



Rapid Determination of Ethanol in Sugarcane Spirit Using Partial Least Squares Regression Embedded in Smartphone

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Abstract

Mobile devices were used to quantify ethanol content—via colorimetry—in *cachaça* samples to great analytical precision. Ethanol from the spirits reacted with ceric ammonium nitrate (CAN) and nitric acid (NA) to form a red-colored complex. Images from the reaction were taken over time using a smartphone device and processed using software PhotoMetrix Pro® via partial least squares (PLS). A priori, Doehlert matrix was used for optimization of reagents concentration in the complexation reaction, with hierarchical cluster analysis (HCA) as estimation method and a flatbed scanner as measuring tool. When compared with the UV-VIS reference method, results from mobile device imaging presented a root mean square error of prediction (RMSEP) of 0.0677% (v/v). A *t* test showed there is no significant difference between methods ($P < 0.05$).

Keywords Ethanol determination · *Cachaça* · Digital image analysis · Doehlert matrix · Partial least squares

Introduction

Brazil is known for the production of distilled spirits made from fermented sugarcane—the most popular of them being *cachaça*. Those spirits usually reach between 38 and 48% alcohol by volume (ABV) at 20 °C. During fermentation, sugar from sugarcane juice breaks into two main substances: ethylic alcohol and carbon dioxide (Baffa Júnior et al. 2011; Granato et al. 2014).

Sugarcane-based spirits, also known as *aguardentes*, are the fourth most consumed distilled beverage in the world. Brazil alone produces ca. 1.5 billion liters annually. Several cases concerning spirits adulteration have been reported, the most common being addition of water, ethanol from external sources, and methanol. The latter being especially toxic. Ethanol—when in higher doses—can alter and/or damage

the central neural system; therefore, it is important to accurately quantify its amount in alcoholic beverages (Sumbhate et al. 2012; Souza et al. 2014; Bortoletto et al. 2015).

There are different methods to determine ethanol in alcoholic beverages, such as densitometry, gas chromatography, infrared spectroscopy, spectrophotometry, and others. The official method designated by the Association of Official Analytical Chemicals (AOAC) for the determination of ethanol in alcoholic beverages is through pycnometer, despite the advantages of this methodology as high precision and the lack of need for calibration, this method is considered laborious, requiring a balance of high accuracy and strict temperature control of the laboratory, thus making routine use difficult. A less laborious and widely used method also recommended by the AOAC is the oxidation with dichromate in an acid medium and quantification via spectrophotometry, despite the advantages of this method, dichromate is carcinogenic, with the interest in alternative methodologies, the use of ceric ammonium nitrate (CAN) for quantification of ethanol becomes a very attractive methodology (Gerogiannaki-Christopoulou et al. 2003; Wang et al. 2003; Pinyou et al. 2011; Benedetti et al. 2015; Debebe et al. 2017). The use of digital imaging through mobile devices—be it for classification or quantification of chemical compounds—has seen increasing use and development. Santos and Pereira-Filho (2013) used digital imaging analysis—through a flatbed scanner—to identify adulteration in milk samples and quantify them via partial least squares (PLS) and principal component

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regression (PCR) methods. Botelho et al. (2014) used digital images acquired through a scanner to quantify Yellow Sunset (YS) currant in orange-based beverages via PLS method. Also, Peng et al. (2017) made use of digital imaging analysis to evaluate iron in food and water.

In their work, Wang et al. (2016) adopted digital images taken from a smartphone to classify different types of catechols in water samples from rivers through various methods such as principal component analysis (PCA), hierarchical cluster analysis (HCA), and linear discriminant analysis (LDA), as well as quantify them via PLS. Levin et al. (2016) made use of smartphones to evaluate fluoride in drinking water samples, correlating RGB values and fluoride concentration values. Helfer et al. (2017) classified Brazilian banknotes through PCA, as well as the amount of iron in vitamin supplements samples, using a smartphone app to build the models.

Design of experiments (DOE) is a powerful tool for optimizing processes; it gives information about the effect variables have upon a system and, therefore, allowing for a more precise tuning of the procedure. DOE may be univariate—when the effect of a single variable is evaluated—or multivariate—when several factors are observed simultaneously (Caldas et al. 2011). Most DOE—such as central composite designs (CCD) and Box-Behnken—uses the same amount of levels for each variable. Doehlert matrix, however, allows the assignment of different levels to each variable. This procedure is especially valuable when the effect of one variable is deemed more important than the others (Ferreira et al. 2007; Khajeh et al. 2010).

This article made use of a Doehlert matrix to assess the influence of the concentration of ceric ammonium nitrate (CAN) and nitric acid (NA) on the complexation reaction of cerium(IV) with ethanol (data was gathered using a flatbed scanner). The optimum condition was then employed in the quantification of ethanol in *cachaça* samples based on digital images taken from a smartphone and processed via PLS by a software application.

Materials and Methods

Reagents and Equipment

Ethanol standards were prepared from ethylic alcohol, absolute grade of 99.5% (C₂H₅OH, Dinâmica, Diadema, Brazil).

Table 1 Experiment planning ethanol determination, where X_1 corresponds to CAN and X_2 to NA concentrations

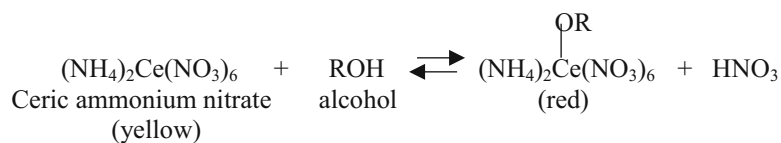
Experiment	X_1 (mol L ⁻¹)	X_2 (mol L ⁻¹)
1	0.15 (0)	0.10 (0)
2	0.15 (0)	0.20 (+ 1)
3	0.20 (+0.866)	0.15 (+ 0.5)
4	0.15 (0)	0 (− 1)
5	0.10 (−0.866)	0.05 (− 0.5)
6	0.10 (−0.866)	0.15 (+ 0.5)
7	0.20 (+0.866)	0.05 (− 0.5)

CAN ((NH₄)₂Ce(NO₃)₆, Dinâmica, Diadema, Brazil) was employed as the complexing agent and acidified by bidistilled NA (HNO₃). Six different samples from *cachaça* were purchased at the local market.

For the DOE, a flatbed scanner (HP Scanjet 300) was used, a volumetric flask with 5 mL capacity was used, the images were captured at 600 dpi, a white mask with a hole in the center was placed in order to acquire the images always in the same position, and the sample was covered with a white plastic cap for uniformity of acquisition and added another black cap to prevent interference of luminosity external. The images were evaluated on software ChemoStat® with a ROI of 64 × 64 pixels. Ethanol quantification was conducted using multivariate PLS calibration through smartphone app PhotoMetrix Pro® on a Samsung GALAXY A3 device (Android 5.0.2, 8 MP camera); a 10-mm-wide cuvette was used and ROI of 64 × 64 pixels. UV-VIS measurements were conducted on a Perkin Elmer Lambda 25 spectrophotometer, 460 nm wavelength and 10 mm wide cuvettes.

Ethanol Quantification

Ethanol quantification in *cachaça* samples was conducted via colorimetric method. CAN reacts with ethanol resulting in a red-colored cerium(IV) alkoxide complex, whose color intensity and RGB values are registered by a digital camera (Shriner et al. 2004).



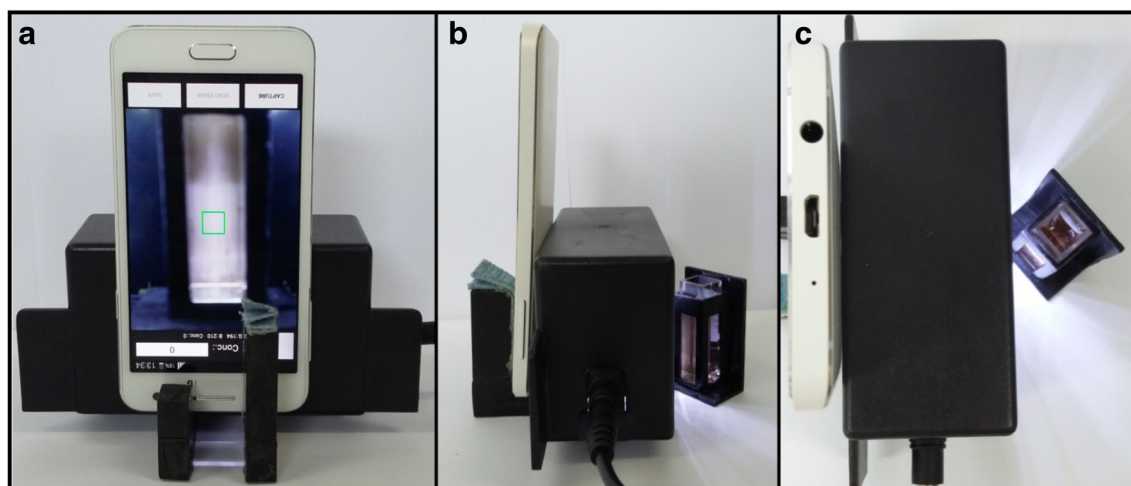


Fig. 1 Illustration of the method of acquisition of the digital images by the smartphone. **a** Front image of the system, **b** side image, and **c** higher image

Design of Experiments

A two variable Doehlert matrix DOE was employed to identify optimum reaction condition. Concentrations of CAN (X_1) and NA (X_2) were varied in three and five levels, respectively. Namely, CAN concentration ranged from 0.10 to 0.20 mol L⁻¹ (0.10, 0.15, and 0.20 mol L⁻¹), while NA concentration ranged from 0 to 0.20 mol L⁻¹ (0, 0.05, 0.10, 0.15, and 0.20 mol L⁻¹). Table 1 summarizes the design.

To each experimental condition, a 5-mL volumetric flask was filled with the appropriate masses of CAN and NA and ultrapure water to completion. Immediately before measurement, 160 μ L of 5% (v/v) ethanol solution were combined with 4 mL of the complexing solution. Images of the reaction were made at each minute for 10 min aiming to evaluate the complex stability.

Calibration Curve and Ethanol Determination from *Cachaça* Samples

After the DOE, a calibration curve was built employing the optimum reaction conditions. Six different ethanol standard solution concentrations were used, namely 0, 1, 2, 3, 4, and 5% (v/v). Again, 160 μ L of the appropriate ethanol solution were combined with 4 mL of the complexing solution.

For ethanol determination, six different *cachaça* samples purchased at the local market were diluted ten times with ultrapure water. One hundred sixty microliters of the sample was combined with 4 mL of the complexing solution. The cuvette containing the mixture was placed in black polyethylene box 10 \times 4 \times 6 cm with controlled light (two led strips with 12 lamps each); the luminosity was controlled from a solarimeter model SL 200 (Kimo) that showed a luminosity of 9 W m⁻² on the sample where images were acquired using the smartphone, as shown in Fig. 1. The images taken were processed within the smartphone through PhotoMetrix Pro® software application.

Digital imaging in both processes (calibration curve and ethanol determination) were conducted in triplicates, and the construction of the PLS curve was based on mean centered histograms.

Results and Discussion

Design of Experiments

Results from the DOE were evaluated through HCA, aiming to determine which experimental conditions are more stable both in relation to the concentration based on the experimental planning and also that this condition is not affected by the time.

Table 2 Example of image acquired by the scanner in the reaction time of 5 min that was used in the HCA analysis

Condition / Time	1	2	3	4	5	6	7
5 min							

The numbers from 1 to 7 correspond to the experiments in Table 1

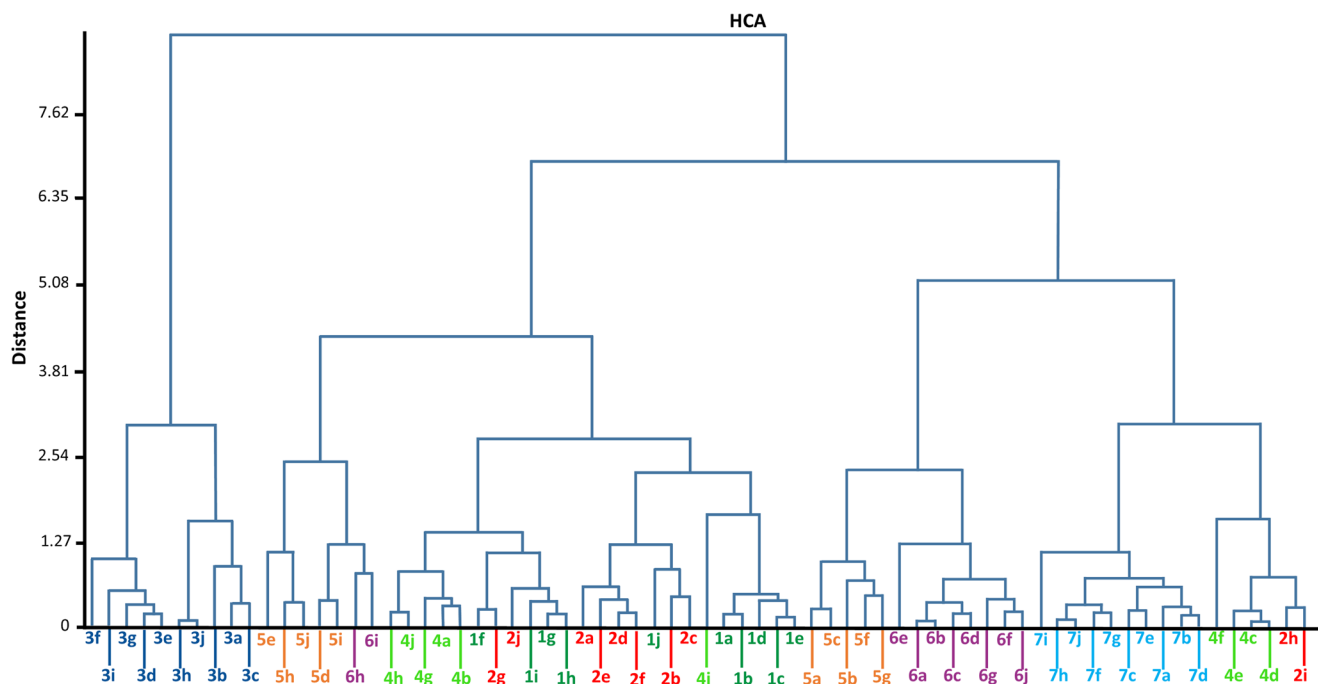


Fig. 2 Dendrogram of the images using the means of the channels R, G, H, V, L, and I

Once a condition is found where the color intensity remains constant, this condition can be differential avoiding any type of variability that the reaction equilibrium may suffer. Table 2 shows examples of the images acquired, with time equal to 5 min, to each experimental condition presented in Table 1.

The dendrogram in Fig. 2 illustrates the arrangement of the clusters produced by HCA. It was obtained evaluating the average autoscaled values of R, G, H, V, L, and I (red, green, hue, value, lightness, and intensity) channels. Euclidean distance is used as the distance metric between clusters. Letters from *a* to *j* represent the time elapsed (from 1 to 10 min, respectively), and numbers from 1 to 7 represent each experimental condition.

Through the analysis of the dendrogram, the variation among replicates can be seen over time. Samples 1, 2, and 4 are seen scattered in the same cluster, which can be attributed to them as having the same CAN concentration. These samples lie in an intermediate position in the DOE.

Samples 5 and 6, which lie in the bottom levels of CAN concentration, can be found scattered in two ways: one cluster formed by images of replicates at the starting time and another formed by images of replicates at the ending time. Hence, it is possible to assign such fluctuation to the amount of NA added, seeing that sample number 6—which has a higher NA concentration—shows less fluctuation.

Samples 3 and 7, which lie in the higher levels of CAN concentration, are the only ones where all replicates exist in the same cluster. The replicates from sample 3, although within the same cluster, show a somewhat high level of scattering. The cluster itself is also farther away from the others. Sample

7 shows lower variance over time when compared with sample 3. The behavior of samples 3 and 7 is opposite to what is seen from samples 5 and 6 concerning the concentration of NA. A possible explanation is the fact that in lower concentrations of CAN, a higher amount of NA ends up regenerating the complexing agent.

Thus, reaction conditions equal to those of sample 7 were chosen as optimum to build the calibration curve, given the lower variance among the outcomes and, also, for presenting one of the highest color intensities, which increases the method accuracy.

Ethanol Quantification

To quantify ethanol content in *cachaça* samples, UV-VIS was used as reference method. Said method presented a coefficient

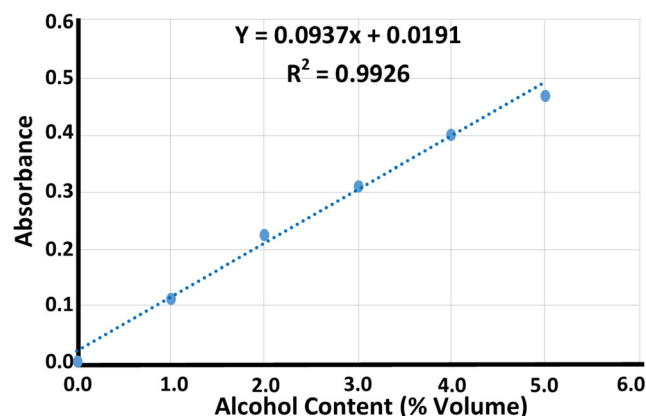


Fig. 3 Calibration curve obtained by UV-VIS data

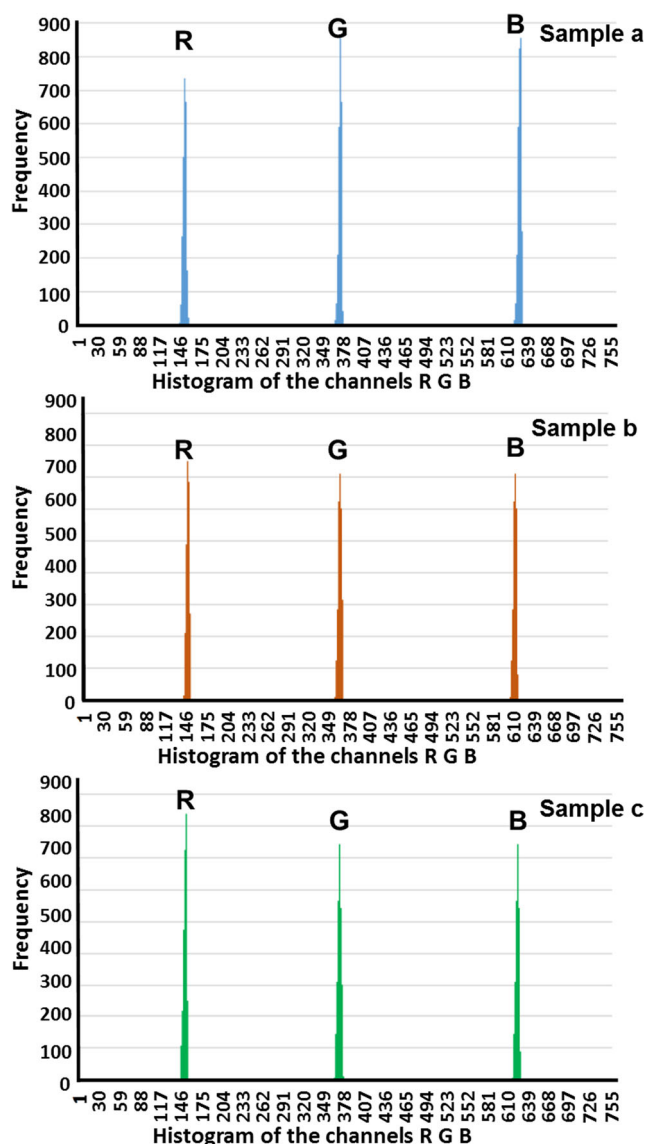
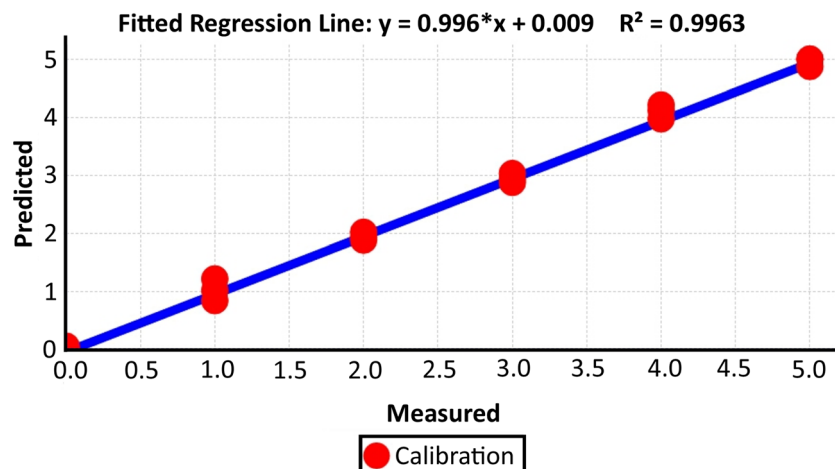


Fig. 4 Histogram of the R G B channels of the sample triplicates channel R (1–256), channel G (257–512), and channel B (513–768)

Fig. 5 Calibration curve obtained by the PLS model using digital image data



of determination equal to 0.9926. Figure 3 shows the calibration curve generated by the method.

The model built from the mobile device's images did so by using PLS. The images were obtained by the smartphone main camera through PhotoMetrix Pro®. The images were unfold in R, G, and B histograms, resulting in one vector of the 768 variables for each channel (256 R, 256 G, and 256 B) per sample. PLS was embedded in the PhotoMetrix Pro® application using NIPALS algorithm and applied to develop the regression model for ethanol determination. It exhibits a coefficient of determination (R^2_{calc}) equal to 0.9963 and root mean square error of calibration (RMSEC) of 0.1073% (v/v). Figure 4 illustrates RGB histograms from replicates of one of the samples. Figure 5 shows the calibration curve generated by the software application. Red points correspond to standards.

A comparison between data obtained from UV-VIS spectrogram and PLS-calibrated digital imaging can be found in Table 3. The last column presents the difference between both techniques, expressed as relative error that was calculated considering as reference the values obtained by UV-VIS, and it was below 4% for all samples. Moreover, a paired t test (with a 95% confidence interval) was performed and showed no significant difference between the two methods, indicating that the proposed method is accurate. The relative standard deviation (RSD) (Table 3) is very low (< 0.5%), indicating that both methods are precise.

The RMSEP, calculated by comparing the results obtained through mobile device digital imaging and the reference UV-VIS, is equal to 0.0798% (v/v). This value is very satisfactory, comparing with the work of Benedetti et al. (2015) that used camera for quantification of ethanol in drinks; from the data available in that work, it was possible to calculate the RMSEP of 2.35% (v/v).

The limits of detection (LOD) and quantification (LOQ) for the PLS model using digital imaging were determined through ten consecutive measurements of blank solution. LOD and LOQ were calculated as the mean concentration of the blank

Table 3 Ethanol concentration, expressed as % v/v, obtained by the reference method (UV-VIS) and by PLS image analysis

Sample	UV-VIS		PLS		Relative error (% v/v)
	Ethanol content (% v/v)	RSD (% v/v)	Ethanol content (% v/v)	RSD (% v/v)	
A	37.6	< 0.1	37.6	0.2	0.0
B	39.2	< 0.1	40.5	0.1	3.4
C	38.9	< 0.1	38.4	0.1	− 1.4
D	39.3	< 0.1	39.7	0.1	− 3.6
E	37.9	< 0.1	38.5	0.2	1.5
F	36.3	< 0.1	35.2	0.1	− 3.0

Relative standard deviation (RSD) for $n = 3$

measures plus 3.3 and 10 times the standard deviation of these ten measures, respectively (IUPAC 2006). The LOD and LOQ values were 0.19% (v/v) and 0.62% (v/v), which are comfortably lower than the minimum ethanol amount required in *cachaça*, according to legislation (see the “Introduction” section).

The proposed method presented lower error (RMSEP), good accuracy, and higher analytical frequency when compared with the reference methodology (pycnometer) and lower toxicity compared with similar methodologies such as quantification of ethanol by dichromate, thus being considered an alternative for the quantification of ethanol in *cachaças*.

Conclusions

The obtained results show the efficiency of digital imaging techniques using mobile devices to optimize experiments and/or quantify chemical species. Optimization of experiments using the HCA method proved to be a fast, cheap, and efficient methodology. Results from ethanol quantification employing smartphones displayed high accuracy and precision, proving to be a valuable analytical technique to quantify chemical compounds.

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Compliance with Ethical Standards

Conflict of Interest Fernanda C. Böck declares that she has no conflict of interest. Gilson A. Helfer declares that he has no conflict of interest. Adilson B. da Costa declares that he has no conflict of interest. Morgana B. Dessuy declares that she has no conflict of interest. Marco F. Ferrão declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human or animal subjects.

Informed Consent Publication has been approved by all individual participants.

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