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Evaluation of the potential of FTIR and chemometrics for separation between defective and non-defective coffees

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ABSTRACT

The objective of this work was to evaluate the potential of Fourier transform infrared spectroscopy (FTIR) for the discrimination of defective and non-defective coffee beans. Defective (black, immature and sour) and non-defective Arabica coffee beans were submitted to FTIR analysis by transmittance readings employing KBr discs and reflectance readings employing attenuated total reflectance (ATR) and diffuse reflectance (DR) accessories. Multivariate statistical analysis (PCA, clusters) was performed in order to verify the possibility of discrimination between defective and non-defective coffee samples. A clear separation between defective and non-defective coffee beans was observed, based on both PCA and cluster analysis of the reflectance spectra (ATR and DR accessories) and of the first derivatives of the transmittance spectra (KBr discs). Such results indicate that FTIR analysis has the potential for the development of a fast and reliable analytical methodology for the discrimination between defective and non-defective coffee beans.

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1. Introduction

The presence of defective coffee beans depreciates the quality of the coffee beverage consumed worldwide (Mancha Agresti, Franca, Oliveira, & Augusti, 2008). The intrinsic defects (sour, black and immature beans) are the ones that, when roasted, contribute the most to the depreciation of the coffee beverage quality. According to Clarke and Macrae (1987), black beans are usually associated with a heavy flavour, sour beans contribute to sour and oniony tastes, while immature beans will impart astringency to the beverage. The negative effect that such beans have on coffee quality can be associated with specific problems that occur during harvesting and post-harvest processing operations. Black beans result from dead beans within the coffee cherries or from beans that fall naturally on the ground by action of rain or over-ripening (Mazzafera, 1999). The presence of sour beans can be associated with ‘overfermentation’ during wet processing and with improper drying or picking of overripe cherries, whereas immature beans come from immature fruits (Clarke & Macrae, 1987; Mendonça, Franca, Oliveira, & Nunes, 2008). Defective beans represent about 20% of the total coffee produced in Brazil and, although they are separated from the non-defective beans prior to commercialisation in external markets, the majority of these beans are dumped on the Brazilian internal market. Thus, the roasting industry in Brazil has been using these defective beans in blends with healthy ones, and overall, a low-grade roasted coffee is consumed in the country (Oliveira, Franca, Mendonça, & Barros-Junior, 2006).

Colour sorting is the major procedure employed for separation of defective and non-defective coffee beans prior to roasting. In Brazil, manual sorting is usually employed for bean quality classification and electronic sorting is employed in farms and cooperatives of producers for the actual removal of defective beans. In the electronic sorters, coffee beans pass, one by one, by an electronic eye or camera system, and depending on wavelength measurements, the bean is either allowed to pass or it is shot with a puff of air into a reject pile (Franca & Oliveira, 2008). However, such procedure is not efficient for the separation of sour and immature beans. Actually, in order make sure that such defects are effectively removed from a specific coffee lot, colour sorting machines are usually set up to allow non-defective coffees to be also removed if their colour is similar to that of sour or immature beans. As a consequence of this, the coffee lots that are rejected as defective may present a high percentage of good coffee, as pointed out in studies employing machine sorted mixtures or low quality Arabica coffees from different origins and crops (Farah, Monteiro, Calado, Franca, & Trugo, 2006; Franca, Mendonça, & Oliveira, 2005; Franca, Oliveira, Mendonça, & Silva, 2005; Vasconcelos, Franca, Glória, & Mendonça, 2007). The same problem is present if separation by sieving is employed (Franca, Oliveira, et al., 2005; Mendonça, Franca, & Oliveira, 2009).

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Recent studies have shown that some chemical parameters could be employed for the separation between defective and non-defective green coffee beans of a given variety (Arabica or Robusta). Examples include levels of histamine, determined by high performance liquid chromatography – HPLC (Vasconcelos et al., 2007) and electrospray-ionisation mass spectrometry (ESI-MS) profiles (Mendonça et al., 2008). However, most of the employed instrumental techniques and analytical procedures are time demanding, expensive and involve a considerable amount of manual work. Recent studies have also shown that FTIR-based methods, in combination with chemometric techniques, can be successfully applied in the food industry, in association with food quality evaluation (Rodriguez-Saona & Allendorf, 2011). FTIR-based methods are fast, reliable, simple to perform and do not require sample pre-treatment. Such technique provides simple and reproducible means of handling food products with nondestructive analyses, with the sampling/analysis procedure usually taking only a few minutes.

There are a few studies that have focused on FTIR applied to coffee analysis, employing either roasted coffee or aqueous extracts (e.g. coffee beverage). The specific applications were discrimination between Arabica and Robusta varieties (Kemsley, Ruault, & Wilson, 1995), detection of glucose, starch or chicory as adulterants of freeze-dried instant coffees (Briandet, Kemsley, & Wilson, 1996), evaluation of roasting conditions (Lyman, Benck, Dell, Merle, & Murray-Wijelath, 2003), geographical discrimination (Wang, Jun, Bittenbender, Gautz, & Li, 2009) and separation between decaffeinated and regular roasted coffees (Ribeiro, Salva, & Ferreira, 2010). Thus, the objective of this work was to evaluate the potential of Fourier transform infrared (FTIR) spectroscopy in the characterisation and discrimination between defective and non-defective coffee beans prior to roasting.

2. Materials and methods

Arabica green coffee samples, acquired from Café Fino Grão (Contagem, MG), were comprised of coffee beans obtained from different cooperatives located in Minas Gerais State, Brazil, that were rejected by colour sorting machines. Black, sour (separated into light and dark coloured), immature and non-defective beans were manually picked to constitute separate sampling lots. Colour measurements were performed using a tristimulus colorimeter (HunterLab Colorflex 45/0 Spectrophotometer, Hunter Laboratories, VA, USA), with standard illumination D_{65}, and colorimetric normal observer angle of 10°, employing both whole and ground coffee samples. Colour measurements were performed thrice for each sample.

A Shimadzu IRAffinity-1 FTIR Spectrophotometer (Shimadzu, Japan) with a DLATGS (Deuterated Triglycine Sulphate Doped with L-Alanine) detector was used in the measurements that were all performed in a dry atmosphere at room temperature (20 ± 0.5 °C). For the transmittance readings, ground coffee samples (particle diameter <0.5 mm) were mixed with potassium bromide at a 1/50 ratio (w/w). This mixture (0.1 g) was then compressed into a thin KBr disc under a pressure of 7845 kPa for 5 min. The spectrum of a clean KBr disc (without coffee) was used for subtraction (background spectrum). For the reflectance readings, both diffuse and attenuated modes were employed. Diffuse reflectance (DR) measurements were performed in diffuse reflection mode with a Shimadzu diffuse reflectance sampling accessory (DRS8000A). The ground coffee sample (1 mg, particle diameter <0.15 mm) was mixed with KBr (100 mg) and then 23 mg of this mixture was placed inside the sample port. Pure KBr was employed as reference material (background spectrum). For the attenuated reflectance measurements (ATR-FTIR), a horizontal ATR sampling accessory (ATR-8200HA) equipped with ZnSe cell was employed. Although the ATR-FTIR technique has been mostly applied for analysis of liquid samples, there are recent studies that employ ATR for direct readings on solid food products (e.g. cheese, meats), given that it requires minimal sample preparation and variations in sample thickness have been shown not to affect the intensity of the bands (Argyri, Panagou, Tarantilis, Polysiou, & Nychas, 2010; Koca, Rodriguez-Saona, Harper, & Alvarez, 2007). In order to obtain a constant sample mass, a small metal recipient 2.4 mm thick and presenting an aperture of the same size of the ATR accessory (79 mm long and 10 mm wide) was placed over the ZnSe ATR crystal. The ground coffee sample (2 g, particle diameter <0.39 mm) was then placed inside the metal recipient and pressed with a spatula in order to obtain the best possible contact with the crystal. The empty recipient was used to obtain the background spectrum. The approximate total times required for sample preparation were 40 min (transmittance readings), 20 min (DR readings) and 5 min (ATR readings). Regardless of the sample preparation procedure, all spectra were recorded within a range of 4000–700 cm^{-1} with a 4 cm^{-1} resolution and 20 scans. Spectra treatment consisted of background subtraction, baseline correction and normalisation. The statistical package XLSTAT Sensory 2010 (Addinsoft, New York) was employed for the chemometric calculations.

3. Results and discussion

The average values of measured colour parameters for non-defective and defective coffee samples are shown in Table 1. The measurements were based on the CIE $L^*a^*b^*$ three dimensional cartesian (*xyz*) colour space, represented by: Luminosity (L^*), ranging from 0 (black) to 100 (white) – z axis; parameter a^*, representing the green–red colour component – x axis; and parameter b^*, representing the blue–yellow component – y axis. However, chromaticity can be better represented and discussed in terms of polar coordinates, so a^* and b^* values were converted to chroma (c^*) and hue angle (h), since these parameters can be directly associated to colour intensity or saturation (c^*) and to colour tone (h):

$$c^* = [a^{*2} + b^{*2}]^{1/2} \quad (1)$$

$$h = \tan^{-1}[b^*/a^*] \quad (2)$$

The results presented in Table 1 representing measurements performed on whole coffee beans, i.e., evaluation of the bean surface colour, show that black and dark sour beans presented lower luminosity values than non-defective, immature and light sour ones, indicating that this parameter can be successfully employed only to separate black and dark sour defects prior to roasting. Such results are in agreement with previous studies on physical attributes of defective coffee beans (Mendonça et al., 2009). It can also be observed that non-defective, immature and black beans presented higher values of hue angle in association with a greenish tone, whereas the lower values of hue angle observed for sour beans are associated to a yellowish brown tone. Black and dark sour beans presented the lowest values of colour saturation. Colour measurements taken for ground samples represent an average colour of the material, taking into account both the surface and interior. Luminosity values were higher for ground beans compared to whole ones, as a consequence of the fact that the bean surface is darker than its core. Values for colour parameters for both whole and ground samples were similar to those reported on previous studies employing defective coffees from different crops and origins (Franca, Oliveira, et al., 2005; Mendonça et al., 2009).

The results presented in Table 1 for whole beans indicate that the monochromatic colour separation procedure commonly employed in farms and cooperatives will only be effective in the case of black and dark sour defects. This can be corroborated by

Table 1
Average colour attributes of non-defective and defective coffee samples.

Sample	Whole beans					Ground beans				
	L^*	a^*	b^*	h	c^*	L^*	a^*	b^*	h	c*
Non-defective	46.1 ± 1.1[a]	2.7 ± 0.3[c]	19.0 ± 0.8[b]	81.8 ± 1.0[b]	19.2 ± 0.8[a]	59.6 ± 1.3[b]	2.3 ± 0.6[c]	19.4 ± 0.6[b]	83.3 ± 1.5[c]	19.6 ± 0.6[b]
Immature	43.1 ± 1.1[b]	1.8 ± 0.3[d]	20.3 ± 0.8[a]	84.9 ± 0.6[a]	20.4 ± 0.8[b]	61.2 ± 1.1[a]	0.5 ± 0.2[d]	21.7 ± 0.9[a]	88.7 ± 0.4[b]	21.7 ± 0.9[a]
Sour (light)	37.1 ± 2.1[c]	6.5 ± 1.0[a]	16.8 ± 0.9[c]	68.8 ± 3.4[c]	18.1 ± 0.8[c]	55.7 ± 0.6[c]	4.1 ± 0.3[a]	18.1 ± ± 0.5[c]	77.3 ± 0.7[e]	18.6 ± 0.6[c]
Sour (dark)	29.6 ± 2.0[d]	3.7 ± 0.4[b]	10.2 ± 1.4[d]	70.0 ± 1.8[c]	10.9 ± 1.4[d]	48.2 ± 0.5[d]	3.5 ± ± 0.1[b]	18.0 ± 0.2[c]	78.9 ± 0.4[d]	18.4 ± 0.2[c]
Black	27.6 ± 1.3[d]	0.9 ± 0.2[e]	6.7 ± 0.8[e]	82.4 ± 1.4[b]	6.8 ± 0.8[e]	36.0 ± 0.9[e]	−1.3 ± 0.4[e]	10.9 ± 0.9[d]	97.0 ± 2.1[a]	11.0 ± 0.9[d]

Average ± standard deviation. Average values followed by the same letter in the same column do not differ significantly by the Tukey test at 5% probability.

the score plots obtained for PCA analysis of colour parameters of whole beans (see Fig. 1). Data matrices for PCA analysis were assembled so that each row corresponded to a sample and each column to a colour parameter. The first two principal components (PCs) explained 66% and 32% of the data variance, respectively. Four distinct groups can be perceived, separated by quadrant: light sour (positive PC1, positive PC2); dark sour (negative PC1, positive PC2); black (negative PC1, negative PC2); and non-defective/immature (positive PC1, negative PC2). The first component allowed for the separation between darker and lighter samples, being mostly affected by luminosity values. Separation by the second component can be associated to black, immature and non-defective beans presenting a greenish tone as opposed to the yellowish hue of sour beans. Such results indicate that even sorting systems that employ bi-chromatic light measurements will not be able to completely separate immature and non-defective coffee beans.

Typical FTIR spectra obtained for green coffee samples are shown in Fig. 2. A full assignment of the spectral bands is quite challenging problem and is not the scope of this work. Furthermore, FTIR literature data on coffee is only available for roasted samples, so a direct comparison cannot be done. Nonetheless, a few qualitative aspects of the spectra can be discussed. The spectra obtained by transmission and reflectance are similar from a qualitative point of view, in the sense that the most significant bands can be viewed in both types of spectrum. Also, higher intensity of peaks can be observed in the spectra that employed KBr (transmission and diffuse reflectance, Fig. 2a and b, respectively), in the 1800–800 cm^{-1} range. The two sharp bands that can be viewed in the 3000–2800 cm^{-1} range (2924–2922 and 2852 cm^{-1}) have also been reported for both Arabica and Robusta roasted coffee samples, but no identification was attempted (Kemsley et al., 1995). Nonetheless, studies of FTIR analysis of caffeine on soft drinks have also reported two sharp peaks at 2882 and 2829 cm^{-1}, with the latter one being correlated with the asymmetric stretching of C—H bonds of methyl (—CH_3) group in the caffeine molecule and the peak region being successfully used to develop predictive models for quantitative analysis of caffeine (Paradkar & Irudayaraj, 2002). The sharp band at 1743 cm^{-1} has been also observed on FTIR studies of roasted coffee (Kemsley et al., 1995; Lyman et al., 2003; Wang et al., 2009). Kemsley et al. (1995) reported that a band at 1744 cm^{-1} was larger in Arabica in comparison to Robusta sample and attributed this to the carbonyl (C═O) vibration associated to the ester group in triglycerides. The study by Lyman et al. (2003) also associated the bands in that region to aliphatic esters (1755–1740 cm^{-1}). A band at 1658 cm^{-1} appears in the spectra obtained by KBr transmission, as can be seen in Fig. 2a, and it is also associated to caffeine absorption (Lyman et al., 2003). Ribeiro et al. (2010) reported that wavenumbers in the range of 1700–1600 cm^{-1} are highly related to chlorogenic acids and caffeine concentration in coffees. We confirmed the identification of the bands previously associated to caffeine (2922, 2852 and 1658 cm^{-1}) by FTIR-ATR analysis of aqueous extracts of non-defective coffee spiked with caffeine. There was a significant increase in peak intensity with the increase in caffeine concentration (spectra not shown).

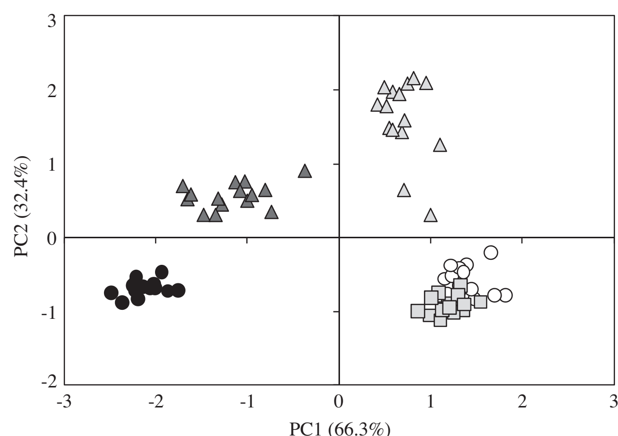

Fig. 1. PCA scores scatter plot of $L^*a^*b^*$ colour parameters for whole coffee beans (PC1 vs. PC2). ○, non-defective; □, immature; △, sour (light); ▲, sour (dark); ●, black.

Other bands that appear at lower intensity can be viewed in the range of 1600–1000 cm^{-1}. According to Kemsley et al. (1995), Briandet et al. (1996), and Lyman et al. (2003), chlorogenic acids present strong absorption in the region of 1300–1150 cm^{-1}. Chlorogenic acids correspond to a large family of esters formed between quinic acid and one to four residues of certain *trans*-cinnamic acids, most commonly caffeic, *p*-coumaric and ferulic (Clifford, Kirkpatrick, Kuhnert, Roozendaal, & Salgado, 2008). Axial C—O deformation of the quinic acid occurs in the range of 1085–1050 cm^{-1} while O—H angular deformation occurs between 1420 and 1330 cm^{-1}. The C—O—C ester bond also absorbs in the 1300–1000 cm^{-1} range (Silverstein, Webster, & Kiemle, 2005). Thus, the bands at 1381–1376, 1161–1153 and 1053 cm^{-1} could be associated to chlorogenic acids. The wavenumber range of 1400–900 cm^{-1} is characterised by vibrations of several types of bonds, including C—H, C—O, C—N and P—O (Sablinskas, Steiner, & Hof, 2003; Wang et al., 2009). Carbohydrates have been previously shown to exhibit several absorption bands in this region (Briandet et al., 1996; Kemsley et al., 1995), so it is expected that this class of compounds will also contribute to the several bands appearing in this region.

PCA analysis of the KBr transmission spectra, employing (a) baseline correction and normalisation and (b) first derivatives is displayed in Fig. 3. The analysis was based on a 24 × 1192 data matrix assembled so that each row corresponded to a sample and each column represented the spectra data at a given wavelength. For these specific analyses, dark and light coloured sour beans were grouped together, because the sampling preparation required a reasonable amount of time. In the case of PCA based on the spectra (Fig. 3a), the two first components accounted for 80.2% of the total sample variance. A certain amount of sample separation can be observed, mainly for non-defective (negative PC1, positive

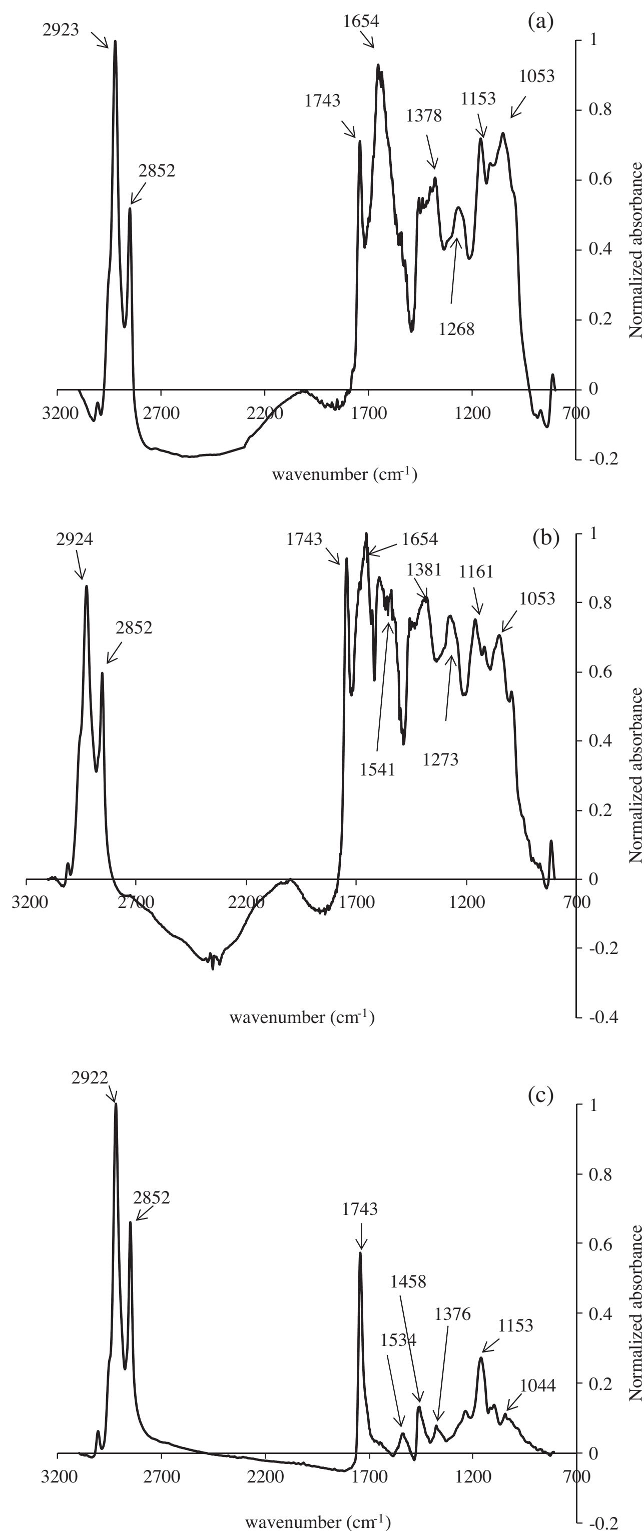

Fig. 2. Typical green coffee bean FTIR spectra obtained by (a) transmittance readings employing KBr discs and reflectance readings employing (b) DR and (c) ATR accessories (spectra manipulation consisted of baseline correction and normalisation).

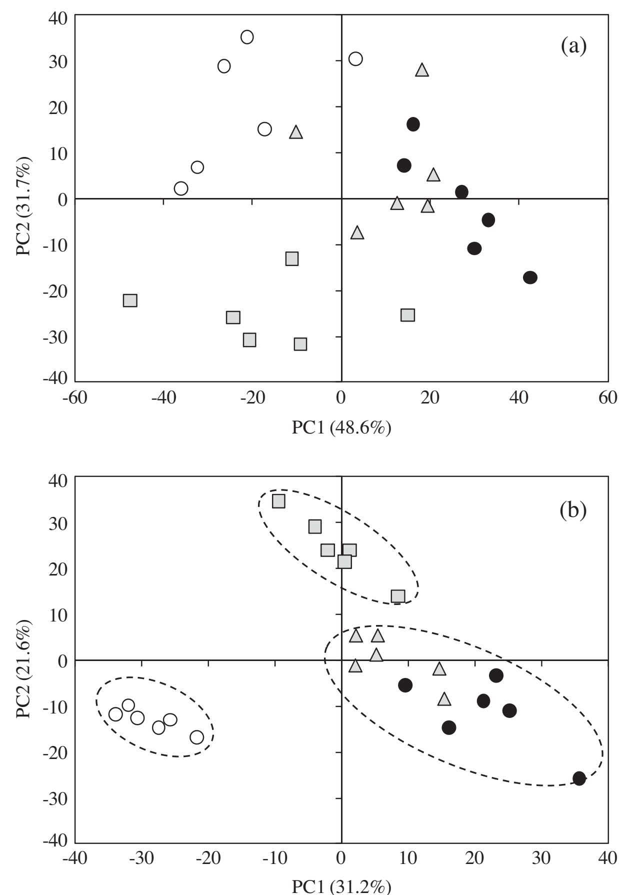

Fig. 3. PCA scores scatter plot (PC1 vs. PC2) of KBr transmission FTIR spectra submitted to (a) normalisation and baseline correction and (b) first derivatives. (○, non-defective; ▢, immature; △, sour (light and dark mixed); ●, black).

PC2), immature (negative PC1, negative PC2), and fermented (sour/black positive PC1), but there was a significant scattering of the samples. In the case of PCA based on the first-derivative of the spectra (Fig. 3b), the first and second principal components accounted for 31.2% and 21.6% of the total sample variance, respectively. In this case, sample scattering diminished considerably and there was a clear separation between non-defective and defective coffee beans. Three separate groups can be identified: (i) non-defective, (ii) immature and (iii) black/sour (fermented). Evaluation of the PC1 and PC2 loadings plots (not shown) did not indicate specific regions of the spectra that could be directly associated to group separation.

PCA analysis of the DR spectra, employing baseline correction and normalisation is displayed in Fig. 4a. Analysis was based on a 45×1188 data matrix assembled so that each row corresponded to a sample and each column represented the spectrum data at a given wavelength. The two first components accounted for 94.2% of the total sample variance. A clear separation between defective and non-defective coffees can be observed, with four major groups: non-defective (positive PC1, negative PC2), immature/light sour (positive PC1, positive PC2), dark sour (negative PC1, positive PC2), and black (negative PC1, negative PC2). Clustering of immature and light sour defects was also observed by Mendonça et al. (2008) for analysis of the ESI(+)-MS profiles. Evaluation of the loadings plot (not shown) indicated that the spectral ranges that presented the highest influence on sample grouping were 2980–2850 and 1560–800 cm^{-1} corresponding to immature/light sour samples, 1700–1570 cm^{-1} corresponding to non-defective beans, 3100–3000, 1980–1760 in reference to dark sour, and 2000–1985 cm^{-1} corresponding to black beans. Group separation was not enhanced by taking derivatives of the spectra.

PCA analysis of the ATR reflectance spectra, employing baseline correction and normalisation is displayed in Fig. 4b. Analysis was based on a 54×1188 data matrix assembled so that each row cor-

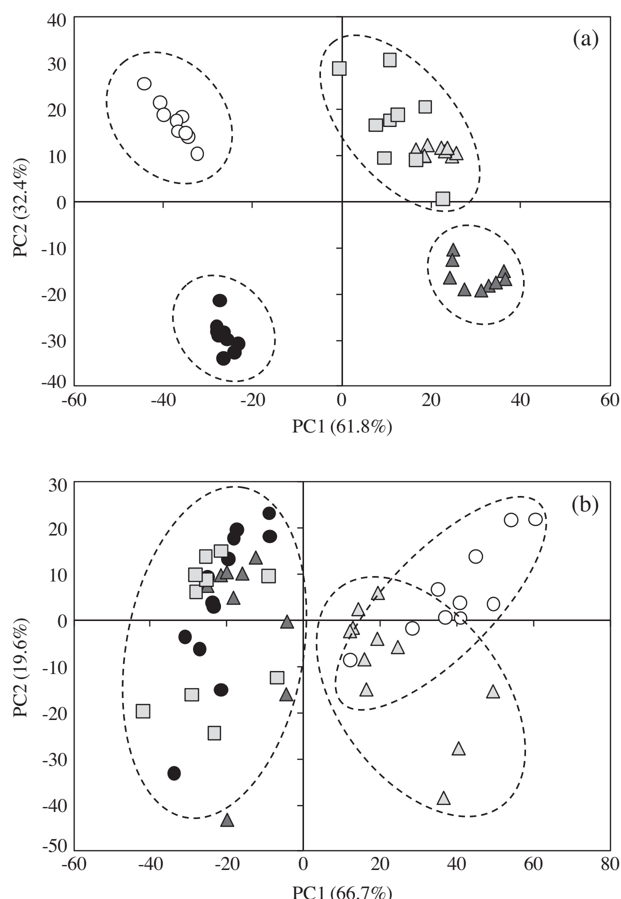

Fig. 4. PCA scores scatter plot (PC1 vs. PC2) of reflectance spectra submitted to normalisation and baseline correction (a) DRIFTS and (b) ATR-FTIR (○, non-defective; □, immature; △, sour (light); ▲, sour (dark); ●, black).

responded to a sample and each column represented the spectra data at a given wavelength. The two first components accounted for 86.3% of the total sample variance. The first component provided separation of the evaluated samples into two major groups: non-defective/light sour (positive PC1) and black/dark sour/immature (negative PC1). Evaluation of the loadings plot (not shown) indicated that the spectral ranges that presented the highest influence on PC1 values in association with the black/dark sour/immature group were the following: 1554–1482, 1797–1776 and 3100–3020 cm^{-1}. The only significant band that can be observed in the ATR spectra (Fig. 2b) in those ranges is the one at 1534 cm^{-1}. It was also present in the spectra obtained by Lyman et al. (2003) for aqueous extracts of roasted coffee, regardless of roasting conditions, although no identification was attempted. We herein infer that this band is most likely associated to C=C stretch in a nitrogen based ring, such as caffeine or trigonelline, both present in significant amounts in raw and roasted coffees. In the case of PCA based on the first-derivative of the spectra, the first and second principal components accounted for 28.6% and 12.6% of the total sample variance, respectively. No group separation could be observed.

To verify the similarity between the samples and to single out some classes, agglomerative hierarchical clustering (AHC) was applied to the set of variables employed for PCA. Ward's hierarchical clustering method and chord distance were employed to establish clusters and calculate dissimilarity coefficients, respectively. The resulting dendrograms are displayed in Fig. 5. Group clustering confirmed the PC analysis. In the case of transmittance spectra obtained with KBr discs (Fig. 5a), a clear separation between non-defective and defective coffees can be observed, in three major clusters: non-defective, immature and fermented (black/sour). Diffuse reflectance spectra (DR) also provided complete separation between non-defective and defective coffee beans, as can be seen in Fig. 5b. Four major clusters can be viewed, in reference to black, dark sour, non-defective and immature/light sour. In this case overall clustering can be also related to sample surface colour, with the darker samples (black and dark sour) being completely separated from the remaining lighter samples. ATR spectra did not provide a complete separation between defective and non-defective coffees, as shown in Fig. 5c. Three major clusters can be viewed, the first one comprised of non-defective and light sour coffees and the other two containing immature, black and dark sour coffees. Grouping of black and dark sour coffees has been previously reported for analysis of ESI(+)-MS profiles (Mendonça et al., 2008), in association with lower sucrose levels of such beans in comparison to non-defective ones. This may also be the case here, since reduction in sucrose levels will probably occur after fermentation. Also, in the case of immature beans, low sucrose levels can be associated to the bean maturity state whereas for black and sour beans, sucrose reduction is probably due to fermentation. Grouping of black and immature beans was also observed in the analysis of the volatile profile of roasted coffees (Mancha Agresti et al., 2008), in association to the occurrence of black beans being associated to the fermentation of immature ones. The same study also reported grouping of non-defective and sour beans (no separation between dark and light sour), in association to sour beans corresponding to non-defective coffees that underwent fermentation during handling and processing after harvest.

The results obtained in the present study showed that FTIR-based methods seem an attractive alternative for developing a fast routine method for discrimination of non-defective and defective coffees. Derivatives of the spectra based on transmittance readings employing KBr discs allowed grouping according to the specific type of defect. However, we foresee two major disadvantages with this particular sampling technique. The first one is related to the time and care required during sample preparation. The second one is related to the small amount of coffee (0.002 g) that is employed for each analysis. DRIFTS also provided a clear separation between defective and non-defective coffees, with the advantage of less sample preparation, i.e., ground coffee is just mixed with KBr and directly placed in the DR accessory to be analysed, without the further need of pressing and preparation of a clear disc (~20 min). Nonetheless, the amount of coffee employed for analysis is even smaller (0.00023 g). Although the previous sampling methods provided satisfactory separation between defective and non-defective coffees, the fact that the sample mass is quite small could be a problem if quantitative analysis is needed, i.e., determining the amount of defective beans that are present in a given coffee sample. It is noteworthy to point out that, although ATR-FTIR did not provide complete separation between non-defective and light sour coffees, such results can still be deemed satisfactory, given that the main problem with colour sorting is the separation of immature and non-defective beans. Thus, ATR-FTIR could be viewed as a complementary procedure to be employed after colour sorting and, among the evaluated sampling techniques, it is the one that allows the use of larger samples (2 g), and thus will probably be more appropriate for quantitative evaluation.

The method used by coffee traders for coffee classification is based on the types and amount of defective beans present in a sample (usually 300 g out of a bag of about 60 kg). The professional sorter is trained to identify the defective and non-defective beans solely by their appearance, with colour being the most effective characteristic contributing for the differentiation (others would be size, shape, etc.). The methodology herein studied, employing the ground beans, could be used to replace the professional sorters

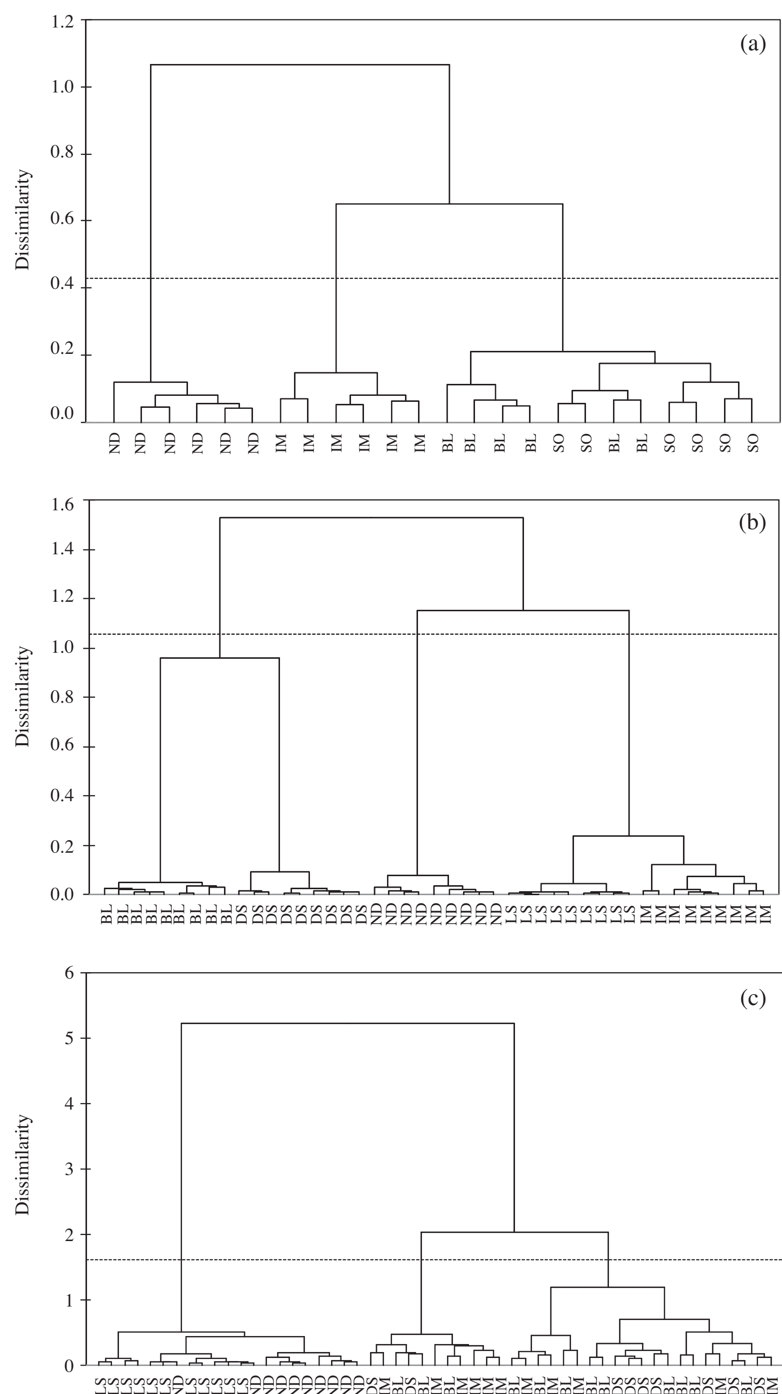

Fig. 5. Hierarchical cluster analysis (HCA) of (a) first derivatives of FTIR spectra obtained by transmittance readings employing KBr discs and normalised FTIR spectra obtained by reflectance readings employing (b) DR and (c) ATR accessories (ND, non-defective; SO, sour; LS, light sour; DS, dark sour; IM, immature; BL, black).

in the process of classification of coffee samples for commercialisation, thus eliminating the subjectivity of the current procedure.

4. Conclusions

The feasibility of employing FTIR as a methodology for the separation between defective and non-defective coffees was evaluated and successfully demonstrated. PCA and AHC results indicated that non-defective and defective coffee samples could be separated into distinct groups, based on transmittance or reflectance spectra obtained by mixing the coffee samples with KBr, i.e., transmittance readings employing KBr discs and DRIFTS readings. PCA and AHC results based on normalised ATR-FTIR reflectance spectra indicated the separation of the samples into two major groups: non -defective/light sour and black/dark sour/immature. The results obtained in the present study confirm that FTIR analysis presents the potential for the development of an analytical methodology for the discrimination between defective and non-defective coffee beans. Further studies will be conducted employing larger sets of samples in order to develop predictive models. The methodology will be also tested for roasted coffee samples.

It is noteworthy to point out, however, that FTIR-based methodologies are devised for dealing with particles, liquids or solids of

large smooth surfaces, making them inappropriate for use with whole coffee beans. Thus, the methodology proposed herein does not allow for the actual separation of the single defective beans in an automated production processes, but represents a first step towards its achievement, in the sense that infrared spectral ranges that presented the highest influence on group separation were identified. When the beans are ground, the chemical makeup of the bean surface will remain embedded in the sample and thus will contribute to the makeup of the FTIR spectra. Also, with the exception of the lipid fraction, which presents a somewhat non uniform distribution within the beans (the wax fraction is only found in the outer layer of the bean), all the other classes of compounds are evenly distributed throughout the bean, including its surface. Thus, one should expect the spectra for the whole beans to be similar to those for the ground beans. Considering the suitability of the method for ground samples shown in this study, the proposal for future implementation in automatic sorting of single defective beans would be to employ infrared radiation at specific wavelengths, in a way similar to that of the colour sorting machines.

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