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Coffee protein profiles during fermentation using different yeast inoculation methods

Abstract – The objective of this work was to evaluate the protein profiles of natural and semidry fermented *Coffea arabica*, either subjected to treatments with different yeast inoculation methods with starter culture or to an uninoculated control. *Saccharomyces cerevisiae* CCMA 0543 and *Candida parapsilosis* CCMA 0544 were separately inoculated into coffee by directly spraying the cherries on a terrace or in buckets, for 16 hours before sun drying. Protein quantification showed a significant difference between the protein profiles of the samples collected after natural dry fermentation. The MALDI-TOF MS analysis generated a list of 96 peaks with different mass-to-charge ratios (m/z) in the samples collected at the beginning and the end of fermentation. The highest number of peaks in the natural dry coffee was observed at the end of fermentation in the samples inoculated with *S. cerevisiae* CCMA 0543, in bucket, and in *C. parapsilosis* CCMA 0544 sprayed on the terrace. However, in the semidry processed coffee, the highest number of peaks was observed in the initial fermentation, with a decrease in the peptide peaks after fermentation. The fermentation with different microorganisms, processing types, and inoculation methods affects m/z profiles, influencing the types of proteins found in coffee.

Index terms: *Candida parapsilosis*, *Saccharomyces cerevisiae*, MALDI-TOF MS, starter culture.

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Perfis proteicos de café durante a fermentação por diferentes métodos de inoculação de leveduras

Resumo – O objetivo deste trabalho foi avaliar o perfil proteico de café arábica (*Coffea arabica*) fermentado por via natural e semisseco, submetido ou a tratamentos com diferentes métodos de inoculação com culturas iniciadoras ou a um controle sem inoculação. *Saccharomyces cerevisiae* CCMA 0543 e *Candida parapsilosis* CCMA 0544 foram inoculadas separadamente no café, por pulverização direta nos frutos-cereja, em um terreiro suspenso, ou em baldes, por 16 horas antes da secagem ao sol. A quantificação de proteínas mostrou uma diferença significativa no perfil proteico das amostras colhidas, após fermentação seca natural. A análise MALDI-TOF MS gerou uma lista de 96 picos com diferentes proporções massa/carga (m/z), nas amostras colhidas no início e ao final da fermentação. O maior número de picos no café processado seco ao natural foi observado ao final da fermentação nas amostras com inoculação de *S. cerevisiae* CCMA 0543, no balde e nas amostras de *C. parapsilosis* CCMA 0544 pulverizadas no terraço. No entanto, no café processado semisseco, o maior número de picos foi observado no tempo inicial de fermentação, com diminuição dos picos dos peptídeos após a fermentação. A fermentação com diferentes microrganismos, tipos de processamento e métodos de inoculação afeta os perfis m/z e influencia os tipos de proteínas encontradas no café.

Termos para indexação: *Candida parapsilosis*, *Saccharomyces cerevisiae*, MALDI-TOF MS, cultura iniciadora.

Introduction

The composition of coffee is complex and diverse. Coffee (*Coffea arabica* L.) cherries contain a matrix of fibers, pectin, minerals, lipids, free amino acids, reducing and non-reducing sugars, tannins, organic acids, chlorogenic acids, and other components. These chemical compounds may be influenced by pre- and post-harvest factors, inoculation methods, and starter cultures (Sunarharum et al., 2014; Bressanello et al., 2017). Basically, coffee can be processed by three different methods, as follows: natural or dry processing, by which coffee fruit is harvested and taken directly to drying; wet processing, by which coffee fruit is pulped and/or demucilated in water tanks; and semidry processing, by which coffee fruit is pulped and taken to drying (Evangelista et al., 2014a).

During coffee processing, a large microbial diversity is present, favoring the natural fermentation in coffee. In addition, several studies show that the use of selected starter cultures of coffee itself helps control fermentation, improves sensory characteristics (such as aroma and flavor), decreases the drying time, and inhibits the growth of some toxigenic fungi (Silva et al., 2013; Vaughan et al., 2015; Bressani et al., 2018; Pereira et al., 2019).

In this sense, some studies showed that the inoculation of potential yeasts – *Saccharomyces cerevisiae*, *Candida parapsilosis*, and *Torulaspora delbrueckii* – in coffee resulted in positive responses by obtaining caramel, cashew, and floral notes (Evangelista et al., 2014a, 2014b; Martinez et al., 2017; Ribeiro et al., 2017; Bressani et al., 2018). However, in general, *C. arabica* – from different regions, altitudes, and varieties – inoculated with *S. cerevisiae* yeast has shown better sensory notes and different desirable volatile compounds.

The inoculation method (direct to suspended terrace or bucket) was also tested by Martinez et al. (2017) and Bressani et al. (2018), in order to verify the population behavior of these yeasts, and whether they would influence the compounds formed during fermentation. The inoculation method in bucket produced a positive effect on the final product, and allowed of the verification of precursor compound production in the development of volatile compounds.

However, variations in proteins, peptides, and free amino acids have not been sufficiently explored in fermented coffee, although they participate as potential flavor precursors in other reactions (such as the Maillard one) (Hwang et al., 2012). The study of these compounds is important to identify the role of proteins in fermented coffee and their possible influence on the formation of volatile compounds.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been successfully used to investigate and identify proteins and peptides in several applications, including the taxonomic identification of microorganisms, the genotyping and analysis of DNA-free polymorphisms, and in investigations of post-transcriptional modifications without RNA (Marvin et al., 2003; Croxatto et al., 2012).

The study of coffee protein profiles with MALDI-TOF MS during starter culture fermentation can provide new insights and discoveries. Analysis of samples with MALDI-TOF MS allows of the rapid identification of molecular masses from organic compounds through ionization and fragmentation (Tanaka et al., 1988; Santos et al., 2015; Miguel et al., 2017), besides providing high-sensitivity (Cho et al., 2013).

The objective of this work was to evaluate the protein profiles of natural and semidry fermented *Coffea arabica*, either subjected to treatments with different yeast inoculation methods with starter culture or to an uninoculated control.

Materials and Methods

Ripe fruit of *Coffea arabica* 'Catuaí Amarelo IAC-62' were manually harvested on a farm located in the municipality of Lavras, in the southeastern region of the state of Minas Gerais (MG), Brazil, at 750–800 m altitude. According to the Köppen-Geiger's classification, the climate in Lavras is characterized as Cwa – temperate rainy (mesothermal) with dry winter and rainy summer, subtropical, with dry winter and warmer month temperature greater than 22°C (Dantas et al., 2007). The ambient temperature ranged from 10.2 to 28.7°C, and the relative humidity, from 56 to 90% (INMET, 2019).

Coffee fruit were processed using the dry and semidry methods. In the present study, the dry method consisted of mature coffee fruit spread as a thin layer on suspended terraces, and allowed to dry in the sun to obtain 11% moisture; and the semidry method consisted of the coffee mature fruit mechanically pulped in a horizontal machine model BDSV-04 (Pinhalense, São Paulo, SP, Brazil). After removing the pulp, berries were transferred to suspended terraces and let to dry in the sun to obtain 11% moisture (Vilela et al., 2010; Martinez et al., 2017).

Saccharomyces cerevisiae CCMA 0543 and *Candida parapsilosis* CCMA 0544 from the culture collection of agricultural microbiology (CCMA) of the Departamento de Biologia, Universidade Federal de Lavras (UFLA), Lavras, MG, Brazil, were used as starter culture. The yeast species were selected based on a previous work (Silva et al., 2013) due to their following characteristics: high-pectin lyase activity on a culture media containing coffee pulp and skin; ability to hydrolyze pectin present in coffee; presence of desirable metabolic products; and fermentative capacity. The strains were reactivated in 10 mL YEPG broth (20 g L^{-1} glucose, 10 g L^{-1} yeast extract; and 10 g L^{-1} soya peptone; pH 3.5), at 28°C for 24 hours, until a concentration of 10^9 cells mL^{-1} for inoculation was achieved. Yeast cells were then centrifuged and resuspended in 500 mL distilled water (which is enough for inoculation without increasing the coffee's moisture content) (Martinez et al., 2017; Bressani et al., 2018).

The yeast suspension (above described) was inoculated in the coffee cherries and beans (10 kg), resulting in 10^7 cells g^{-1} concentration. Yeast was then inoculated in both dry and semidry processing treatments using either the direct method (D) or polystyrene buckets (B). In method D, the yeast suspension was sprayed on coffee cherries that had already been spread on the suspended terraces. For method B, the yeast suspension was transferred to a polystyrene bucket, homogenized with coffee cherries, and left for 16 hours before being transferred to the suspended terraces. The control treatments (without inoculation) were performed for both the dry and semidry processing treatments. For all applications of the yeast suspension, the same volume of water (500 mL) was used and handled under identical conditions (Martinez et al., 2017; Bressani et al., 2018). All coffee treatments were fermented and sun-dried three times a day, until 11% moisture content was obtained.

The coffee samples processed by the natural dry method were evaluated at the beginning (T0) and at the end (400 hours) of fermentation (FT), while the semidry processed coffee samples were evaluated at the beginning (T0) and at end (352 hours) of fermentation (FT). At each time point, an aliquot of the sample (0.5 g) was ground into a fine powder using an IKA A11 analytical mill (IKA Werke, Staufen, Breisgau, Germany) and liquid nitrogen. Each sample was homogenized in a Falcon tube with 2 mL of 25% formic acid by vortexing at room temperature for 5 min. The extracts were then centrifuged at 12,745 *g*, at 4°C for 5 min. The supernatant was removed and frozen for analysis.

The calibration solution was made according to the Bradford method (Bradford, 1976) with some modifications, using 1 mg mL^{-1} bovine serum albumin solution (BSA) (0 to 100 µL) and distilled water (0 to 100 µL). From this calibration curve, 10 µL of BSA were removed and added to 790 µL of distilled water and 200 µL of Bradford reagent. For the standard, 800 µL of distilled water and 200 µL of Bradford reagent were used. For the coffee samples, 790 µL of distilled water, 10 µL of the sample, and 200 µL of Bradford reagent were used. The calibration test was performed in triplicate. After the calibration curve was calculated, the samples were homogenized for 5 min with a vortex. Then, 300 µL of each solution were placed in the wells and read in a microplate photometer Multiskan FC (Thermo Fisher Scientific, Vantaa, Finland) at 595 nm. Protein quantification was performed in triplicate. After testing in MALDI-TOF MS, the optimal amount of protein for each sample was verified, and the final protein concentration was 0.1 mg mL^{-1}.

A coffee sample (1 µL) was transferred to a 96-well MALDI flex-target plate (Bruker Daltonik, Bremen, Germany). When the liquid phase was almost evaporated, 1 µL of matrix solution, comprising a saturated solution of α-cyano-4-hydroxycinnamic acid (CHCA) in 50% acetonitrile/2.5% trifluoroacetic acid was added, and the solution was gently mixed. The samples were then analyzed in a MALDI-TOF microflex LT spectrometer (Bruker Daltoniks, Bremen, Germany) (Pereira et al., 2014; Oliveira et al., 2015), in positive ion reflection mode. Mass spectra

were formed, and each peak was assigned a mass-to-charge ratio (m/z) value. A standard with *Escherichia coli* was also analyzed. Raw data (spectra in mzXML format) were preprocessed and analyzed using the MASCOT distiller software following the protocol (Matrix Science, 2020). The m/z value of each peak was identified.

Results and Discussion

There was an increase of the protein concentration in the natural dry coffee (the most difference observed was 0.64 mg of protein g^{-1} of extract), suggesting the formation of proteins; and, for the semidry method, the concentration decreased, suggesting degradation of the same proteins (Table 1). According to Hwang et al. (2012), higher-protein concentrations in coffee are correlated with increases of pyrazine, characterized by nut, almond, and sweet flavors (Bressanello et al., 2017; Bressani et al., 2018), as well as an increase of sucrose degradation after roasting. In this context, the higher formation of protein peaks in the CCMA 0543 bucket treatment may be related to pyrazines (Bressani et al., 2018).

In green coffee beans, free amino acids, peptides, and proteins represent potential volatile nitrogen precursors. Coffee proteins act as activators in the induction of sugar degradation, and as ammonia or hydrogen sulfate donors, after the degradation of amino acid residues, suggesting that they are essential to produce volatile compounds (Hwang et al., 2012).

The MASCOT distiller software (Matrix Science, 2020) processed the spectra obtained by the MALDI-TOF MS analysis, and determined the minimum and maximum molecular weights, as well as the m/z values of the peaks, in each analyzed sample. At the end of the fermentation, the number of peaks increased in most of the coffee samples processed by the natural dry method (Table 2), corroborating the results (Table 1), which showed an increased protein concentration at the end of fermentation. Livramento et al. (2017) observed a higher-protein content in natural coffee,

Table 1. Bradford method for protein concentration (PC) quantification of inoculated and noninoculated coffee cherries of *Coffea arabica* 'Catuaí Amarelo IAC-62', at the beginning and at the end of the natural dry and semidry fermentation processes[(1)].

Inoculation method	PC at the beginning (mg g^{-1} extract)	PC at the end of the fermentation process (mg g^{-1} extract)		
	Control T0	Control FT	CCMA 0543	CCMA 0544
Natural - bucket	0.40±0.005a	1.04±0.013b	0.80±0.030c	0.94±0.007c
Natural - direct	0.40±0.005a	0.93±0.006c	0.89±0.007c	0.80±0.020c
Semidry - bucket	0.32±0.004a	0.30±0.008a	0.29±0.006a	0.31±0.01a
Semidry - direct	0.32±0.004a	0.24±0.01a	0.25±0.02a	0.24±0.06a

[(1)]Means followed by equal letters do not differ, by Scott-Knott's test, at 5% probability. Mean values ± standard deviation. Inoculation methods: natural bucket, coffee cherries in polystyrene bucket; natural direct, coffee cherries spread on suspended terraces; semidry bucket, coffee cherries in polystyrene bucket; semidry direct, coffee cherries spread on suspended terraces; control T0, beginning of fermentation; control FT, end of fermentation, without inoculation; CCMA 0543, inoculation with *Saccharomyces cerevisiae*; CCMA 0544, inoculation with *Candida parapsilosis*.

Table 2. Minimum and maximum mass-to-charge ratios (m/z), and the total number of protein peaks identified at the beginning and end time of fermentation of inoculated and noninoculated natural dry coffee (*Coffea arabica* 'Catuaí Amarelo IAC-62').

Fermentation time (h)	Inoculation method(1)		Lowest m/z	Highest m/z	Number of protein peaks
0		Control T0	2,144.037	9,354.792	14
400	Direct	Control FT	2,144.739	9,356.028	18
		CCMA 0543	2,144.205	9,356.222	13
		CCMA 0544	2,476.336	9,354.848	22
400	Bucket	Control FT	2,143.434	9,353.634	21
		CCMA 0543	2,143.434	9,353.634	25
		CCMA 0544	2,143.434	9,353.634	18

Direct, coffee spread on suspended terraces; bucket, coffee spread in polystyrene bucket; control T0, beginning of fermentation; control FT, end of fermentation, without inoculation; CCMA 0543, inoculation with *Saccharomyces cerevisiae*; CCMA 0544, inoculation with *Candida parapsilosis*.

after sun drying on platforms, in comparison to drying in mechanical dryers. However, the authors of that study did not evaluate the protein content before drying. The CCMA 0543 bucket treatment registered the highest-peak number, suggesting an increase of protein synthesis (Table 2).

According to Lee et al. (2017), the samples of fermented coffee using different starter cultures showed different protein profiles from each other and from the control. Therefore, it may be suggested that in addition to fermentation, the microorganism specie used as the starter culture may alter the protein profile of the samples, which may interfere in the formation of the volatile compounds, altering the sensorial perception of the samples.

The MASCOT Distiller software generated a list of 96 peaks with different m/z values. From this list, 27 peaks had an area greater than 100 and a Rho (correlation coefficient) greater than 0.85 (Table 3). Out of these, ten peaks were found in all bucket and direct method of natural dry processed treatment samples (2,476.519, 2,507.767, 2,632.853, 2,664.387, 3,091.855, 4,664.383, 4,678.641, 7,383.585, 9,325.823, and 9,354.792); five peaks were found in all bucket and direct method semidry processed treatment samples (2,478.418, 2,509.552, 2,665.585, 9,325.823, and 9,354.792); and two peaks were found in all treatment samples (9,325.823 and 9,354.792).

Different m/z peaks were detected in the direct inoculation samples taken from the beginning and end

Table 3. List of 27 peaks with different mass-to-charge ratios (m/z) values of fermented coffee (*Coffea arabica*), inoculated with *Saccharomyces cerevisiae* CCMA 0543 and *Candida parapsilosis* CCMA 0544, and the control (uninoculated) generated from the MASCOT Distiller software with Rho (correlation coefficient) higher than 0.85.

Peaks	Rho	m/z	Bucket method				Direct method			
			Control T0	Control FT	CCMA 0543	CCMA 0544	Control T0	Control FT	CCMA 0543	CCMA 0544
1	0.977	2,050.000	- o	- -	- -	- -	- o	- -	- -	- -
2	0.971	2,225.154	- o	- -	- -	- -	- o	- -	- -	- -
3	0.991	2,476.519	x -	x -	x -	x -	x -	x -	x -	x -
4	0.987	2,478.418	- o	- o	- o	- o	- o	- o	- o	- o
5	0.982	2,507.767	x -	x -	x -	x -	x -	x -	x -	x -
6	0.976	2,509.552	- o	- o	- o	- o	- o	- o	- o	- o
7	0.976	2,516.312	- -	- o	- -	- -	- -	- -	- -	- -
8	0.990	2,517.121	- o	- -	- -	- -	- -	- -	- -	- -
9	0.987	2,600.818	- o	- -	- -	- -	- -	- -	- -	- -
10	0.985	2,632.853	x -	x -	x -	x -	x -	x -	x -	x -
11	0.987	2,664.387	x -	x -	x -	x -	x -	x -	x -	x -
12	0.995	2,665.585	- o	- o	- o	- o	- o	- o	- o	- o
13	0.997	2,714.550	- o	- -	- -	- -	- o	- -	- -	- -
14	0.992	3,091.855	x -	x -	x -	x -	x -	x -	x -	x -
15	0.876	3,591.487	- -	- -	x -	- -	- -	- -	x -	- -
16	0.987	4,664.383	x -	x -	x -	x -	x -	x -	x -	x -
17	0.980	4,678.641	x -	x -	x o	x -	x -	x -	x -	x o
18	0.973	5,614.395	x -	x o	x -	x -	- -	- o	- -	- -
19	0.979	5,616.307	- -	- -	- -	- -	- -	x -	x -	x -
20	0.983	5,633.808	- -	x -	x -	x -	- -	- o	- -	- -
21	0.881	5,634.149	- -	x o	x o	x -	- -	- -	- -	- -
22	0.973	5,635.693	- -	- -	- -	- -	x -	x -	- -	x -
23	0.910	5,798.640	- -	x -	x -	x -	- -	- -	- -	x -
24	0.914	7,383.585	x -	x -	x -	x -	x -	x -	x -	x -
25	0.886	8,632.044	- -	- -	- -	- -	- -	- -	- -	x -
26	0.932	9,325.823	x o	x o	x o	x o	x o	x o	x o	x o
27	0.941	9,354.792	x o	x o	x o	x o	x o	x o	x o	x o

Bucket, coffee spread in polystyrene bucket; Direct, coffee spread on the suspended terraces; Control T0, beginning of fermentation; Control FT, end of fermentation without inoculation. x, peaks present in natural dry treatments; o, peaks present in semidry treatments; -, peak not detected.

of fermentation. The m/z value 5,616.307 was only found in samples at the end of the bucket method in natural dry fermentation. The m/z value 8,632.044 was only found in the CCMA 0544 natural dry fermentation samples by direct method. For the bucket method in natural fermentation samples, the only notable m/z value difference from the other treatments was observed in the CCMA 0543 samples, at the end of the fermentation process. These samples (CCMA 0543) had an m/z value of 3,591.487, which might be related to the high note this coffee obtained in the sensory analysis performed by Bressani et al. (2018), and, for the direct method, only m/z 5,635.693 was not found in the CCMA 0543 treatment. For semidry processing, many peptides found at the beginning of the fermentation were not detected at its end. Peptides with m/z 2,517.121, 2,600.818, 2,672.579, and 2,673.797 were found only in the treatment of initial control of the bucket method. The peptide m/z 5,634.149 was found at the end of the fermentation only in the control and CCMA 0543 treatments by the bucket method (Table 3), suggesting the formation of new proteins.

The results suggest that fermentation with different microorganisms and different inoculation methods affected the m/z peak profiles, potentially influencing the types of proteins found in the coffee, as well the interaction of these proteins with the coffee volatile compounds (Table 3). These findings corroborate those obtained by Bressani et al. (2018), in which different volatile compounds were found for each sample.

In the semidry coffee fermentation, the control T0 showed the highest number of m/z peaks after 352 hours of fermentation (Table 4). Samples of the CCMA 0543 direct and bucket treatment had the lowest number of m/z peaks, while the highest number of m/z peaks were found in the CCMA 0544 bucket treatment. The highest m/z values for all samples were similar, with little variation. The lowest m/z value was found in the control T0 sample. The CCMA 0543 treatment resulted in different m/z values from the corresponding control samples, after 352 hours of fermentation.

The present work show that variations in post-harvest processing (processing type, use of starter cultures, and inoculation methods) influenced the intensity, number, and abundance of the m/z peaks in the analyzed samples. Results obtained by Livramento et al. (2017) also showed changes in protein abundance, depending on the processing method and drying used. Both the presence of different proteins and the variations in protein concentration can generate significant changes in the beverage quality due to different amino groups, peptides, or free amino acids.

Similarities were identified between the protein profiles of the treatments according to the agglomerative hierarchical clustering (AHC) (Figure 1). In the natural -processed coffee, protein similarities were mainly grouped according to the method of yeast inoculation (the bucket method samples were grouped together, and the direct method samples had more similar profiles) (Figure 1 A). In the bucket treatment, the CCMA 0544 samples taken from the end of the fermentation were farther from the CCMA 0543 samples and closer to the control samples. In the direct treatments, the CCMA 0544 and CCMA 0543 samples were near and far from the control samples, respectively.

In contrast, protein similarities in the semidry processed coffee were more related to either the type

Table 4. Minimum and maximum mass-to-charge ratios (m/z), and the total number of m/z peaks found at the beginning and end of fermentations of inoculated and noninoculated semidry coffee (*Coffea arabica* 'Catuaí Amarelo IAC-62').

Fermentation time (h)	Inoculation method		Lowest m/z	Highest m/z	Number of protein peaks
0		Control T0	2,049.205	9,352.683	33
352	Direct	Control FT	2,478.001	9,354.392	23
		CCMA 0543	2,479.043	9,357.212	10
		CCMA 0544	2,478.667	9,355.307	22
352	Bucket	Control FT	2,478.158	9,353.329	21
		CCMA 0543	2,478.138	9,354.616	11
		CCMA 0544	2,478.913	9,356.073	27

Direct, coffee spread on suspended terraces; bucket, coffee in polystyrene bucket; control T0, beginning of fermentation; control FT, end of fermentation, without inoculation; CCMA 0543, inoculation with *Saccharomyces cerevisiae*; CCMA 0544, inoculation with *Candida parapsilosis*.

of inoculating yeast or to the control (Figure 1 B). These results confirmed that the type of processing and fermentation (with, or without starter cultures) can influence the coffee protein profile during the fermentation and drying processes. With the AHC, it is possible to notice that the inoculated treatments with the same yeast are very distant and show different behaviors in relation to the method of inoculation.

Starter cultures and processing types influence the coffee protein profile during the fermentation and drying processes. Only the treatment inoculated with CCMA 0543 showed a smaller grouping between the bucket and direct inoculation methods, indicating that *S. cerevisiae* behaved differently according to the method of inoculation (bucket and direct) applied, for both processed coffees by the dry and semidry methods, proving a correlation between yeast, inoculation method, and protein profile.

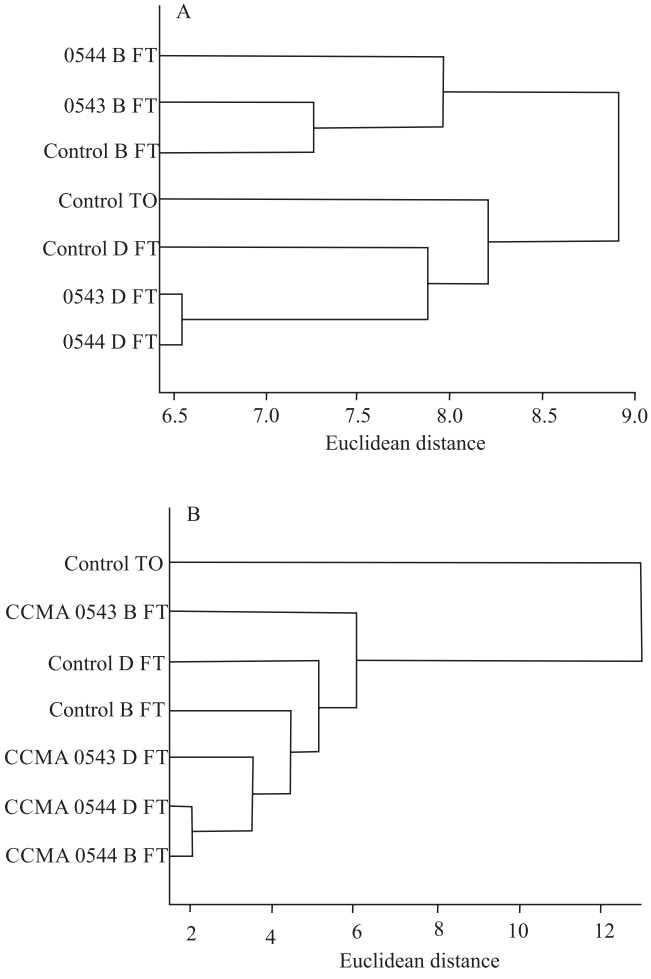

Figure 1. Agglomerative hierarchical clustering (AHC) by presence and absence of protein peaks with different mass-to-charge ratio (m/z) values. A, natural dry fermented coffee (*Coffea arabica*); B, semidry fermented coffee. In the Y axis: control T0, beginning of fermentation; control FT, end of fermentation, without inoculation; D, coffee spread on suspended terraces; B, in polystyrene bucket; CCMA 0543, inoculation of *Saccharomyces cerevisiae*; and CCMA 0544, inoculation of *Candida parapsilosis*.

Conclusions

1. The matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis shows that it is a fast and appropriate methodology to evaluate the protein profile of fermented coffee (*Coffea arabica*) samples.

2. The starter cultures and the type of processing influence the coffee protein profile during the fermentation and drying processes; 'Catuaí Amarelo IAC-62' inoculated with *Saccharomyces cerevisiae* CCMA 0543 in bucket shows the highest peak number in natural fermented coffee, suggesting an increase of protein synthesis, and showing a smaller grouping between the inoculation methods in bucket and direct, which indicates that the yeast behavior is different according to the methods of inoculation for both coffees processed by the method natural dry and semidry, proving a correlation between yeast, inoculation methods, and protein profiles.

3. 'Catuaí Amarelo IAC-62' inoculated with *Candida parapsilosis* CCMA 0544 shows no difference for the number of protein peaks between natural and semidry processing by the direct inoculation method.

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References

BRADFORD, M.M. A Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, v.72, p.248-254, 1976. DOI: https://doi.org/10.1016/0003-2697(76)90527-3.

BRESSANELLO, D.; LIBERTO, E.; CORDERO, C.; RUBIOLO, P.; PELLEGRINO, G.; RUOSI, M.R.; BICCHI, C. Coffee aroma: chemometric comparison of the chemical information provided by three different samplings combined with GC–MS to describe the sensory properties in cup. **Food Chemistry**, v.214, p.218-226, 2017. DOI: https://doi.org/10.1016/j.foodchem.2016.07.088.

BRESSANI, A.P.P.; MARTINEZ, S.J.; EVANGELISTA, S.R.; DIAS, D.R.; SCHWAN, R.F. Characteristics of fermented coffee inoculated with yeast starter cultures using different inoculation methods. **LWT - Food Science and Technology**, v.92, p.212-219, 2018. DOI: https://doi.org/10.1016/j.lwt.2018.02.029.

CHO, Y.-T.; SU, H.; HUANG, T.-L.; CHEN, H.-C.; WU, W.-J.; WU, P.-C.; WU, D.-C.; SHIEA, J. Matrix-assisted laser desorption ionization/time-of-flight mass spectrometry for clinical diagnosis. **Clinica Chimica Acta**, v.415, p.266-275, 2013. DOI: https://doi.org/10.1016/j.cca.2012.10.032.

CROXATTO, A.; PROD'HOM, G.; GREUB, G. Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. **FEMS Microbiology Reviews**, v.36, p.380-407, 2012. DOI: https://doi.org/10.1111/j.1574-6976.2011.00298.x.

DANTAS, A.A.A.; CARVALHO, L.G. de; FERREIRA, E. Classificação e tendências climáticas em Lavras, MG. **Ciência e Agrotecnologia**, v.31, p.1862-1866, 2007. DOI: https://doi.org/10.1590/S1413-70542007000600039.

EVANGELISTA, S.R.; MIGUEL, M.G. da C.P.; CORDEIRO, C. de S.; SILVA, C.F.; PINHEIRO, A.C.M.; SCHWAN, R.F. Inoculation of starter cultures in a semi-dry coffee (*Coffea arabica*) fermentation process. **Food Microbiology**, v.44, p.87-95, 2014a. DOI: https://doi.org/10.1016/j.fm.2014.05.013.

EVANGELISTA, S.R.; SILVA, C.F.; MIGUEL, M.G.P. da C.; CORDEIRO, C. de S.; PINHEIRO, A.C.M.; DUARTE, W.F.; SCHWAN, R.F. Improvement of coffee beverage quality by using selected yeasts strains during the fermentation in dry process. **Food Research International**, v.61, p.183-195, 2014b. DOI: https://doi.org/10.1016/j.foodres.2013.11.033.

HWANG, C.-F.; CHEN, C.-C.; HO, C.-T. Contribution of coffee proteins to roasted coffee volatiles in a model system. **International Journal of Food Science and Technology**, v.47, p.2117-2126, 2012. DOI: https://doi.org/10.1111/j.1365-2621.2012.03078.x.

INMET. Instituto Nacional de Meteorologia. **Gráficos [das Condições Registradas]**. Available at: <http://www.inmet.gov.br/portal/index.php?r=tempo/graficos>. Accessed on: Dec. 5 2019.

LEE, L.W.; TAY, G.Y.; CHEONG, M.W.; CURRAN, P.; YU, B.; LIU, S.Q. Modulation of the volatile and non-volatile profiles of coffee fermented with Yarrowia lipolytica: II. Roasted coffee. **LWT - Food Science and Technology**, v.80, p.32-42, 2017. DOI: https://doi.org/10.1016/j.lwt.2017.01.070.

LIVRAMENTO, K.G. do; BORÉM, F.M.; JOSÉ, A.C.; SANTOS, A.V.; LIVRAMENTO, D.E. do; ALVES, J.D.; PAIVA, L.V. Proteomic analysis of coffee grains exposed to different drying process. **Food Chemistry**, v.221, p.1874-1882, 2017. DOI: https://doi.org/10.1016/j.foodchem.2016.10.069.

MARTINEZ, S.J.; BRESSANI, A.P.P.; MIGUEL, M.G. da C.P.; DIAS, D.R.; SCHWAN, R.F. Different inoculation methods for semi-dry processed coffee using yeasts as starter cultures. **Food Research International**, v.102, p.333-340, 2017. DOI: https://doi.org/10.1016/j.foodres.2017.09.096.

MARVIN, L.F.; ROBERTS, M.A.; FAY, L.B. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in clinical chemistry. **Clinica Chimica Acta**, v.337, p.11-21, 2003. DOI: https://doi.org/10.1016/j.cccn.2003.08.008.

MATRIX SCIENCE. Available at: <http://www.matrixscience.com/>. Accessed on: Feb. 19 2020.

MIGUEL, M.G. da C.P.; REIS, L.V. de C.; EFRAIM, P.; SANTOS, C.; LIMA, N.; SCHWAN, R.F. Cocoa fermentation: microbial identification by MALDI-TOF MS, and sensory evaluation of produced chocolate. **LWT - Food Science and Technology**, v.77, p.362-369, 2017. DOI: https://doi.org/10.1016/j.lwt.2016.11.076.

OLIVEIRA, M.M.E.; SANTOS, C.; SAMPAIO, P.; ROMEO, O.; ALMEIDA-PAES, R.; PAIS, C.; LIMA, N.; ZANCOPÉ-OLIVEIRA, R.M. Development and optimization of a new MALDI-TOF protocol for identification of the Sporothrix species complex. **Research in Microbiology**, v.166, p.102-110, 2015. DOI: https://doi.org/10.1016/j.resmic.2014.12.008.

PEREIRA, G.V. de M.; CARVALHO NETO, D.P. de; MAGALHÃES JÚNIOR, A.I.; VÁSQUEZ, Z.S.; MEDEIROS, A.B.P.; VANDENBERGHE, L.P.S.; SOCCOL, C.R. Exploring the impacts of postharvest processing on the aroma formation of coffee beans – a review. **Food Chemistry**, v.272, p.441-452, 2019. DOI: https://doi.org/10.1016/j.foodchem.2018.08.061.

PEREIRA, L.; DIAS, N.; SANTOS, C.; LIMA, N. The use of MALDI-TOF ICMS as an alternative tool for *Trichophyton rubrum* identification and typing. **Enfermedades Infecciosas y Microbiología Clínica**, v.32, p.11-17, 2014. DOI: https://doi.org/10.1016/j.eimc.2013.01.009.

RIBEIRO, L.S.; RIBEIRO, D.E.; EVANGELISTA, S.R.; MIGUEL, M.G. da C.P.; PINHEIRO, A.C.M.; BORÉM, F.M.; SCHWAN, R.F. Controlled fermentation of semi-dry coffee (*Coffea arabica*) using starter cultures: a sensory perspective. **LWT - Food Science and Technology**, v.82, p.32-38, 2017. DOI: https://doi.org/10.1016/j.lwt.2017.04.008.

SANTOS, C.; VENTURA, J.A.; COSTA, H.; FERNANDES, P.M.B.; LIMA, N. MALDI-TOF MS to identify the pineapple pathogen *Fusarium guttiforme* and its antagonist *Trichoderma asperellum* on decayed pineapple. **Tropical Plant Pathology**, v.40, p.227-232, 2015. DOI: https://doi.org/10.1007/s40858-015-0027-7.

SILVA, C.F.; VILELA, D.M.; CORDEIRO, C. de S.; DUARTE, W.F.; DIAS, D.R.; SCHWAN, R.F. Evaluation of a potential starter culture for enhance quality of coffee fermentation. **World Journal of Microbiology and Biotechnology**, v.29, p.235-247, 2013. DOI: https://doi.org/10.1007/s11274-012-1175-2.

SUNARHARUM, W.B.; WILLIAMS, D.J.; SMYTH, H.E. Complexity of coffee flavor: a compositional and sensory perspective. **Food Research International**, v.62, p.315-325, 2014. DOI: https://doi.org/10.1016/j.foodres.2014.02.030.

TANAKA, K.; WAKI, H.; IDO, Y.; AKITA, S.; YOSHIDA, Y.; YOSHIDA, T. Protein and polymer analyses up to m/z 100 000 by laser ionization timeofflight mass spectrometry. **Rapid Communications in Mass Spectrometry**, v.2, p.151-153, 1988. DOI: https://doi.org/10.1002/rcm.1290020802.

VAUGHAN, M.J.; MITCHELL, T.; GARDENER, B.B.M. What's inside that seed we brew? A new approach to mining the coffee microbiome. **Applied and Environmental Microbiology**, v.81, p.6518-6527, 2015. DOI: https://doi.org/10.1128/AEM.01933-15.

VILELA, D.M.; PEREIRA, G.V. de M.; SILVA, C.F.; BATISTA, L.R.; SCHWAN, R.F. Molecular ecology and polyphasic characterization of the microbiota associated with semi-dry processed coffee (*Coffea arabica* L.). **Food Microbiology**, v.27, p.1128-1135, 2010. DOI: https://doi.org/10.1016/j.fm.2010.07.024.