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Short communication

# Authenticity of roasted coffee using $^1$H NMR spectroscopy

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ABSTRACT

Coffee is globally one of the most widely used food products and is vital to the economies of countries involved in its production and export. Due to its commercial importance, the detection of impurities and foreign matters has been a constant concern in fraud verification, especially because it is difficult to perceive adulterations with the naked eye in samples of roasted and ground coffee. Until now, there has been no comprehensive methodology for identification of the many types of coffee adulterants. This work describes, for the first time, a fast and effective technique using $^1$H NMR to check for adulteration in roasted coffee. The samples used were a set of commercial Brazilian arabica blends produced from good cup quality beans, and four adulterants (corn, coffee husks, barley, and soybean). The methodology was effective for all the adulterants analyzed in 31 commercial samples.

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## 1. Introduction

Fraud in the food sector has grown considerably in recent years, especially in the case of products with high aggregate value and physical characteristics that favor adulteration (Shears, 2010; Cai et al., 2016). Coffee is globally one of the most popular beverages (EMBRAPA, 2016; ICO, 2016 ICO, 2016) and Brazil is the largest producer as well as one of the largest consumers of coffee (USDA, 2016). The consumption of coffee has increased in recent years, stimulated by advances made in terms of product quality (ABIC, 2016). However, the adulteration of roasted coffee is a strategy used to reduce costs, and is both frequent and varied. It can involve the quality of the beans (considering species, geographical origin, and defective beans), as well as the addition of other substances (coffee husks and stems, corn, barley, chicory, wheat middlings, brown sugar, soybean, rye, triticale, and açaí) to coffee blends in order to make them less expensive (Pauli et al., 2014; Toci et al., 2016; Winkler-Moser et al., 2015).

Adulteration in coffee impairs its sensory quality and can cause harm to the consumer, whether economic or health-related (ABIC, 2016). The presence of impurities affects the quality of the ground and roasted coffee, causing undesirable effects in terms of flavor, aroma, acidity, bitterness, and the final sensorial characteristics of the drink (Tavares et al., 2012; Toledo et al., 2016). The materials used to adulterate coffee are usually low cost, available in large quantities, and similar in appearance to roasted coffee after grinding (Lopez, 1983). In order to ensure good coffee quality, special care is required during the pre-harvest, harvest, and post-harvest phases, because many factors can cause changes that influence product quality (Oliveira et al., 2009; Toledo et al., 2016).

The conventional methods that are most widely used in laboratories to identify the adulteration of roasted and ground coffee involve the use of optical and electron microscopy (Menezes Júnior and Bicudo, 1958). Complementary physicochemical analyses include measurements of moisture content, mineral residues, ether-extractable substances, and caffeine. Analyses based on microscopy are frequently slow and subjective, and can produce conflicting results (Toci et al., 2016; Toledo et al., 2016).

The development and application of fast and accurate methods for the detection and quantification of contaminants can help to minimize or even eliminate coffee fraud. To this end, various techniques have been studied in order provide more secure, reproducible, and wide ranging analyses of different types of adulteration. In addition to optical microscopy, other important analytical techniques such as chromatography and infrared spectroscopy have been studied for the identification of

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adulteration in roasted and ground coffee (Ebrahimi-Najafabadi et al., 2012; Domingues et al., 2014; Aquino et al., 2014; Reis et al., 2013).

In this context, nuclear magnetic resonance spectrometry (NMR) coupled with multivariate statistics has been shown to be a useful procedure for the determination of adulterants in different food matrices (Consonni and Cagliani, 2010). This technique provides information on the structure and chemical composition of the major chemical constituents of the sample (Tavares and Ferreira, 2006). Such applications include the study of wine (Ogrinc et al., 2003), beer (Lachenmeier et al., 2005), fruit juices (Belton et al., 1998), meat (Jakes et al., 2015; Ritota et al., 2012; Shintu et al., 2007 Ritota et al., 2012; Shintu et al., 2007), fish (Erikson et al., 2012; Martinez et al., 2009), dairy products (Brescia et al., 2005), honey (Bertelli et al., 2010), and oils (Agiomyrgianaki et al., 2010; Šmejkalová and Piccolo, 2010). The NMR technique has the advantage of using small amounts of sample, without any need for pretreatment. It avoids the production of toxic waste, is non-destructive, and generates multiple data from a single spectrum.

The present work describes, for the first time, the use of $^{1}$H NMR for identification and quantification of the main adulterants in roasted and ground coffees, including barley, corn, soybean, and coffee husks. Principal components analysis of the spectral profile was used, in order to improve the reliability of the results. The findings should assist coffee producing countries in adopting measures to protect their markets.

## 2. Materials and methods

### 2.1. Samples and roasting procedure

Nineteen batches of coffee beans were harvested at different farms in Brazil located in Guaxupé (south of Minas Gerais state), Franca (north of São Paulo state), and Paraná state. The samples used were a set of commercial Brazilian arabica blends produced from good cup quality beans, classified as 'strictly soft' and 'soft', and four adulterants (corn, coffee husks, barley, and soybean) purchased locally. The samples were roasted in a spouted bed roaster (IRoast, Gurnee, IL), using a temperature program from 196 °C (3 min) to 235 °C (2 min), resulting in a light-medium roast, according to the Roast Color Classification System (AGTRON—SCAA, USA, 1995). In order to ensure reliability of the results, 24 commercial coffee blends from different regions of Brazil were analyzed. Analysis was also performed on seven samples certified by ABIC (Associação Brasileira da Indústria de Café). Electron microscopy was used as a comparative method, following the procedure described by Lopez (1983). The results were compared with those obtained using the proposed methodology (Table 1).

### 2.2. Sample extraction

Portions (150 mg) of the roasted and ground materials (coffee, corn, coffee husks, barley, and soybean) were weighed into 2-mL Eppendorf tubes, followed by addition of 1000 μL of deuterated water. The tubes were vortexed for 1 min at room temperature and centrifuged at 87,965 rad/min (14000 rpm) for 10 min (Universal model 320R). Subsequently, 750-μL aliquots of supernatant were collected with an automatic volumetric pipette and transferred to 5-mm NMR tubes for analysis. The extractions were performed in triplicate.

**Table 1**
Comparison of ABIC method with proposed method.

| sample | ABIC method | proposed method |
|---|---|---|
| 3 | 0.62–6.75% barley | adulterated with barley |
| 25 | 2.39% corn | adulterated with corn |
| 9 | pure | pure |
| 10 | pure | pure |
| 21 | 7.35% coffee husks | adulterated with coffee husks |
| 26 | 3.69–10.16% barley | adulterated with barley |
| 30 | 2.60% coffee husks | adulterated with coffee husks |

### 2.3. $^{1}$H NMR analyses

$^{1}$H NMR spectra were acquired using a 600 MHz BrukerAvance III spectrometer equipped with a PABBO BB/19F-1H/D probe head with gradients, automated tuning, BVT-1000 and BCU-1 accessories for temperature control, and a Sample Express autochanger. Samples were measured at 298 K in manual mode. Automated tuning, locking, shimming, and calibration of 90° pulses were performed using standard Bruker routines. Two 1D experiments were performed in sequence:

Experiment 1: The first experiment consisted of a simple single pulse procedure (zg30) with 2 s relaxation delay (RD), 3.6 s acquisition time (AQ), recording of 64 free induction decays (FID) to form a 64k complex data point vector, spectral window of 20.0276 ppm, and detector gain (RG) of 18. After Fourier transformation (without apodization), automatic peak selection was applied to determine the residual water signal at 4.70 ppm. The values at 4.70 ppm were used as the band-selective saturation field in the next experiment. The phase and baseline were adjusted using TOPSPIN 3.2.5 software (Bruker Biospin).

Experiment 2: The second 1D experiment employed the ZGCPPR suppression pulse sequence, acquisition time (AQ) of 2.76 s, relaxation delay (RD) of 2 s, 128 free induction decays (FID), and water pre-saturation with low power radiofrequency irradiation at 48.05 dB, using the residual water signal at 4.70 ppm. An exponential function with LB = 0.3 was applied before Fourier transformation. The spectra were aligned for bucket integration of the (trimethylsilyl)-propionic-2,2,3,3-d4 acid (TSP) signal at 0 ppm, and were reduced by integration in 30 regions (buckets) from 0.15 to 10 ppm. Data analysis and integration of the signals was performed using TopSpin v. 3.2.5 spectrometer software (Bruker Biospin), or alternatively MestRenova v. 8.1.

### 2.4. Statistical analyses

A data matrix was constructed by integration of the $^{1}$H NMR data signals. Subsequently the data were treated and submitted to exploratory analysis using unsupervised pattern recognition, employing principal component analysis (PCA). The statistical analyses were performed with Pirouette v. 4.0 software.

## 3. Results and discussion

The $^{1}$H NMR spectra (Fig. 1) proposed as fingerprints showed that the chemical composition of the roasted coffee differed significantly from the compositions of the barley, soybean, corn, and coffee husk samples. Some of the compounds identified in coffee by Ribeiro et al., 2014 are indicated in spectrum 1A, among which stand out chlorogenic acid, caffeine, trigonelline, *N*-methylpyridine, and formic acid in the region from 6.0 to 9.3 ppm, and lipid compounds, amino acids, and aliphatic fatty acids in the region from 0.7 to 3.0 ppm. The adulterants studied (soybean, corn, and barley) are seeds in which the main components are carbohydrates. This characteristic was maintained even after the roasting process, as can be seen in the spectra shown in Fig. 1B–E (chemical shift region 5–3 ppm).

For the identification of an adulterant using this technique, it was necessary to find peaks that were present in the adulterant spectra but not in the coffee spectrum. The selection of these

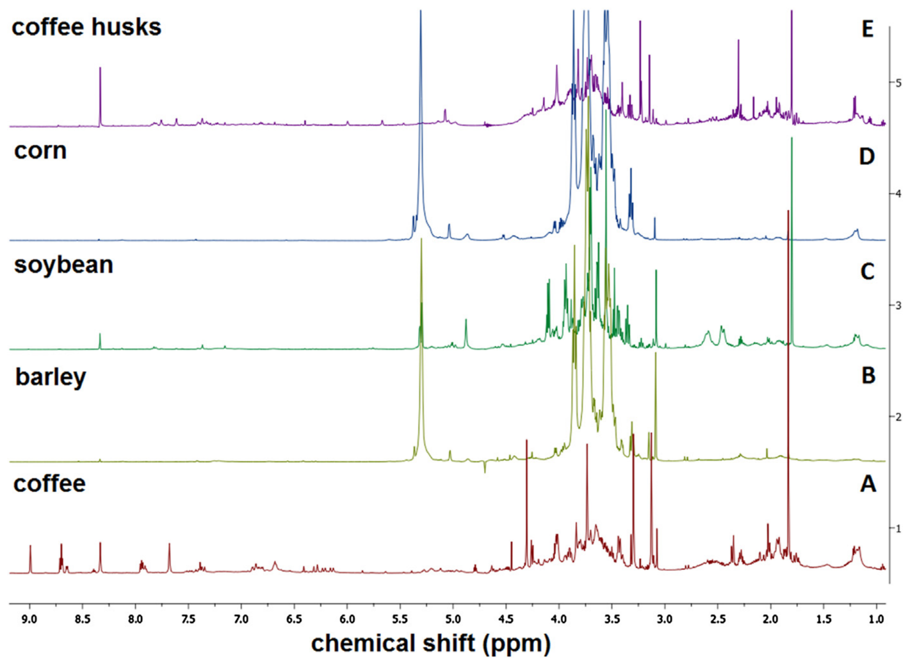


**Fig. 1.** $^{1}$H NMR fingerprints of roasted coffee (A), barley (B), soybean (C), corn (D), and coffee husks (E).

**Fig. 2.** Main adulterant marker peaks obtained by overlap with the fingerprint of roasted coffee: barley (A), soybean (B), corn (C), and coffee husk (D).

marker peaks considered two parameters, namely intensity and location, because there must be no interfering peaks. After superposition of the adulterant spectra with the fingerprint of roasted coffee, several peaks that could be used as markers were identified (Fig. 2). For indication of the presence of corn, a high intensity singlet chemical shift at 5.03 (s) was selected, corresponding to the signal of hydrogen attached to the anomeric carbon of a specific carbohydrate. Signals at 5.30 (s) and 3.15 (s) were chosen to indicate the presence of barley. For soybean, the marker signals were those that presented chemical shifts at 5.30 ($J$ = 4.15 Hz) (dd) and 4.87 (t). The coffee and coffee husk spectra showed considerable similarity, with various compounds present in the coffee that could also be observed in the husk. Signals at 5.08 ($J$ = 5.92 Hz) (d) and 4.98 (s) were identified as markers for the presence of coffee husk in the analyses of the commercial coffee samples.

Quantitative assessment of adulterants was also performed using blends of adulterants and roasted coffee. Using different peak markers, it was observed that increasing percentages (30, 50, and 70%) of adulterants in coffee blends resulted in increased intensity of the marker compound peaks (Fig. 3), ensuring that the analyses were quantitative.

Five of the 31 samples analyzed showed adulteration due to the presence of corn, barley, or coffee husk. Fig. 4 provides an overlay of the spectra for samples of pure coffee, corn, and commercial coffee, clearly revealing adulteration of the commercial coffee sample by corn, indicated by the corn signal at 5.30 ppm in the $^{1}$H NMR spectrum. It is noteworthy that most studies employing the NMR technique to study coffees have focused on the identification of individual substances or small groups of substances present in extracted coffee fractions. The approach adopted in this work considered the spectrum in its entirety.

### 3.1. Statistical analysis

Before performing the chemometric analysis, the data were analyzed visually in order to identify differences between the spectra. It is important to perform this analysis of NMR data, in order to confirm that spectral differences actually originate from differences between the compositions of samples, rather than from problems in the spectroscopic measurements associated with

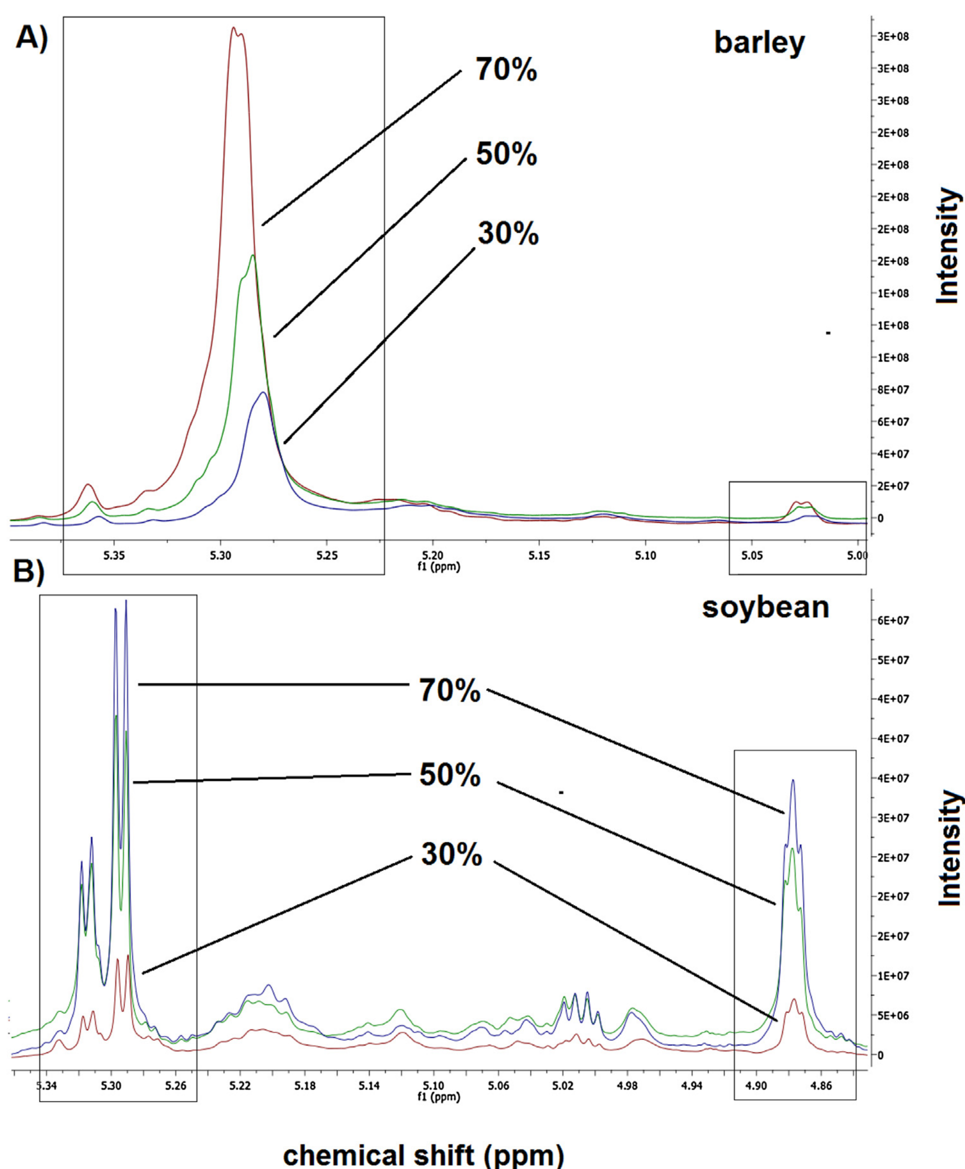


**Fig. 3.** Marker peak intensities according to adulterant percentage: barley (A) and soybean (B).

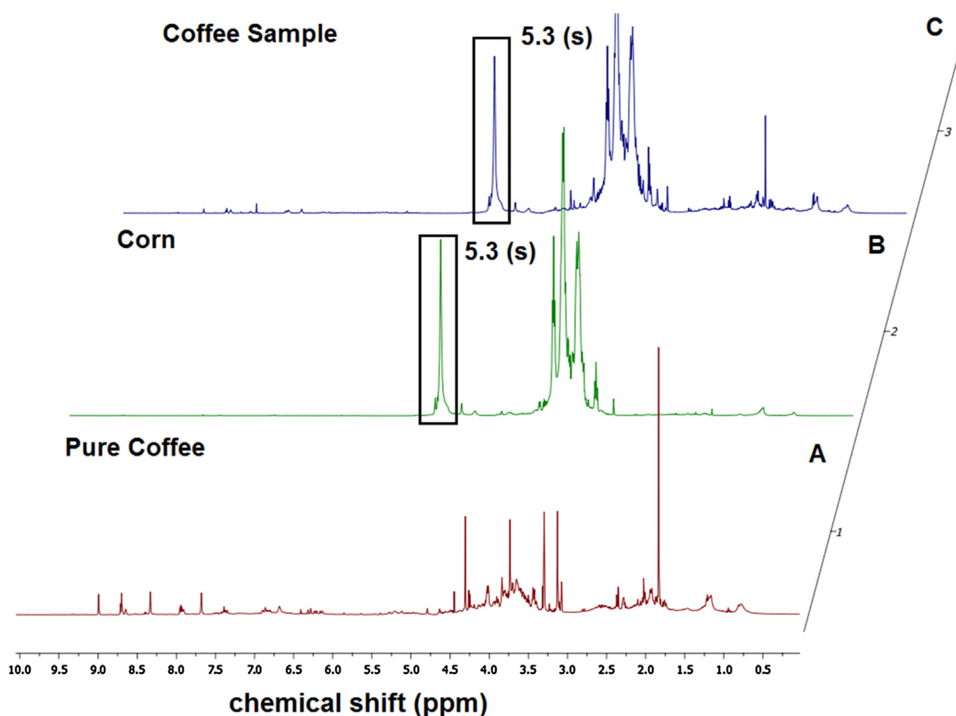


**Fig. 4.** Overlay of $^{1}H$ NMR spectra for pure roasted coffee (A), corn (B), and a commercial coffee sample (C).

factors such as shimming, signal suppression due to $H_2O$/HDO, phase shifts, and baseline corrections. Care must be taken that variations in these parameters do not lead to errors in the analysis, because when there are differences between the spectra, whether due to differences in the compositions of samples or to other factors, the chemometric analysis will distinguish between them, classifying them into many different groups. However, the data in question showed no visible problems.

Firstly, the analysis was performed using the entire spectrum, except the region of the TSP signal corresponding to the hydrogens of the reference and water ($H_2O$/HDO). The coffee extracts were analyzed without any pretreatment. Principal component analysis (PCA) was applied using the results for the 31 commercial coffee samples and the four adulterants, with autoscaling of the spectra. Three factors explained 47.20% of the total variance.

Two-dimensional graphs were used to assist interpretation of the PCA results. Scores graphs represent the sample data projected onto new axes corresponding to the main components, enabling identification of similarities and discrimination of different classes. Loadings graphs indicate those variables of the original set that contribute to the discrimination observed in the scores graphs, showing the differences in the chemical compositions of the samples. The scores plot of the samples with standard pretreatment (Fig. 5) showed good separation of main components 1 and 2 (PC1 × PC2). Some coffee samples (3, 25 and 26) appeared to be grouped with adulterants 8 (barley), 17 (corn), and 18 (soybean). However, samples 30 and 22 showed a tendency to group together with adulterant 7 (coffee husk). All the other pure samples showed two distinct groups resulting from the roasting processes and the geographical origins.

It could be seen from the loadings plot that discrimination between the samples was provided by the signals in the spectral region from 5.1 to 9.5 ppm, which provided the best differentiation of substances present in the coffee samples and the adulterants (Fig. 6). The results obtained by the proposed $^{1}H$ NMR method (Table 1) were compared with the results obtained by the ABIC method (electron microscopy), showing excellent agreement. It should be noted that this methodology could be applied for rapid evaluation of the presence of a larger number of adulterants.

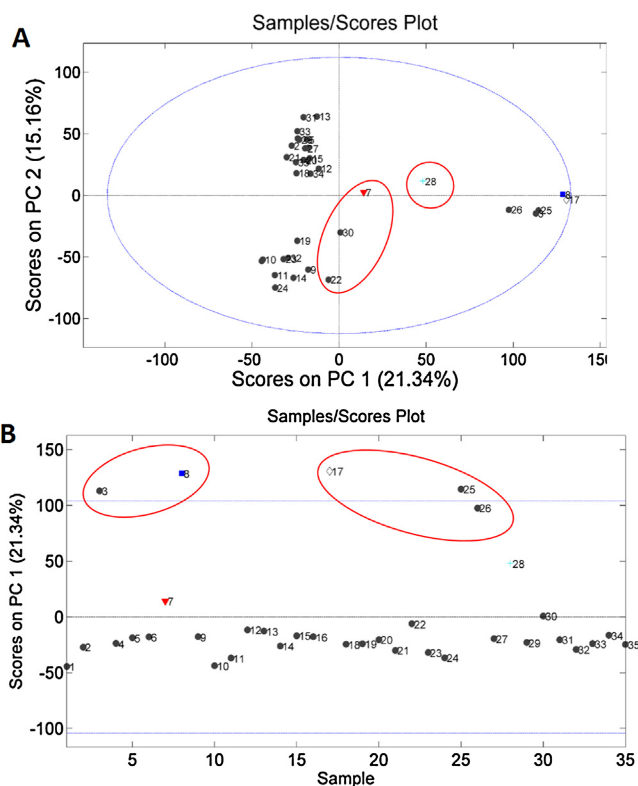


**Fig. 5.** Scores graphic for PC1 × PC2 (A) and scores graphic for PC1 (B).

The proposed procedure using the $^{1}H$ NMR technique proved to be highly effective for the identification and quantification of the main types of adulterants in roasted coffee. The next steps include identification of metabolites suitable for distinguishing the various

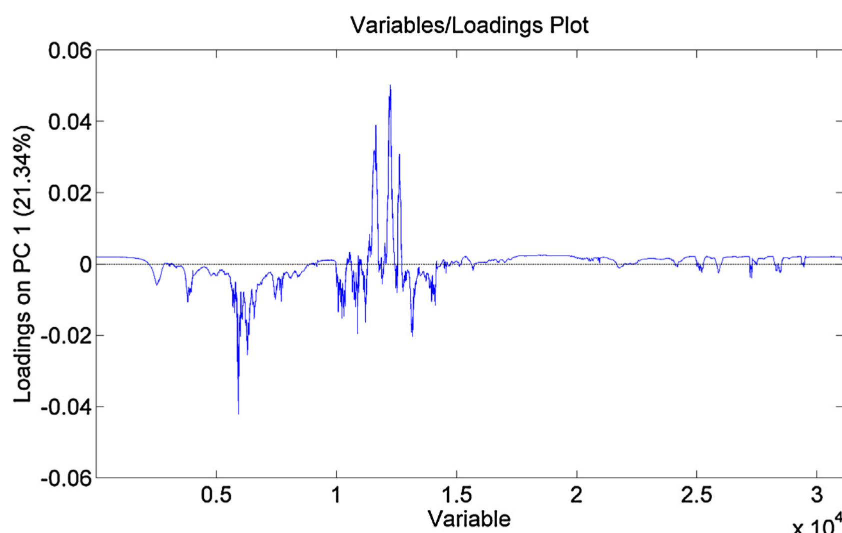


**Fig. 6.** Loadings graph obtained for PC1.

types of seeds and other adulterants, enabling elucidation of the main differences among them. This will pave the way for the elaboration of a broader approach involving a wider range of seed types found less frequently, as well as the influence of geographical variability on defective beans and adulterants.

The use of discriminant analysis enabled refinement of the results, making them more reliable and effective, since observation of the spectrum alone could not unequivocally identify the nature of the adulterant. Additional refinements of the technique will be made in the future, including considering other adulterants and different degrees of roast, as well as lower percentages of adulterants in the samples. With the identification of fraud in coffee marketed nationally, the methodology should help to facilitate addition of value to products by means of quality certification. It could be used in the adoption of more effective measures for the detection of adulterants (authenticity), as well as in certification of origins (traceability). In Brazil, these issues are currently the subject of government and commercial incentives.

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