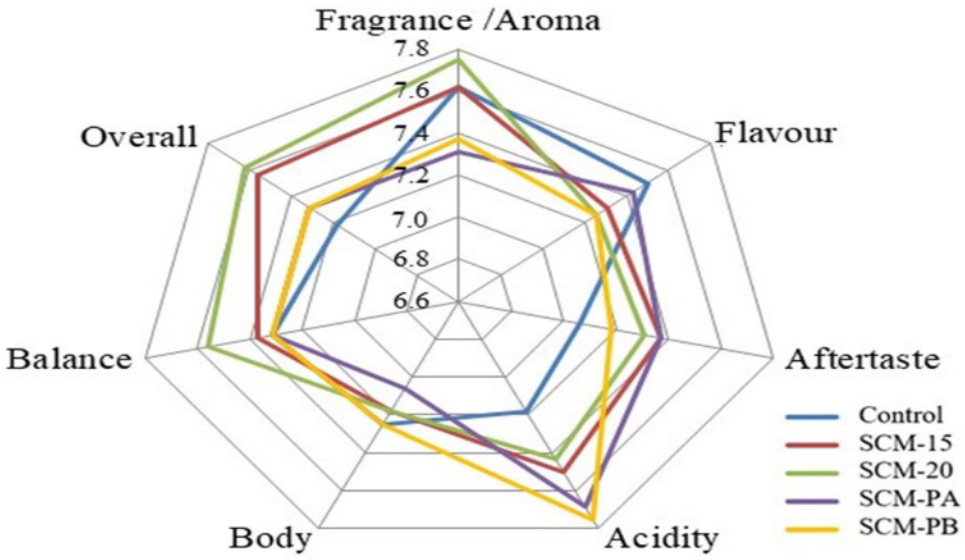

Figure 2: Spider graphical presentation of quantitative descriptive sensory analysis from the five different SCM processes: pulped natural process (control); at 15 °C for 10 days (SCM-15); at 20 °C for 10 days (SCM-20); with 0.01 g.L^{-1} pectinases A (polygalacturonase, pectin esterase and pectin lyase) at 20 °C for 5 days (SCM-PA); and with 0.03 g.L^{-1} pectinases B (polygalacturonase, pectin esterase, pectin lyase and ß-glucanase) at 20 °C for 5 days (SCM-PB).

The pyrazines are the second most abundant class of aromatic compounds and contribute to the roasted, walnut, cereal, cracker, or toast-like flavors in coffee (Coffee Research Institute, 2001). The different SCM processes increase most pyrazine amount as compared with the control, excepted for 2, 6-dimethylparazine, 2-ethylpyrazine and 2,3-dimethypyrazine. The high concentration was predominant for 2,5-dimethylpyrazine, 2,5-dimethyl-3-ethylpyrazine and pyrazinecarboxamide in the SCM-20 process, 2-ethyl-6-methylpyrazine, 2-ethyl-5-methyl pyrazine, 2-ethyl-3-methyl pyrazine, 2, 3, 5 trimethylpyrazine and 2,6 diethylpyrazine in the SCM-PA process, and 2-methylpyrazine in the SCM-PB process. These compounds originated from the Maillard reaction, which is strongly dependent on the roasting conditions and the green bean composition. As the thermal conditions were kept constant, any changes to the attributes of coffee beans is caused by the SCM process, which creates flavour precursors in the Maillard reaction, particularly amino acids and the reduction of sugar.

Thus, in a coffee bean where the limiting reactant is naturally found at higher concentration, higher levels of Maillard reaction products are expected after roasting (Caporasoa, 2018). It indicates that the precursors generated in the semi-carbonic maceration green bean samples influenced the generating of aromatic compounds.

The pyrroles are responsible for the sweet, caramel, and mushroom-like aromas in coffee (Coffee Research Institute, 2001). The most dominant were 1-methyl-1H-pyrole in the SCM-15 process, 1H-pyrrole in the SCM-PB, and 1-methyl-2-formylpyrrole, 1-furfurylpyrrole, 2-acetylpyrrole and 2-formylpyrrole in the SCM-PA process. These compounds are interdependence such as 1-methyl-1H pyrrole which contributes for smoky, woody and herbal might originate from different degrees of breakage of the original intermediate molecule. 1-Methyl-1H-pyrrole might also emerge when 3-ethylpyridine loses a methyl group (Caporasoa, 2018).

The contributing class of aldehydes is generally added a fruity and green aroma (Coffee Research Institute, 2001). They are considered as key component with a high aromatic impact for coffee. The most predominant compounds were furfural and 5-methylfurfuralin the SCM-PA process, and benzaldehydeinthe SCM-15 process, respectively. They are expected to be higher at a more intense roasting (Moon; Shibamoto, 2009). However, the coffee samples were roasted under the same time-temperature profile. It indicates that the levels of volatile precursors in the green bean have been influenced by the semi-carbonic maceration process, combined with specific enzymes. Furthermore, furfuryl alcohol also contributes for fruity prominence in the SCM-PA process. The furfurylmethylsulphide, which were found mostly in the SCM-20 process but not as much the SCM-PA and SCM-PB processes, contributes for onion, garlic, sulfuraceous and vegetable notes in coffee. The isobutyl methyl ketone, and 2-furyl acetone contributed to the sharp, green, herbal and fruity notes, which were most prominently found in the control sample, as well as in the samples of SCM-15 and SCM-PB. High concentrations of 3-methyl crotonic acid, which is responsible for green, phenolic and dairy notes, were found in the SCM-PA sample (The Good Sense Company, 2019).

Pyridine is a decomposition product of trigonelline, an alkaloid found in green beans (Yeretzian, 2002), which is related roasting time: high pyridine concentrations are produced at the initial stage of roasting, which is followed by continuous decrease at longer times (Baggenstoss et al., 2008). High levels of pyridine in roasted coffee can influence the overall coffee aroma negatively (Rizzi; Sanders, 1996). However, in this study the roasting time has been the same in every sample, thus differences in pyridine concentration have to be attributed to differences in the initial levels of green coffee bean aroma precursors. The samples of SCM-15, SCM-20 and SCM-PB produced pyridine which is responsible for the sour, putrid and fishy notes (Caporasoa, 2018). It indicates that using SCM process combined with pectinases (SCM-PA) can decrease pyridine concentration.

The volatile compounds mentioned above reacted interdependently during the roasting steps. The Maillard reaction initially resulted in Amadori products which further degrades into sugar fragmentation products, followed by reactions of dehydration, fragmentation, cyclisation and polymerization (van Boekel, 2006). The production of furfural arises from the rearrangement of Amadori products, in particular from deoxyosones, when the sugar is a pentose. It can also be produced by oxidation of furfuryl alcohol, where the furfuryl alcohol is a product of the reaction of (deoxy) ribose or sucrose with cysteine/methionine (Hertz-Schünemann, 2013). In the case of hexoses, hydroxymethylfurfural and 5-methylfurfural are likely to arise from this reaction. Pyrroles, pyranones and furanones are generated from sugar fragmentation of deoxyosones, followed by a reduction reaction. When other amino acids participate in the reaction, the Strecker reaction of aldehydes with amino ketones, followed by heterocyclization gives a series of aroma-active volatile compounds, such as pyridines, pyrazines, thiazoles, pyrroles, etc (Caporasoa, 2018).

Phenols are negative contributors (smoky and medicinal notes), to the aroma of brewed coffee. The three phenolic compounds in coffee, guaiacol, 4-ethylguaiacol and 4-vinylguaiacol, were clearly recognized as the sulfurous and strong odor notes, all of which are considered undesirable odors (Czerny; Mayer; Grosch, 1999). The 2, and 3-methylphenol and 4-vinylguaiacol were highly concentrated in the SCM-PB process, and guaiacol in the SCM-15 and SCM-20 processes. During the roasting process phenol compounds can exist in different forms, such as 4-vinylguaiacol, guaiacol and phenol (Dorfner, 2003). The degradation of 5-feruloylquinic acid has been reported as the origin of melanoidins and phenolic volatile compounds in coffee, due to hydrolysis, polymerization and oxidation. The 4-vinylguaiacol, guaiacol and phenol concentration is strictly interrelated, as ferulic acid degradation generates 4-vinylguaiacol. Degradation of chlorogenic acids causes the appearance of a wider series of neo-formation products (Kamiyama, 2015).

4.4 Sensory evaluation

The cup quality results, evaluated by the tasters, revealed the attributes of uniformity, clean cup and sweetness with top scores (score of 10.00) on all treatments which concur with a previous report (Rebeiro et al., 2017).

According to the SCAA, coffee beverages that receive scores above 80 can be considered specialty coffee. Specialty coffee, a specific term used to describe the end product of excellent and meticulous care to maintain the standards and excellence from start to finish (SCA, 2015). For these coffees, each step, from the location, to choosing coffee cherries, harvesting, fermentation, drying, storage, to roasting (Dinnella; Masi; Naes, 2013) and preparation of the beverage, are handled with the best possible expertise.

These results show the different biochemical interactions between the sensory and chemical profiles of coffee subjected to SCM process. Another factor which influenced the fermentation process is the amount and types of microbials present during the fermentation (Brioschi et al., 2021), which is related to the sensory profiles of the final product, such is observable during the wine fermentation process (Lai et al., 2019). The coffee from SCM processes had greater potential in terms of active-aroma attributes than the ones from dry process (Jitjaroen; Chaisri; Panjai, 2023). These complex and diverse factors are directly correlated to the increase in the sensory profiles of coffee.

5 CONCLUSIONS

The five different types of SCM influence the chemical composition of the green bean and roasted coffee. The relationship between temperature and enzymes promote differentamount of acidity andreducing sugar, resulting in a change in sensorial, and volatile compounds of coffee. The volatile profile of roasted coffee has been analyzed by SPME/GC-MS to understand variability in the SCM process. Fifty-one tentative compounds belonging to 12 chemical classes were identified. The results clearly indicate that the total peak area of volatile compounds was changed depending on temperature levels, and types of enzymes used in the SCM process.Positive compounds (such as pyrazine, furan, aldehyde) and negative compounds (such as pyrroles, pyridine and phenol) have a high impact on the overall coffee aroma, regardless of whether they will increase or decrease after going through the SCM process. Furthermore, comparative sensory evaluation indicates that the application of SCM process at 15 and 20 °C obtained the highest score for fragrance, flavor, aftertaste, body, balance and overall impression, while the enzyme treatment attained the highest scores for acidity. All treatments resulted in a final score of above 80, which qualified all of them as specialty coffee. A deeper study shall be developed in order to know the interactions of some other parameters, like coffee

variety, and roasting profile. Comparative sensory evaluation of semi-carbonic maceration coffee and the identification of aroma descriptor by olfactory method can be subjected to investigation in the future.

6 AUTHORS' CONTRIBUTION

WJ wrote the manuscript, supervised and performed the experiment, RK performed the experiment and conducted all statistical analyses, and LP performed the experiment, co-work the manuscript, reviewed and approved the final version of the work.

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