






Chemical and sensory perception of robusta coffees under wet processing

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ABSTRACT

The quality of the coffee is determined by the sensorial analysis, using subjective methods and it is known that this quality is related to the chemical constituents of the grains. The present study used infrared spectroscopy to correlate the chemical composition of the grain with the final quality of the product and to assist in the current evaluation method, generating more reliability in the process. This study applied the spontaneous and induced fermentation with starter cultures (yeast) to evaluate the sensory potential from the impacts generated by the types of processes adopted. The experiments were conducted in a randomized block design with five replicates, in a 3x4 factorial scheme, with three fermentation times: 24, 48, and 72 hours and four wet processes: Washed, Yeast fermentation, Fully washed without yeast, and Fully washed with yeast. Infrared spectra were taken in the medium region (FTIR-ATR) of the 34 samples generated by the treatments. Given that the method of determining the quality of coffee is subjective, based on scores given by cuppers, the FTIR-ATR spectra were used in the construction of chemometric models to predict sensory ratings given to the attribute 'acidity'. The mean error of prediction of the model constructed for the sensory score was 0.11. Sensory results indicated a higher score for the acidity attribute, in dry fermentation with yeast at the time of 48 hours, suggesting a new strategy for the production of quality robusta coffee.

Key words: Fourier transform infrared spectroscopy; chemometrics; fermentation; acidity.

1 INTRODUCTION

Data from the US Department of Agriculture shows that the world consumes approximately 9 million tons of coffee per year (USDA, 2017). Coffee is an important product for the Brazilian economy, with Brazil being the largest producer and exporter of coffee in the world, having in its production chain two species of economic importance: *Coffea arabica* L. (arabica coffee) and *Coffea canephora* (robusta coffee). Arabica coffee accounts for 81% of the coffee area cultivated in the country and robusta 19% (CONAB, 2018).

Robusta coffee has sensory attributes that normally present neutrality as to the sweetness and acidity; it has a remarkable aroma of roasted cereal and it stands out for its body more pronounced than the arabica coffee. The final quality of the product that is offered to the consumer is determined by the sensory analysis performed by cuppers, using subjective methods and cupping experience accumulated over the years (Di Donfrancesco; Gutierrez Guzman; Chambers, 2014).

This task is often complex because Q-Graders should be able to balance and define the sensory routes that best fit the reality of the consumer market that the coffee will be destined for because the nuances of flavor and aroma are what make the evaluation of coffee a complicated task (Bhumiratana; Adhikari; Chambers, 2011)

The chemical constituents, volatile or not, present in the coffee are responsible for giving it distinctive flavor and aroma, and these constituents vary both qualitatively and

quantitatively when compared to coffees of different qualities (Carrera et al., 1998).

From the sensory perspective, it is known that *Coffea arabica* L. coffees usually receive better scores on flavor, aroma, sweetness, and body than coffees from warmer regions (Da Silva et al., 2014). These factors are widely discussed among researchers who study the behavior of these variables for arabica coffee (Joët et al., 2010; Vaast et al., 2006). In the case of *Coffea canephora* PIERRE culture, these factors are beginning to be perceived as potential quality indicators. Apart from that fact, the quality of robusta coffee needs more studies that relate the sensory specifications to the chemical compounds responsible for them (Wei et al., 2014).

The final quality of the coffee is related to the chemical constituents of the roasted grains, due to the wide chemical interaction that occurs during the roasting stages (Ribeiro et al., 2014). From the chemical perspective, it is already known that the rates of caffeine, chlorogenic acids, and reducing sugars are different depending on genetics and the groups, arabica or robusta (Alonso-Salces et al., 2009; Liu et al., 2012; Tfouni et al., 2012; Wei et al., 2010).

In addition to the search for more robust methods of analysis, new processing has been applied in the production of specialty coffees to reduce climatic and technological disparities. Such approach is based on the effect of microorganisms present in the processing, such as yeasts: *Debaryomyces* ssp., *Pichia* ssp., *Candida* ssp., *Pichia kluyveri*, and *Saccharomyces cerevisiae* (Pereira et al., 2014; Silva et

al., 2008; Silva; Batista; Schwan, 2008); as well as bacteria, such as *Lactobacillus* spp., *Bacillus* spp., *Arthrobacter* spp., *Acinetobacter* spp., *Klebsiella* spp., and *Weissella* spp. (Evangelista et al., 2014a; Evangelista et al., 2014b; Massawe; Lifa, 2010; Velmourougane, 2013), once their fermentation capacity can generate new optimization strands of coffee quality.

Studies have been carried out in an attempt to correlate the chemical composition of the grain with the final quality of the product, and thus help the current evaluation method, reducing its subjectivity (Correia et al., 2018; Ribeiro et al., 2014; Tfouni et al., 2012; Wei et al., 2014). The chemical compounds of coffee concerning sensory perception are given by their subjectivity in the process (Bhumiratana; Adhikari; Chambers, 2011; Di Donfrancesco (Di Donfrancesco; Gutierrez Guzman; Chambers, 2014; Seo; Lee; Hwang, 2009). Infrared spectroscopy, compared to other techniques, has the advantage of being a simple, rapid, inexpensive, and non-degrading analytical method, as well as providing a spectrum with many relevant pieces of information, such as functional groups that may be related to compounds present in the coffee responsible for their quality.

The infrared spectra of the coffee contain numerous absorption bands reflecting its great chemical complexity. Therefore, to explore more information of this data, multivariate analysis methods have been applied to extract as much information as possible, and thus correlate a chemical composition with sensory attributes.

In this paper, MID-infrared spectroscopy FTIR-ATR (Fourier Transform Infrared Attenuated Total Reflectance) was applied on the chemical class to evaluate the coffee beans subjected to the robusta wet-processing technology, to evaluate the effectiveness on improving the quality of robusta coffee through the prediction model. For this purpose, PLS (Partial Least Squares) regression models were constructed from the mean infrared spectra of the coffee samples, aiming to estimate acidity scores that reflect the sensorial descriptor of the robusta coffee.

2 MATERIAL AND METHODS

2.1 Sample preparation: wet processing

The raw materials selected to perform the experiments were composed of coffees from *Coffea canephora* PIERRE species - *Conilon Vitoria* variety. All samples were processed according to the same specifications as described: coffee pulp, husk from the peeling process, water, and yeast (*Saccharomyces cerevisiae*). Fifteen kilograms of coffee were harvested per experimental plot in all experiments, presenting 95% of ripe fruits. After harvest, the fruits were processed according to established procedures.

For the four proposed methods, a must from the patent process BR1020160040531 was prepared with yeast culture (*Saccharomyces cerevisiae*) Fleischmann brand. The yeast was acquired in the local market, 100 grams were sterilized in falcon tubes with the aid of precision scales, after these procedures, husk was added on the must to conduce the fermentation processing.

The four methods consisted of the following processes:

- Process 1: fermentation wort with water (*washed*), 10 kg of pulped coffee cherries (pulp), 5 kg of husk and 5 liters of water.
- Process 2: yeast starter culture (*Saccharomyces cerevisiae*), 1% (¹p/v) of the must, 10 kg of pulped coffee cherries (pulp), 5 kg of husk, 5 liters of water.
- Process 3: dry fermentation must (*fully washed without yeast*), 10 kg of pulped coffee cherries (pulp) and 5 kg of husk, without additional water on the process.
- Process 4: dry fermentation wort with yeast (*fully washed with yeast*), fermentation must with yeast starter culture (*Saccharomyces cerevisiae*), 1% (²p/v) of the must, 10 kg of pulped coffee cherries (pulp), 5 kg of husk, without added water.

To perform the experiments, must 1 and 2 were submitted to the treatment methods at 38 °C, remaining immersed in fermentation tanks, for 24, 48 and 72 hours (Pereira et al., 2015). Musts 3 and 4 received husk only, originated from the wet process, and remained in the fermentation process for 24, 48, and 72 hours. Must 3 did not receive addition of external microorganisms.

After the fermentation period, must 1, 2, 3, and 4 were washed and taken to dry on a suspended, covered yard (Pereira; Guarçoni; Cardoso, 2018a).

2.2 Preparation of the samples for sensory analysis

After the resting period of the samples (45 days), the coffees were classified according to the methodology of the Uganda Coffee Development Authority (UCDA, 2010) by type and sieve. The roasting process was performed only with coffee beans that passed through sieve #15 up. Thus, a 15 sieve has holes with a diameter of 15/64 inches or 6 mm (15 × 2.54 / 64).

Samples were prepared according to the Uganda Coffee Development Authority methodology. Roasting was conducted using the Laboratto TGP-2 roaster with the Agrtron-SCA disk set. The roasting point of these samples was set based on grain color among the colors determined by manual disks of Agrtron #65 and #55 for specialty coffees (UCDA, 2010).

Roasting was executed within 24 hours of cupping and it was allowed to rest for 8 hours before grinding. All samples

¹Parts per volume (quantity of *Saccharomyces cerevisiae* in function of the must).

²Parts per volume (quantity of *Saccharomyces cerevisiae* in function of the must).

were roasted from 9 to 10 minutes. After roasting and cooling, the samples remained stored in sealed containers (UCDA, 2010).

After 8 to 24 hours after the roasting process, the coffee was ground in a disc mill (Bunn Coffee Mill, model G3A HD), with granulometry between 70 and 75% of the particles passing through a sieve 20 mesh, US Standards (SCA, 2015). The infusion was performed after the water reached 92-95 °C. The Q-Graders began the evaluation when the temperature of the cups reached 55 °C, respecting the time of 4 minutes from the infusion before the evaluation.

2.3 Sensory Analysis by the UCDA Method

The sensory analysis of the coffees was carried out by a panel of 6 (six) cuppers, all with Q Robusta Grader³ certification. The minimum number of evaluators for the sensory analysis was initially proposed aiming to reduce the subjectivity of the analysis (Pereira et al., 2018b).

2.4 Infrared Analysis

Infrared (IR) spectra in the middle region of the roasted and ground coffee samples were obtained using a Cary 630 FTIR-ATR spectrometer from Agilent Technologies, and a diamond ATR (Attenuated Total Reflectance) accessory with reflection angle of 45°, 1 mm in diameter, 200 µm of active area, and approximately 2 µm of penetration depth, using a Zinc Selenide (ZnSe) reflectance detector. The recorded spectrum was obtained as the mean of 256 consecutive scans, with a resolution of 1 cm⁻¹ in the spectral range of 4000 to 630cm⁻¹. In this work, the spectra of three different aliquots of each sample were recorded.

2.5 Experimental design

The experiments were conducted in a randomized block design with five replications, in a 3 x 4 factorial scheme, with three fermentation times - 24, 48, and 72 hours and four wet treatments - *Washed*, *Yeast fermentation*, *Fully washed without yeast*, and *Fully washed with yeast*. For statistical analysis, variance analysis was performed, the means were compared using the Tukey test.

2.6 Chemometric data treatment

The original spectra were organized in a matrix, where each replica was considered as a sample. All calculations were performed on *Matlab* version R2013a and *Microsoft Excel* version 2016. Before the application of the chemometric tools to the dataset, the multiplicative scatter correction (MSC), or first derivative correction, with a 9-point window and grade 2 polynomial adjustment, was applied to correct problems

with spectra baseline variation. The optimal number of latent variables for each PLS model was determined by the k-fold cross-validation procedure. The models were evaluated for the linear adjustment (R^2 - coefficient of determination) and mean accuracy (RMSEP - Root mean square error of prediction) (Filgueiras et al., 2014; Portela et al., 2016).

3 RESULTS AND DISCUSSION

The acidity in the coffee has still been discussed in function to the fermentative processes that occur in the processing phase, for the composition of the mucilaginous mesocarp of ripe coffee fruits includes sugars, complex polysaccharides, proteins, lipids, and minerals. In detriment of this chemical composition, they become a favorable medium for the growth of bacteria, fungi, and yeasts (Castro; Marraccini, 2006). The variation of these components in the coffee influences its quality, which is given by the sensory analysis. Table 1 shows the sensory results for the acidity attribute that is evaluated according to the Uganda Coffee Development Authority (UCDA, 2010) protocol.

Table 1: Averages of the sensory attribute acidity evaluated in three fermentation times and four processes.

Sensory Variable	Salt/Acid				Mean
	Fermentation Time (h)				
	24	48	72		
1-Washed	7.48 a	7.42 ab	7.20 a	7.36 ab	
2-Yeast fermentation	7.36 a	7.30 ab	7.45 a	7.37 ab	
3-Fully washed without yeast	7.43 a	7.18 b	7.25 a	7.29 b	
4-Fully washed with yeast	7.57 a	7.53 a	7.39 a	7.49 a	
Mean	7.46	7.36	7.32	7.38	
Standard diversion -	0.193781				
Coefficient of variation	2.63%				

¹ Means followed by at least one repeated letter vertically do not differ from each other by the Tukey test at 5% probability.

The data of the variable *acidity* indicated that for 24 and 72 hours of fermentation, the treatments did not show statistical differences between themselves by the Tukey test at 5% of significance. In the fermentation time of 48 hours, treatment 4 is superior to treatment 3 but does not differ from the other treatments by the Tukey test at 5% of significance. The same behavior was observed on the mean values for acidity.

Low-quality coffee is associated with high acidity indexes, mainly due to harmful fermentation (Mazzafera, 1999). There is a direct relationship between the increase of acidity and the reduction of coffee quality. However, the results of this study indicate that increased acidity is perceived as a positive factor for Q-Graders.

³Q Robusta Graders, formerly known as R-Graders, are skilled, credible Robusta coffee cuppers certified by the Coffee Quality Institute (CQI).

This fact suggests that the metabolic processes generated during the yeast-induced fermentation phase had a significant effect on the production of some compounds that provide a higher acidity load to coffee. The use of yeasts (*Saccharomyces cerevisiae*) improves the acidity of coffees (Ribeiro et al., 2017). These results reinforce the discussion about fermentation, considering that desirable attributes can be optimized during wet processing (Lee et al., 2015).

The absence of experimental control can be a factor that contributes to the existence of different positions regarding acidity. The understanding that microorganisms can produce compounds that give acidity and that it can migrate to the grain is common to the scientific community (Nguyen; Byun, 2013); (De Bruyn et al., 2017).

The components responsible for coffee acidity (phosphoric acid, chlorogenic acids, quinic acid) generate information at the molecular level that can be captured in infrared spectra in the medium region. The infrared spectra of the coffee samples, after treated, are shown in Figure 1. There are numerous absorption bands in the FTIR-ATR spectra of the coffees. Some of which are more significant, such as bands related to C—O (1300-1000 cm^{-1}) and O—H (mainly in the 3650-3100 cm^{-1} region) present in carboxylic acids and alcohols (that appear in sugar molecules in the coffee), and

C—H bond (2840-3350 cm^{-1}) present in virtually all coffee compounds, since they are organic compounds, among others.

The FTIR-ATR spectra were used to estimate the acidity scores of the coffees (sensory values attributed to acidity by the cuppers, shown in Table 1) using the PLS regression method (Figure 2). The 34 samples were divided into subsets: calibration and prediction, using the Kennard-stone method, where approximately 70% of the samples were previously selected for the calibration set, while the remaining 30% were for the prediction set.

The choice of the spectral range used to construct the PLS models was based on the regions of greater variance after the analysis of the original data. Such range lies within the interval of 4000 to 2750 cm^{-1} (Lopes; Fascio, 2004), corresponds to O—H stretches present in alcohols and carboxylic acids, N—H of amines and amides, in addition to C—H stretches present in hydrocarbons. This variance is due to the different compositions obtained for the samples after the four treatments applied at three different periods. Amines are nitrogenous organic bases that serve as important indicators of quality in a wide variety of food products, these organic compounds are usually formed by free amino acid decarboxylation as a consequence of physical-chemical, and biochemical processes that take place during coffee bean processing (Kurniawan et al., 2017). Thus, this

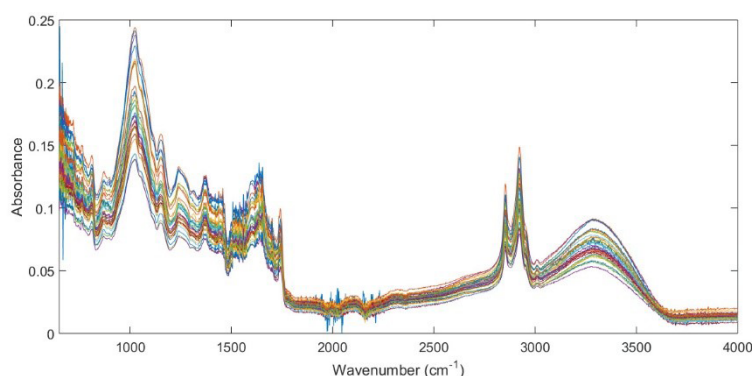


Figure 1: Pretreated Infrared spectra of the coffee samples.

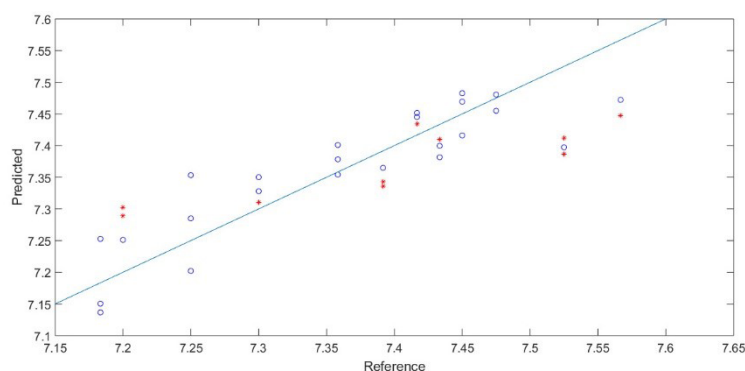


Figure 2: Relation between reference (o) and predicted (*) values of the score for the sensory attribute acidity of the coffee samples.

result suggests that the fermentation process applied resulted in the chemical change, which reflected in the sensory aspect of the coffee, according to results observed by (Evangelista et al., 2014a; Evangelista et al., 2014b; Pereira et al., 2015; Ribeiro et al., 2014).

The results for the best PLS models are presented in Table 2, where the most accurate model is highlighted in bold. RMSEC (Root Mean Square Error of Calibration) and RMSEP (Root Mean Square Error of Prediction) correspond to the mean errors of calibration and prediction, respectively.

Table 2: Results of the PLS models in determining the sensory attribute Coffee Acidity.

Preprocessing	Statistical Parameters					
	VL	RMSEC	R ² _{calibration}	RMSECV	RMSEP	R ² _{prediction}
MSC	3	0.07	0.6591	0.09	0.14	0.3128
Derivative	5	0.01	0.9877	0.08	0.16	0.4915
Derivative	4	0.03	0.9370	0.09	0.14	0.5336
Derivative	3	0.06	0.7837	0.09	0.11	0.7566

For the highlighted model, the relationship between the values predicted by the PLS regression method and those determined by the Uganda Coffee Development Authority (UCDA, 2010), for the calibration samples and the test samples, can be observed in Figure 2. The diagonal line represents the identity curve, the closer a sample is to this line the better its estimate. There is a fine concordance between the reference values and those predicted by the PLS model with a coefficient of determination above 0.75 (Table 2, preprocessing using the derivative with 3 latent variables) and accuracy of 0.06 and 0.11 for the data of the calibration and prediction sets, respectively.

The residuals of the models did not present evidence of the presence of systematic errors or tendency to the level of significance of 5% (T-Test) (Filgueiras et al., 2014). The values provided by the experts compared to those predicted by

the PLS model for the 10 samples from the prediction set are shown on Table 3.

Table 3: Values measured by the specialists and values predicted by the regression model.

Sample	Measured	Predicted
1	7.39	7.34
2	7.43	7.41
3	7.57	7.45
4	7.39	7.34
5	7.30	7.31
6	7.20	7.30
7	7.52	7.41
8	7.42	7.43
9	7.52	7.39
10	7.20	7.29

The method used as a reference for coffee evaluation does not present a numerical error, thus reinforcing the need for new methods that can bring more reliability to this evaluation, functioning as an auxiliary tool. Infrared spectroscopy combined with chemometrics proved to be effective due to the low error (0.11) for the acidity score.

The most significant variables in the construction of the PLS model are highlighted in Figure 3, where the VIP values of the spectral variables are found. In this graph, the higher the VIP value of a variable, the greater its importance in the construction of the PLS model.

The spectral band of 3000 to 2840 cm⁻¹ shows absorptions relative to symmetric and asymmetric stretches of the C—H bond of alkyl groups. The band of 3200 to 2400 cm⁻¹ shows absorptions of O—H stretches present in carboxylic acids, the range of 3650 to 3600 cm⁻¹ shows bands referring to O—H stretches of alcohols, which may be present in coffee sugars (Pavia et al., 2001; Silverstein; Webster; Kiemle, 2005).

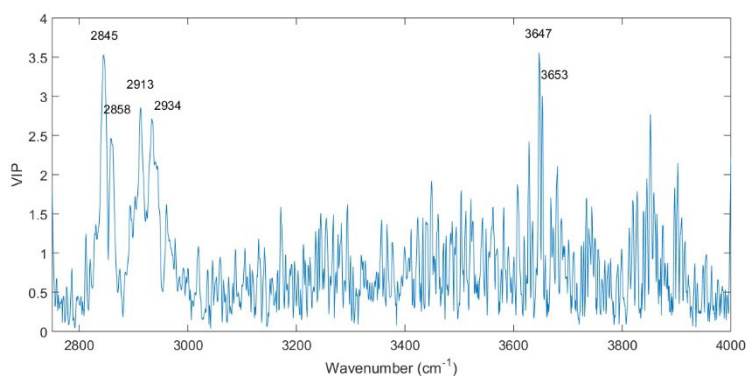


Figure 3: Graphic of the weight of the variables (VIP) in the constructed PLS model.

The acidity of coffee is due to phosphoric acid, chlorogenic acids, quinic acid, and organic aliphatic acids present in roasted coffee. It was possible to observe in the bands mentioned above, absorptions that have their origin in the bonds present in the organic acids (O—H and C—H) (Ribeiro; Ferreira; Salva, 2011). Furthermore, the perceived acidity depends on the final quantity of acids present (and further extracted), which is mainly related to the roasting degree (Santos et al., 2016). Although it is not clear which compounds are responsible for the perceived acidity, it is known that citric, malic, acetic, quinic, and mainly chlorogenic acids, are the most abundant acid species in coffee beans and may be responsible for this organoleptic characteristic (Balzer, 2001).

The influence of the chlorogenic acids can be justified by the possible decomposition that occurs during the roasting process and the decomposition of compounds that affect coffee acidity. These catabolic processes of organic substances oxidation, especially sugars, which are transformed into energy and simpler compounds, such as ethanol, acetic acid, lactic acid, and butyric acid, are caused by bacteria and yeasts and are the result of fermentation dependent on the set of bacteria and yeasts present during the processing stages (Quintero; Mejía; Betancur, 2012).

The results highlight the potential of the creation of sensory routes for robusta coffees through fermentation induction with microbial culture. The sensory potential and prediction potential of process optimization from chemometric treatments were evidenced.

4 CONCLUSIONS

Sensorial results indicated a higher score for the acidity attribute, in dry fermentation with yeast at the time of 48 hours, indicating that the induced fermentation can act in the construction of sensory routes, becoming a new strategy for the production of quality robusta coffee.

The study indicates that it is possible to estimate the score of the sensory attribute *acidity* of the robusta coffee submitted to different processes and fermentation times using infrared spectra in the medium region associated with the PLS regression. The selected band (2800 to 4000 cm^{-1}) was able to capture the information related to the acidity attribute. The proposed methodology presents a mean error of 0.11 in the acidity score, being considered an acceptable value for this sensory analysis.

The considerable stability of the models allowed us to establish a correlation between the chosen spectral region and the sensory attribute *acidity*, since it is a chemical property with direct influence on the spectral profile, due to the presence of chlorogenic acids.

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