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Fermented natural coffee followed by pulping: Analysis of the initial sensory quality and after six months of storage

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ABSTRACT

In recent years, different methods of fermentation have emerged for coffee, with the intention of adding complexity to its flavor. To be able to clearly identify the information from sensory analysis, tools capable of detecting small differences are needed. One such tool is multiple factor analysis (MFA). Thus, the objective of this experiment was to evaluate the effects of fermentation time and storage on the quality of sensory attributes using MFA. The coffee (*Coffea arabica* L.) samples collected for the study were from the Serra da Mantiqueira region – Brazil. In the present study, two natural coffee fermentation methods were evaluated, one using natural coffee microbiota (NF) and the other using a starter culture (Y), along with different times of anaerobic fermentation (0, 24, 48, 72, and 96h), followed by the pulping of the samples without the use of water. Sensory analysis of fermented coffee samples was performed immediately after drying and after six months of storage in permeable packaging in a refrigerated environment. Thus, the experiment was conducted in an intirely randomized design with a 2 x 5 x 2 factorial scheme (2 fermentation treatments; 5 fermentation times; 2 storage times). The highest scores and the attributes described in higher quality coffees, such as sweetness, acidity, and aftertaste, were attributed to coffees fermented for 96 hours. Results indicated that inoculation of the yeast *Saccharomyces cerevisiae* CCMA 0543 was responsible for maintaining the sensory qualities of the coffee fermented for 96 hours after 6 months of storage.

Key words: Sensory attributes; anaerobic fermentation; starter culture; specialty coffee; post-harvest processing.

1 INTRODUCTION

Coffee quality involves many aspects, such as the growing environment, the genetic characteristics inherent to different varieties, and the technology used for post-harvest processing (Scholz et al., 2018). The classification of the beverage is a task that requires knowledge, sensory ability, practice, and good olfactory and gustatory memory. These are requirements to perceive both the defects and the qualitative aspects of the beverage accurately (Malta et al., 2008).

The quality of the coffee beverage is mainly linked to the physical and chemical characteristics of the green coffee bean. In addition to changes caused by roasting (Bastian et al., 2021) and by the beverage preparation method (Cordoba et al., 2020), coffee beverage quality can be determined by the combination of three factors: the growing environment, genetic composition, and crop management, all of which are relevant and interact with each other (Sanz-Uribe et al., 2017; Williams et al., 2022). It is extremely important for producers and professionals in the field who intend to produce high-quality coffees to understand these factors.

Among the various factors that affect coffee quality, the post-harvest step is of considerable importance because it alters or even adjusts the intrinsic quality of the bean, modifying characteristics such as acidity, body, and sweetness (Borém et al., 2014; Hameed et al., 2018; Sanz-Uribe et al., 2017).

In fermentation, which is a post-harvest process, the solubilization of complex polysaccharides (pectins) that are present in the coffee pulp and mucilage can facilitate drying. This fermentation allows the development of microorganisms that produce enzymes such as pectin lyases and polygalacturonases that are necessary for depolymerizing and hydrolyzing the pectin present in the mucilage (Siridevi et al., 2019; Suhaimi et al., 2021).

Recently, studies using yeasts as starter cultures to improve the sensory quality of coffee from dry and wet processing showed metabolic activity and effects on the final fraction of volatiles in roasted beans and on the sensory quality of the beverage (Evangelista et al., 2014; Mahingsapun at al., 2022; Martinez et al., 2019; Pereira et al., 2015).

Coffee storage is an extremely important step because, if properly performed, product quality can be ensured. This protects the producer from price fluctuations and depreciation in times of excess inventory. There are various studies on storage of specialty coffees, but none have analyzed the impact of induced fermentation over long periods on stored coffee (Abreu et al., 2019; Borém et al., 2013; Borém et al. 2019; Donovan; Foster; Salinas, 2019; Rendón; Salva; Bragagnolo, 2014).

Big advances in the area of coffee sensory analysis in recent decades have enabled the development of evaluation protocols, lexicons, and flavor and aroma wheels. Several forms of sensory analysis are used to elucidate the characteristics of the beverage. These include temporal dominance of sensations (TDS) analysis, quantitative descriptive analyses (the Specialty Coffee Association (SCA) Protocol and Cup of Excellence (CoE)), and others.

For the processing of sensory analysis data, multiple factor analysis (MFA) is an extension of principal component analysis (PCA); and it is able to collect more relevant information on the same set of observations. According to Abdi, Williams and Valentin (2013), other objectives of MFA are 1) to determine the relationship between the groups of variables, 2) to provide a set of measures common to all factors, and 3) to project each original dataset to analyze similarities and discrepancies.

The aim of the experiment conducted in this study was to analyze the effects of fermentation time, the inoculation of the starter culture (*Saccharomyces cerevisiae* CCMA 0543), and storage for 6 months in a cold chamber on the sensory quality of coffee samples using multiple factor analysis (MFA).

2 MATERIAL AND METHODS

2.1 Experimental setting

The material chosen for the study was *Coffea arabica* L., variety Mundo Novo (red color), harvested in the 2017-2018 season at the Condado Farm, in the municipality of Santa Rita do Sapucaí (Minas Gerais, Brazil) in the Serra da Mantiqueira region at an altitude of 890-960 m.

Mundo Novo coffee fruit was harvested with the highest possible percentage of ripe fruit and subsequently sent to a hydraulic separator for separation of beans with different densities. After that, the coffee fruit that sank was placed on a raised bed for manual separation and selection of only ripe fruit. The coffee fruit was then sent to the Laboratory for Processing of Agricultural Product (*Laboratório de Processamento de Produtos Agrícolas* - LPPA), Federal University of Lavras (Universidade Federal de Lavras - UFLA), in the city of Lavras, Minas Gerais, for the next steps.

2.2 Harvest and post-harvest procedures used for sample collection and preparation

The selected coffee fruit was divided into two lots. In one lot, spontaneous fermentation was performed using the indigenous microbiota of the site, and the other lot was inoculated with the starter culture *S. cerevisiae* CCMA 0543, followed by anaerobic fermentation of the fruit.

The starter culture *S. cerevisiae* CCMA 0543 was obtained from the Agricultural Microbiology Culture Collection (*Coleção de Culturas da Microbiologia Agrícola* - CCMA) of UFLA. The yeast, stored at -80 °C, was reactivated in tubes containing 9 mL of YEPG liquid medium [yeast extract 10 g/L (Merck, USA), bacteriological peptone 10 g/L (Hi Media, India), and glucose 20 g/L (Merck, USA), pH 3.5]. The culture was incubated at 28 °C for 48 hours, transferred to 90 mL of the same medium, and incubated at 28 °C and 150 rpm for 24 hours. The yeast cells were transferred to larger volumes of medium until a sufficient quantity of cells was obtained to inoculate 75 liters of coffee fruit, with approximately 1×10^7 cells/g.

The procedure for inoculation of the starter culture was as follows: the coffee fruit was placed in previously sanitized hermetically-sealed HDPE plastic cylindrical containers so that the fruit was directly inoculated with the cells, and homogenized so that the entire mass was in contact with the starter culture in solution.

Fifteen liters of samples from each sample were obtained at the following time intervals: 0, 24, 48, 72, and 96 hours. The fermentations were performed in 100-L hermetically-sealed cylindrical containers made of high-density polyethylene (HDPE) plastic, with virgin raw material.

After reaching the planned fermentation time, the coffee was pulped with a DCV-183 Advance Line, Penagos. Pulping was performed without water to maintain as much mucilage as possible. This resulted in about 5 to 6 liters of coffee beans, which were spread in a single thin layer on a raised bed exposed to the sun and left to dry until reaching the desired water content (10.8% to 11.5% wet basis).

After drying, the coffees were hulled in a Carmomaq DC1 machine and then the beans were separated on the size 16 sieves mentioned above. Right away separation of the coffee beans by size, the beans with defects (broken beans husk, etc.) were separated. For sensory analysis, 300 g of green coffee without defects was separated for each experimental sample. For sensory analysis, a portion of each lot was roasted and tasted.

2.3 Storage conditions

To evaluate the sensorial quality of the fermented coffees after storage, the samples were placed in a cold chamber with controlled conditions of temperature (10°C) and relative humidity (50%). The green coffee beans were packaged in double-layer paper and plastic packaging (permeable packaging). The samples remained in these conditions for six months of storage. Afterwards, a new sensory analysis was performed, with the same four judges, to evaluate the quality of the stored fermented coffees.

2.4 Sensory analysis

For the roasting process of the coffee samples used in this study, a temperature versus time roasting curve was developed.

This roasting curve was developed with the main objective of standardizing the process for all samples. The total roasting time parameter and final color of the beans parameter were defined following the guidelines of the SCA Protocols – Cupping Specialty Coffee (Lingle, 2011). Thus, the total roasting time was defined as 9 min, and the Agtron disc no. 60 (SCA Roast Color Classification System – Discs) was used to define the final color of the beans.

A total of 100 g of coffee beans from each sample was roasted in a Leogap type TP2 roaster with a standardized initial roasting temperature of 170 °C, a value defined based on pre-tests. Each sample represented a repetition. Sensory analysis was performed in 3 replicates.

The sensory analysis of the sample was performed at the LPPA by four trained graders with Q grader certification, following the cupping protocol developed by the SCA (Lingle, 2011). The characteristics observed and analyzed by the graders to reach the final score include the following attributes: fragrance/aroma, uniformity, sweetness, flavor, acidity, body, aftertaste, balance, and overall impression. The sensory analysis was approved by the Ethics Committee on Human Research of the Universidade Federal de Lavras – UFLA, under the following certificate number for ethical consideration (CAAE): 40641620.8.0000.5148

During the cupping, five cups were tasted per coffee sample, randomly arranged in two cupping rounds, in which 15 samples were tasted in each round. The graders received information about the coffee origin only after the cupping and evaluation of all the samples to prevent any biases that could affect the final evaluation.

The cupping sheet (Figure 1) was designed to contain three forms of descriptive sensory analysis. The first part was performed using a CATA (Check all that apply) questionnaire, containing descriptions that can be found in the SCA and World Coffee Research (WCR) flavor and aroma wheel. The aroma and flavor part were divided into sensory groups (inner part of the wheel), including fruity, floral, sweet, spices, cocoa/nutty, fermented, green/vegetative, and other. In each group, descriptive terms (outer part of the wheel) were added. The other characteristics were acidity (citric, malic, phosphoric, acetic, lactic, and tartaric) and body (creamy, viscous, oily, and silky).

The sheets were filled out by graders who indicated whether the defined attributes matched the characteristics perceived during sample cupping. There were no restrictions on the number of descriptive terms that could be selected.

The second part of the form included an intensity scale. This evaluation was performed simultaneously with the cupping, where the graders used a nonparametric scale ranging from 0-10. The intensities of sweetness, acidity, body, bitterness, uniformity, and aftertaste were evaluated.

The third and last part of the sheet was intended for the grader to give a final score to the sample being evaluated. This score was based on the scoring criteria of the SCA cupping protocol (SCA, 2015). Before the cupping began, training and calibration were performed with the graders so that they could become accustomed to using the cupping sheet.

2.5 Statistical analyses

The experimental design used was completely randomized (CRD) with a 2 × 5 × 2 factorial arrangements [two treatments - natural coffee microbiota for fermentation (NF) and yeast starter culture (Y); five fermentation times - 0, 24, 48, 72, and 96h; two storage times - without storage and stored for six months], with three replications.

After the cupping, the data on the cupping sheets were organized in an Excel file. The data related to the CATA questionnaire were adjusted to the frequency (%) of how many times they were cited throughout the cupping, and all characteristics with less than 20% frequency were discarded. The intensity scale and SCA scores were obtained by averaging the scores from all graders.

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Sample	CATA									SCALE OF INTENSITY							
	AROMA			FLAVOR			ACIDITY			SWEETNESS	ACIDITY	BODY	ASTRINGENCY	BITTERNESS	AFTERTASTE	FERMENTED	PAST CROP
	FRUITY	SPICES	HERBAL	FRUTADO	ESPECIARIAS	HERBÁCEO				10	10	10	10	10	10	10	10
	Red fuit	Pepper	Straw	Red fuit	Pepper	Straw				9	9	9	9	9	9	9	9
	Yellow fruit	Cinnamon	Grassy	Yellow fruit	Cinnamon	Grassy				8	8	8	8	8	8	8	8
	Citric fruit	Anise	Cucumber	Citric fruit	Anise	Cucumber				7	7	7	7	7	7	7	7
	Tropical fruit	Nutmeg	Pea	Tropical fruit	Nutmeg	Pea		Citric		6	6	6	6	6	6	6	6
	Dry fruit	Clove	Herbs	Dry fruit	Clove	Herbs		Phosphoric		5	5	5	5	5	5	5	5
	FLORAL	Tabacco	DEFECTS	FLORAL	Tabacco	DEFECTS		Malic		4	4	4	4	4	4	4	4
	Jasmim	CHOCOLATE	Rubber	Jasmim	CHOCOLATE	Rubber		Acetic		3	3	3	3	3	3	3	3
	Coffee flower	Milk chocolate	Medicinal	Coffee flower	Milk chocolate	Medicinal		Latic		2	2	2	2	2	2	2	2
Roast	Orange flower	Dark chocolate	Earthy	Orange flower	Dark chocolate	Earthy		Tartaric		1	1	1	1	1	1	1	1
	Black tea	CHESNUT	Wooden	Black tea	CHESNUT	Wooden				0	0	0	0	0	0	0	0
	Chamomile	Peanut	Musty	Chamomile	Peanut	Musty											
	SWEET	Hazelnut	Cardboard	SWEET	Hazelnut	Cardboard	BODY										
	Brown sugar	Almounds	Phenolic	Brown sugar	Almounds	Phenolic											
	Baunilha	Walnut	Smoked	Baunilha	Walnut	Smoked											
	Honey	FERMENTED	Saur	Honey	FERMENTED	Saur		Creamy									
	Caramel	Winy	Acetic fermentation	Caramel	Winy	Acetic fermentation		Viscous									
	Molasses	Alcoholic	Rotten	Molasses	Alcoholic	Rotten		Smooth									
	Sugar cane	Coffee pulp	Past crop	Sugar cane	Coffee pulp	Past crop		Liqueur									
	Sucrose	Lacteal	Rancid	Sucrose	Lacteal	Rancid		Watery									

Name:	OBS:	FINAL SCORE:

Figure 1: The cupping sheet.

Multiple factor analysis (MFA) was used to analyze the CATA questionnaire data from the cupping sheet in the R software, version 4.1.0 (R Core Team, 2021). To analyze the data related to the intensity scale and final score, analysis of variance (ANOVA) and the Scott-Knott test were applied with the Sisvar software (Ferreira, 2011). PCA was also used to analyze the intensity scale data, using the Chemoface program, version 1.4 (Nunes et al., 2012).

3 RESULTS

The analysis of variance did not show significance for the inoculation factor and showed significance for the fermentation and storage time factors. That is, variations in attribute intensities and final score were identified due to fermentation time and storage, regardless of whether yeast was inoculated or not for the fermentation process. Thus, due to the non-significance of the inoculation factor, the variation of the intensities of the sensorial attributes and final score due to the fermentation time and storage was performed by analyzing the averages presented by the coffees with natural fermentation (NF) and with starter culture (Y) and the results of the ANOVA with the Scott-Knott test ($P \leq 0.05$) are presented in Table 1.

The coffees tasted in the first storage period showed significantly greater intensity for the sweetness, acidity, body, and aftertaste attributes with 72 and 96 hours of fermentation compared to the shorter anaerobic fermentation times studied (Table 1). Yet, the characteristics that disqualify the drink, such as bitterness and astringency, did not change.

After 6 months of cold storage, the only positive attributes that remained significantly higher were acidity and aftertaste for treatments with 72h of fermentation, regardless of yeast inoculation. The coffees fermented for 96 hours showed a significant reduction in sweetness, acidity, body, and aftertaste; in addition, they showed a significant increase in astringency and bitterness. Consequently, the final score of these samples decreased.

The analysis of the effect of storage on the intensity of the attributes and final score of the coffees fermented for 96 hours is presented in Table 2.

Table 1: Mean intensity scale score and final score according to fermentation time and storage of the samples.

Fermentation time (hours)	Sweetness	Acidity	Body	Astringency	Bitterness	Aftertaste	Score
0	6.83^{b}	6.43^{b}	6.39^{b}	0.46^{a}	0.34^{a}	5.42^{b}	82.77^{b}
24	6.64^{b}	6.19^{b}	5.79^{b}	0.44^{a}	0.34^{a}	5.51^{b}	82.60^{b}
48	7.28^{a}	6.87^{b}	6.42^{b}	0.24^{a}	0.14^{a}	5.97^{b}	83.54^{b}
72	7.70^{a}	7.77^{a}	7.32^{a}	0.22^{a}	0.04^{a}	7.18^{a}	84.98^{a}
96	7.73^{a}	7.31^{a}	7.67^{a}	0.21^{a}	0.16^{a}	7.09^{a}	84.71^{a}
0 (stored)	6.62^{a}	5.60^{b}	6.06^{a}	0.33^{b}	0.35^{b}	5.51^{b}	83.02^{b}
24 (stored)	6.79^{a}	6.50^{b}	6.52^{a}	0.25^{b}	0.12^{b}	5.98^{b}	83.62^{b}
48 (stored)	6.43^{a}	6.00^{b}	6.16^{a}	0.17^{b}	0.25^{b}	5.93^{b}	83.27^{b}
72 (stored)	6.96^{a}	7.04^{a}	6.69^{a}	0.10^{b}	0.19^{b}	6.49^{a}	85.23^{a}
96 (stored)	6.04^{a}	6.29^{b}	6.62^{a}	0.82^{a}	1.04^{a}	5.34^{b}	83.73^{a}

*Means followed by different letters in the columns differ significantly according to the Scott-Knot test ($P \leq 0.05$).

Table 2: Effect of 6months of storage on the intensity of the attributes and final scores of the coffees fermented for 96 hours with and without yeast.

Fermentation time	Sweetness	Acidity	Body	Astringency	Bitterness	Aftertaste	Score
96 hNF	7.83^{a}	7.50^{a}	7.67^{a}	0.16^{b}	0.17^{b}	7.11^{a}	85.08^{a}
96 hNFS	5.71^{b}	5.87^{b}	6.54^{b}	1.04^{a}	1.62^{a}	4.72^{b}	82.50^{b}
96 hY	7.62^{a}	7.13^{a}	7.67^{a}	0.25^{b}	0.16^{b}	7.08^{a}	84.33^{a}
96 hYS	6.37^{b}	6.70^{a}	6.71^{a}	0.58^{b}	0.46^{b}	5.96^{a}	84.96^{a}

* Means followed by different letters in the columns differ significantly according to the Scott-Knot test ($P \leq 0.05$).NF =Natural/spontaneous fermentation from the indigenous microbiota with sensory analysis soon after drying; NFS = natural/spontaneous fermentation from indigenous microbiota with sensory analysis after storage; Y = fermentation with *Saccharomyces cerevisiae* with sensory analysis soon after drying; YS = fermentation with *Saccharomyces cerevisiae* with sensory analysis after storage.

Treatment with the *S. cerevisiae* CCMA 0543 yeast showed that in addition to the score remaining similar after 6 months, the positive attributes, such as acidity, body, and aftertaste, remained significantly high. At the same time, the bitterness and astringency attributes did not show a significant difference due to storage. In contrast, coffee fermented with indigenous microbiota for 96 hours showed a reduction in positive attributes (sweetness, acidity, body, and aftertaste) after 6 months of storage in a refrigerated environment, with a consequent reduction in the final score. In addition, there was a significant increase in astringency and bitterness, thus explaining the reduction in the final score.

To better understand the interaction between the fermentation microbiota, anaerobic fermentation time, and cold chamber storage on the intensities of the sensory attributes, a principal component analysis (PCA) was performed, presented in Figure 2.

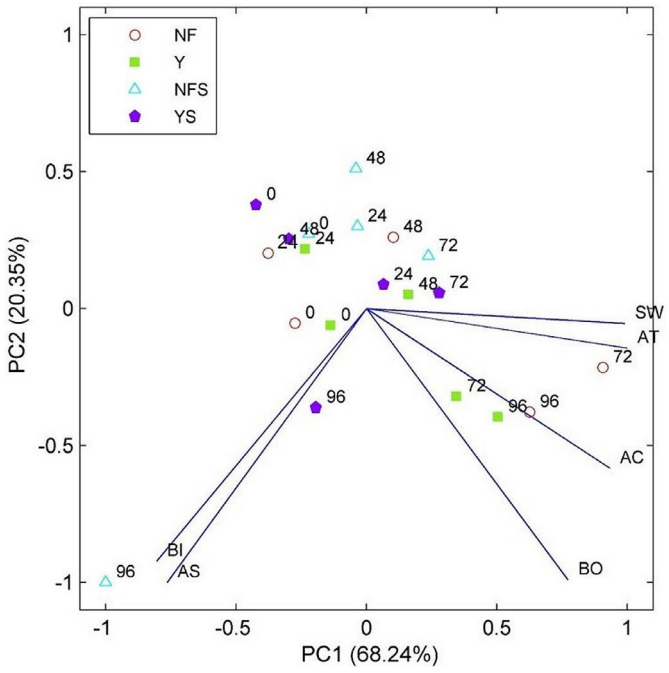

Figure 2: PCA plot of intensities.

Legend: NF =natural/spontaneous fermentation from the indigenous microbiota with sensory analysis soon after drying; NFS = natural/spontaneous fermentation from indigenous microbiota with sensory analysis after storage; Y = fermentation with *Saccharomyces cerevisiae* with sensory analysis soon after drying; YS = fermentation with *Saccharomyces cerevisiae* with sensory analysis after storage; BI = bitterness; AS = astringency; BO = body; AC = acidity; AT = aftertaste; SW = sweetness.

There is a high correlation between sample groups and attributes such as sweetness and aftertaste, responsible for the horizontal spacing of the centroids in the plot, and bitterness and astringency, responsible for the vertical separation of the treatments. Proximity to these vectors provided a clear separation of the samples into 3 groups. The first group is formed by samples inoculated and non-inoculated for 0, 24, and 48 hours, and samples fermented for 72 hours that were stored. The second group is formed by samples fermented both naturally and with the use of starter cultures for 72 and 96 hours, with greater intensity of sweetness, body, acidity, and aftertaste. Finally, group 3 had the greatest relationship with astringency and bitterness, represented by samples fermented for 96 hours and stored for 6 months. This separation shows a positive effect of fermentation times of 72 and 96 hours for coffee before storage. However, it identifies important losses for coffee fermented for 96 hours when stored for 6 months, and the intensity of these losses depends on whether or not yeast is inoculated. Thus, it can be inferred that the inoculation of the *S. cerevisiae* CCMA 0543 yeast better preserved the intensity of the sensory attributes of coffee fermented for 96 hours during storage.

The final scores were evaluated using multiple factor analysis (MFA), presented in Figure 2. The inertia plot of the final scores (Figure 3A) showed the formation of a group of treatments from which only the stored samples from the treatment without yeast inoculation were distanced, thus showing that after 6 months, the scores of this treatment differed from the group formed.

The correspondence graph of the analysis of fermentation times and the analysis of treatments with and without yeast inoculation (Figure 3B) showed that the scores of the treatments fermented for 0, 24, and 48 hours did not differ, only those for 72 and 96 hours. However, the addition of yeast for the 96 hours of fermentation treatment had an effect on maintaining the quality of the stored coffee. The formation of the vector in relation to the centroid of the time of 96 hours shows that the treatment without yeast (that was stored) exhibited a large distance from and opposite to the vector of the treatment with yeast, indicating that for that fermentation time, the samples exhibited antagonistic behavior, i.e., everything that happens positively in one happens negatively in the other. The final score of the 96-hour treatment without yeast after 6 months of storage decreased from 85.08 to 82.50 points on the SCA scale.

The evaluation of aroma descriptors was performed using multiple factor analysis (MFA), and the results are shown in Figure 4. The aroma characteristics, according to the results obtained in the sensory analysis, may explain the interaction of the fermentation time, yeast inoculation, and the types of aromatic notes in two-dimensional graphs, since the proportion of variation explained by the first two components was equal to 76.34%.

Regarding the aroma attributes, in the inertia plot of the groups (Figure 4A), there was grouping of all treatments, indicating that no treatment altered the aromatic characteristics of the samples.

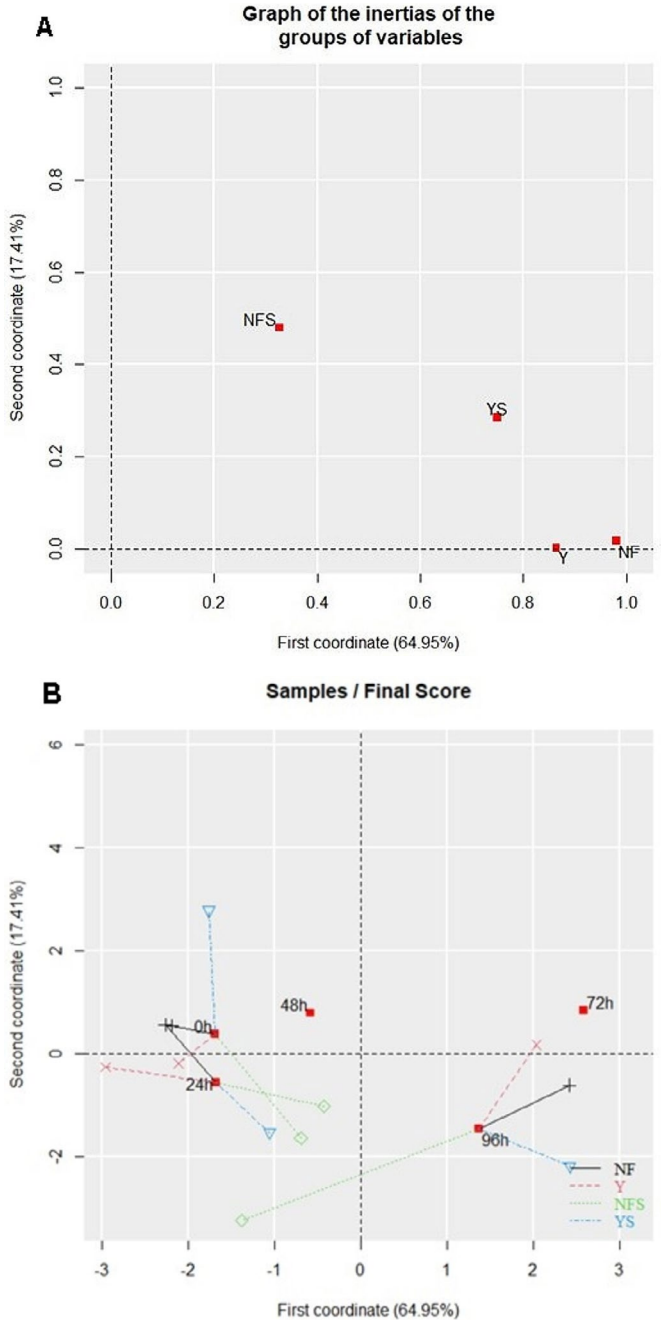

Figure 3: Multiple Factor Analysis (MFA) of the final score of the sensory analysis of fermented natural coffee followed by pulping and storage for 6 months. (A) Inertia of the groups and (B) correspondence graph.

Legend: NF =natural/spontaneous fermentation from the indigenous microbiota with sensory analysis soon after drying; NFS = natural/spontaneous fermentation from indigenous microbiota with sensory analysis after storage; Y = fermentation with *Saccharomyces cerevisiae* with sensory analysis soon after drying; YS = fermentation with *Saccharomyces cerevisiae* with sensory analysis after storage.

Figure 4B shows the plot corresponding to the analysis of fermentation times and analysis of treatments with and without yeast inoculation, indicating the formation of vectors in relation to the centroid for the times of 0, 24, and 96 hours, showing an effect of the yeast on the aromatic characteristics of the coffee, where the times of 0 and 96 hours were located in different quadrants and thus differed. The 48- and 72-hour samples were affected only by the fermentation time. The times of 0, 24, and 48 hours were grouped close together and in the same quadrant, suggesting that fermentation had no effect until 48 hours; whereas the times of 72 and 96 hours were located in different quadrants, suggesting that from that time onwards, changes in the aroma of the samples began to occur.

The correlation circle plot (Figure 4C) indicates through vectors which characteristics of the aroma group defined the position of the centroids. At the initial fermentation times (0, 24, and 48 hours), the coffee mainly exhibited (milk) chocolaty, nutty (almonds), and sweet (honey, caramel, and brown sugar) aromatic notes. Conversely, the times of 72 and 96 hours were separated in the plot by fermented (winey), fruity (yellow and red fruits), floral, and spices notes.

The evaluation of flavor descriptors was performed using multiple factor analysis (MFA), and the results are shown in Figure 5. The flavor attributes, according to the results obtained in sensory analysis, may explain the interaction of fermentation time, yeast inoculation, and flavor characteristics in two-dimensional graphs.

In the inertia plot of the groups for the flavor attribute (Figure 5A), the samples showed the same response; that is, the treatment had no effect on the flavor differentiation of the samples. The correspondence graph for analysis of the fermentation times and the analysis of the treatments with and without inoculation for the flavor attributes (Figure 5B) was similar to that of the aroma attributes, indicating that only the fermentation times of the 72- and 96-hour samples differed in relation to samples of other times and that the 96-hour samples were slightly affected by the treatments with and without yeast inoculation, indicated by the formation of vectors.

From the correlation circle plot of the flavor characteristics (Figure 5C) at 0, 24, and 48 hours of fermentation, the dominant characteristics were sweet (brown sugar, caramel, and honey), nutty (almond), (milk) chocolaty, and vegetative. At 72 and 96 hours, the development of more complex flavors, such as fruity (yellow and red fruits), fermented (winey and alcoholic), and spices, was observed.

Acidity descriptors were analyzed using multiple factor analysis (MFA), and the results are shown in Figure 6. The results obtained in the sensory analysis can explain the interaction of the fermentation time, yeast inoculation, and the characteristics of the acidity types in two-dimensional graphs.

The inertia plot of the groups for the acidity attribute (Figure 6A) showed that the 6 months of storage had an effect on these characteristics based on the distancing between the treatments with and without inoculation of *S. cerevisiae*.

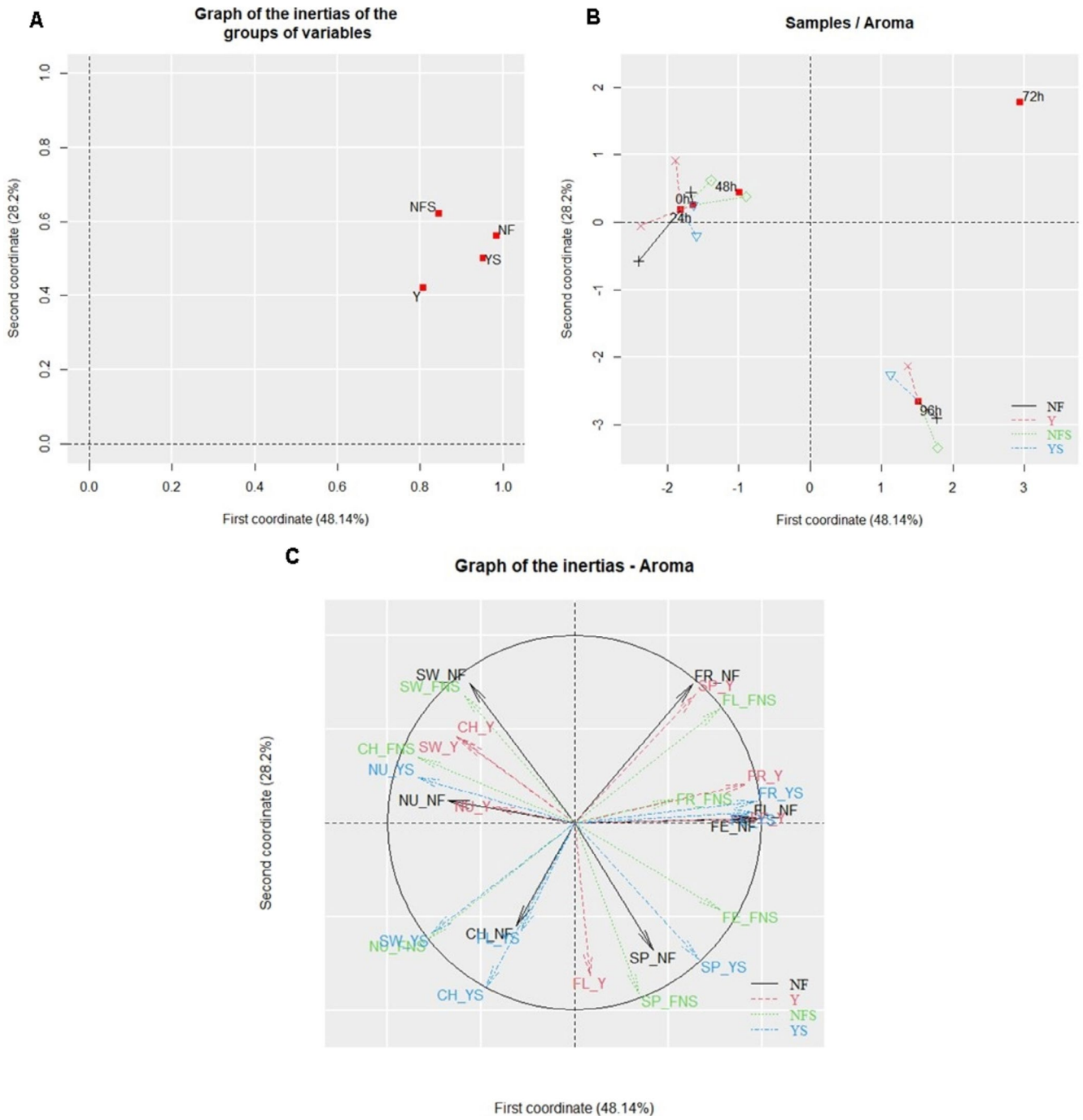

Figure 4: Multiple Factor Analysis (MFA) of the descriptors of aroma of the sensory analysis of fermented natural coffee followed by pulping and storage for 6 months. (A) Inertia of the groups; (B) correspondence graph and (C) correlation circle.

Legend: SW = sweet; NU = nutty; CH = chocolaty; SP = spicy; FE = fermented; FL = floral; FR = fruity; NF =natural/spontaneous fermentation from the indigenous microbiota with sensory analysis soon after drying; NFS = natural/spontaneous fermentation from indigenous microbiota with sensory analysis after storage; Y = fermentation with *Saccharomyces cerevisiae* with sensory analysis soon after drying; YS = fermentation with *Saccharomyces cerevisiae* with sensory analysis after storage.

Regarding the correspondence plot for analysis of the fermentation times and the analysis of the treatments with and without inoculation for the acidity attribute (Figure 6B), the fermentation times of 0, 24, and 48 hours remained grouped, as did the times of 72 and 96 hours, indicating that there was some change in the acidity of the coffees during fermentation. The large distancing of the vectors (treatment with and without yeast + storage) in a manner opposite the centroid of 96 hours suggested a strong effect of the treatment in that time period.

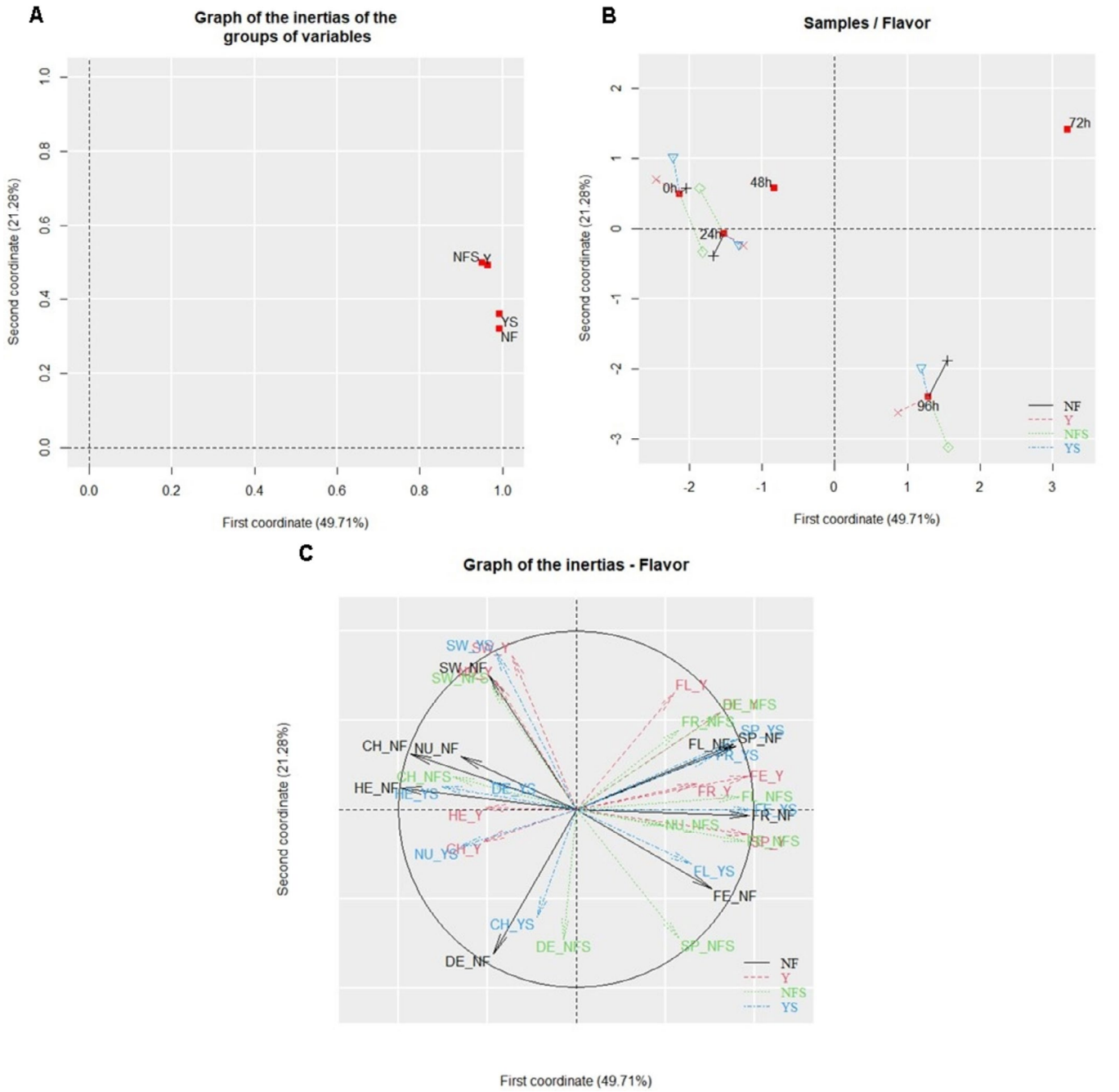

Figure 5: Multiple Factor Analysis (MFA) of the flavor descriptors of the sensory analysis of fermented natural coffee followed by pulping and storage for 6 months. (A) Inertia of the groups; (B) correspondence graph and (C) correlation circle.

Legend: SW = sweet; NU = nutty; CH = chocolaty; SP = spicy; FE = fermented; FL = floral; FR = fruity; NF =natural/spontaneous fermentation from the indigenous microbiota with sensory analysis soon after drying; NFS = natural/spontaneous fermentation from indigenous microbiota with sensory analysis after storage; Y = fermentation with *Saccharomyces cerevisiae* with sensory analysis soon after drying; YS = fermentation with *Saccharomyces cerevisiae* with sensory analysis after storage.

From the correlation circle plot for the acidity characteristics (Figure 6C) of the samples, the fermentation times of 0, 24, and 48 hours had a dominant characteristic of citric acidity. What separated the times of 72 and 96 hours in the quadrants was the development of other types of acidity, such as malic and tartaric, which differentiated the quality of the coffee because these characteristics are linked to the fermentation process.

Body descriptors were analyzed using multiple factor analysis (MFA), and the results are shown in Figure 7. The body attributes, according to the results obtained in the sensory analysis, may explain the interaction of fermentation time,

yeast inoculation, and the characteristics of the body types in two-dimensional.

The inertia plot of the groups for the body attribute (Figure 7A) showed a response different from the other attributes, indicating that there was differentiation in the body of the samples during the storage of the samples inoculated with yeast. In general, the other treatments were grouped, so they did not differ from each other.

Regarding the plot corresponding to the analysis of fermentation times and the analysis of treatments with and without inoculation for the body attributes (Figure 7B), no fermentation times were clearly distanced,

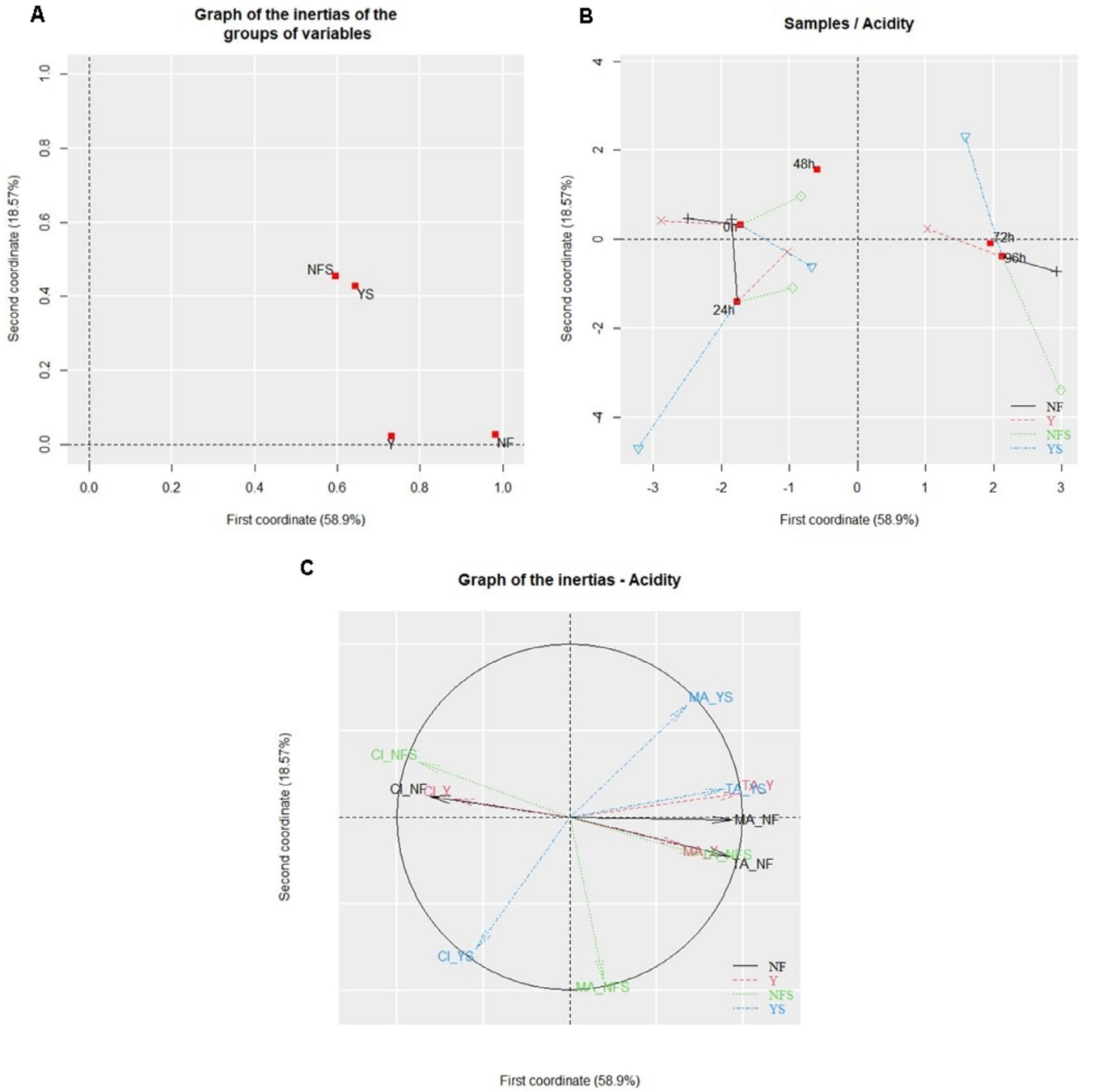

Figure 6: Multiple Factor Analysis (MFA) of the descriptors of acidity of the sensory analysis of fermented natural coffee followed by pulping and storage for 6 months. (A) Inertia of the groups; (B) correspondence graph and (C) correlation circle.

Legend: CI = citrus; MA = malic acid; NF =natural/spontaneous fermentation from the indigenous microbiota with sensory analysis soon after drying; NFS = natural/spontaneous fermentation from indigenous microbiota with sensory analysis after storage; Y = fermentation with *Saccharomyces cerevisiae* with sensory analysis soon after drying; YS = fermentation with *Saccharomyces cerevisiae* with sensory analysis after storage.

indicating that the body characteristics did not change during the fermentation time or with the inoculation of starter culture.

The correlation circle plot of the body characteristics (Figure 7C) showed that the samples of 72 and 96 hours exhibited creamy and silky characteristics, indicating that over the fermentation time, the creaminess of the samples increased, which may be linked to increased complexity and tactile perception of coffee, contributing to a longer and more pleasant aftertaste of the beverage.

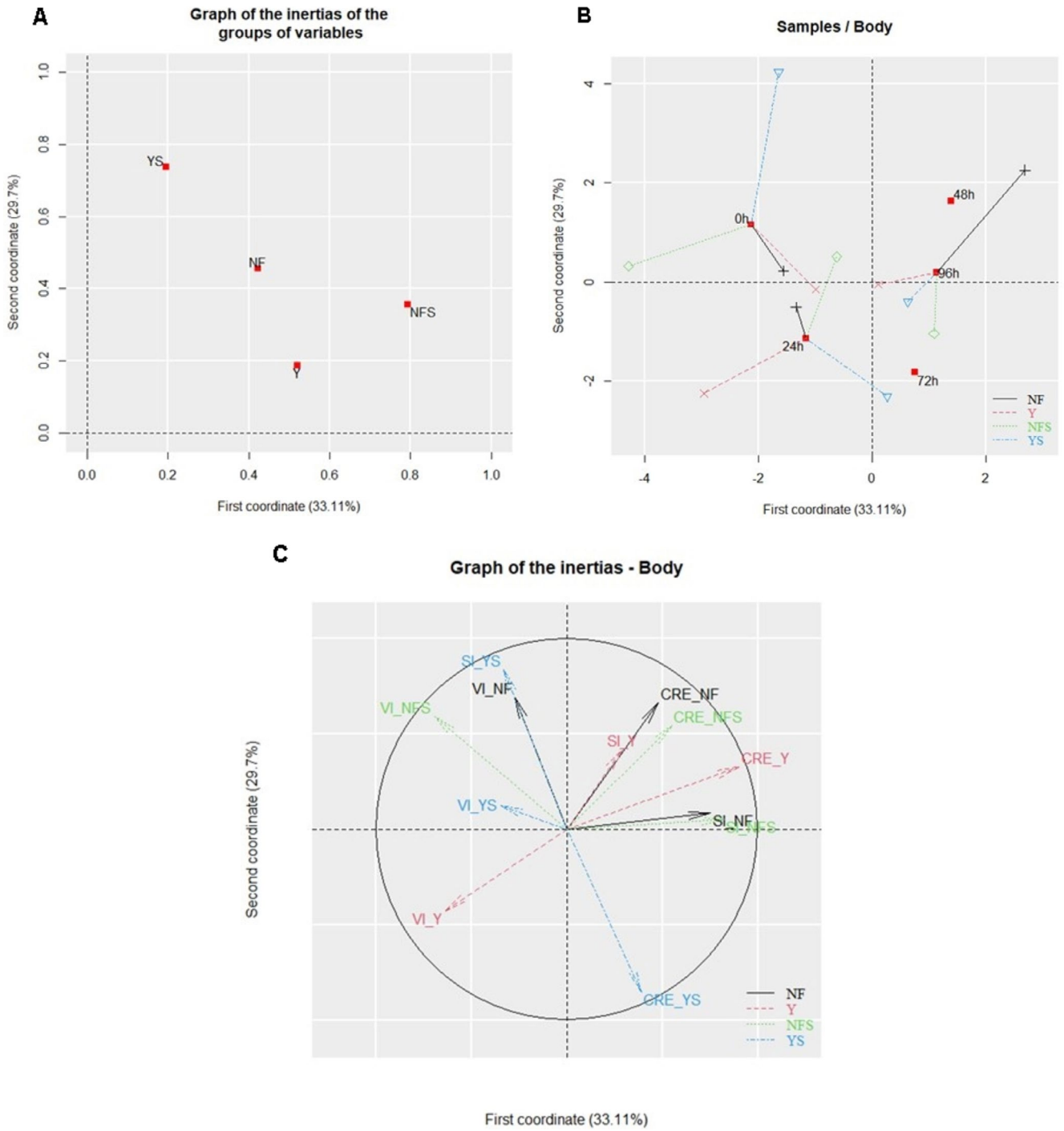

Figure 7: Multiple Factor Analysis (MFA) of the descriptors of body of the sensory analysis of fermented natural coffee followed by pulping and storage for 6 months. (A) Inertia of the groups; (B) correspondence graph and (C) correlation circle.

Legend: LI = liqueur; VI = viscous; CR = creamy; SI = silky; NF =natural/spontaneous fermentation from the indigenous microbiota with sensory analysis soon after drying; NFS = natural/spontaneous fermentation from indigenous microbiota with sensory analysis after storage; Y = fermentation with *Saccharomyces cerevisiae* with sensory analysis soon after drying; YS = fermentation with *Saccharomyces cerevisiae* with sensory analysis after storage.

4 DISCUSSION

According to Evangelista et al. (2015), the use of two strains of *S. cerevisiae* (UFLA YCN724 and UFLA YCN727) resulted in an improvement in sensory quality according to TDS analysis. In pulped coffees, strong acidity and fruity notes were observed, while in natural coffees, characteristics such as caramel, fruity, and fermented notes were observed.

The use of *S. cerevisiae* yeast (CCMA 0543) stressed the intensity of the sweetness and acidity of the samples. In a recent study using the same strain, Mota et al. (2020) concluded that yeast performed better when applied in processing of parchment coffee, mainly influencing characteristics related to the body, acidity, sweetness, and aftertaste of the beverage.

Regarding the distinction of the score according to the SCA methodology, Evangelista et al. (2014) concluded that the wet-processed (parchment) coffee inoculated with *S. cerevisiae* performed better than the control (without inoculation), different from the results of this study, in which there was no differentiation either in the final scores or the sensory quality comparing the control and the use of the same yeast.

Several studies using different strains of *S. cerevisiae* have shown good performance in use in the fermentation process, as well as improvement in sensory quality, adding characteristics such as caramel, fruity, fermented, and buttery to the aroma and flavor of coffee (Evangelista et al., 2015; Evangelista et al., 2014; Pereira et al., 2016; Ribeiro et al., 2017).

Ribeiro et al. (2017) used the same starter culture for their experiment and the same coffee variety (Mundo Novo) and concluded that *S. cerevisiae* (CCMA 0543) was responsible for higher production of volatile aroma precursor compounds, but sensory differentiation in relation to the control was not obtained. Those results differ from the results of the present study, where the yeast exhibited excellent performance in sensory differentiation of the product, though only in combination with fermentation time.

Pereira et al. (2016) observed that higher intensities of the buttery, fruity, and fermented aromas in inoculated coffees may be linked to the total concentration of volatile compounds, such as 2,3-butanedione (buttery flavor), acetaldehyde (fruity flavor), ethanol (alcoholic flavor), and esters (fruity flavors) produced during the fermentation process.

Elhalis et al (2021) comparing the fermented coffee beverage with the unfermented coffee beverage, observed that the fermented coffee beverage was rated significantly higher in flavor, aroma, and uniformity scores, with a notable fruity aroma compared to the beverage made with unfermented beans. In addition, they observed that fermentation promoted higher acidity and body intensities, as occurred in this work in which the coffees fermented for 72 and 96 hours had significantly higher acidity and body intensities.

In this study, the samples of the 96-hour treatment inoculated with *S. cerevisiae* (CCMA 0543) favored the maintenance of the sensory qualities after 6 months of storage because the un inoculated coffee dropped from the excellent (85.08) to the very good (82.5) class of the SCA (2015), as the graders perceived signs of aging, such as vegetative, earthy, rancid, old, and bread notes. That confirms one of the main advantages of using starter cultures in the food industry –to increase product longevity (Corsetti et al., 2012; Holzapfel, 2002).

The sensory profiles of the coffees up to 48 hours of fermentation were characterized by sugary (honey, caramel, brown sugar), (milk) chocolaty, and nutty (almond) aromas and flavors with medium acidity, predominantly citric, and medium body. This profile reflects characteristics that are very common in Brazilian coffees (Hoffmann, 2018) and such coffee is widely used to compose espresso blends.

Conversely, the coffees fermented for 72 and 96 hours showed greater complexity, which may be attributed to the fermentation process and the development of characteristics considered exotic, such as fruity (red and yellow fruits), fermented (winey and alcoholic), and spicy aromas and flavors, as well as high acidity – citric, malic, and tartaric – and a dense body with long aftertaste, due to increased creaminess during fermentation. Notably, all the coffees exhibited high sweetness and had higher final scores compared to coffees with less fermentation time (Table 1) even after 6 months of storage.

5 CONCLUSION

The coffee samples inoculated with the *S. cerevisiae* CCMA 0543 starter culture did not differ in a sensory manner from the samples without inoculation on initial sensory quality, without storage. However, the fermentation time affected the intensity of the sensorial attributes and the final score.

After 6 months of storage in a cold chamber, none of the samples were affected, except for coffee fermented for 96 hours without inoculation of the yeast *S. cerevisiae* CCMA 0543. However, the beverage was still classified as specialty. Further studies addressing the effect of storage on coffee fermented for a long periods of time (more than 96 hours) are needed to better understand this phenomenon.

This study leads to the recommendation to ferment coffee for 72 to 96 hours if the objective is to distinguish the sensory profile, and the use of yeast is recommended when the objective is to store these types of coffee for up to 6 months.

The use of a cupping sheet containing different sensory analysis techniques was found to be very useful for data analysis and definition of the sensory profile of the samples evaluated.

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7 AUTHORS' CONTRIBUTIONS

LGAS wrote the manuscript and performed the experiment, MAC conducted all statistical analyses, FMB supervised the experiment and co-work the manuscript, APCA wrote the manuscript and performed the experiment, JMCP performed the experiment, CMS wrote the manuscript, LH wrote the manuscript, RFS supervised the experiment and MN and RS review and approved the final version of the work.

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