UNIVERSIDADE FEDERAL DE MINAS GERAIS
FACULDADE DE FARMÁCIA

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CLASSIFICATION AND QUANTIFICATION OF DEFECTIVE AND NON-DEFECTIVE COFFEES BY FTIR AND NIR SPECTROSCOPY

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Tese apresentada ao Programa de Pós-Graduação em Ciências de Alimentos da Faculdade de Farmácia da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do título de Doutor em Ciências de Alimentos.

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Orientadora: Dra. Adriana Silva Franca

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CLASSIFICATION AND QUANTIFICATION OF DEFECTIVE AND NON DEFECTIVE COFFEES BY FTIR AND NIR SPECTROSCOPY

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Your work is going to fill a large part of your life, and the only way to be truly satisfied is to do what you believe is great work. And the only way to do great work is to love what you do. If you haven’t found it yet, keep looking. Don’t settle. As with all matters of the heart, you'll know when you find it.

(Steve Jobs)

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RESUMO

A presença de grãos defeituosos é um importante parâmetro diretamente relacionado à qualidade do café, pois é associado a características sensoriais indesejáveis na bebida. Os grãos defeituosos que mais contribuem para a depreciação da bebida são os grãos pretos, ardidos e imaturos. O método convencional empregado na avaliação da qualidade de cafés torrados é baseado na análise sensorial da bebida ou "prova de xícara", que demanda considerável tempo para ser executado, requer provadores treinados e depende de um controle rigoroso do grau de torração. Diante do exposto, este estudo teve como objetivo avaliar o potencial das técnicas espectroscópicas FTIR e NIR para a avaliação da qualidade de cafés com base na presença de grãos defeituosos. Grãos de café foram manualmente separados em cinco classes: sadio, ardido claro, ardido escuro, preto e imaturo. Cada uma das classes foi processada a três temperaturas (220 °C, 235 °C e 250 °C) e três níveis de torração (claro, médio e escuro) obtendo-se nove condições de torração. As amostras de café torrado foram então moídas, peneiradas e analisadas por DRIFTS, ATR-FTIR e NIR em um estudo classificatório. Os resultados de PCA indicaram que, com base nos espectros obtidos por DRIFTS, é possível discriminar as amostras em quatro grupos: (a) sadio, (b) preto, (c) ardido escuro e (d) ardido claro, com café imaturo dispersado entre os cafés ardidos. ATR-FTIR proporcionou a discriminação das amostras, apesar de não efetivamente, em dois principais grupos: (a) sadio e ardido claro, e (b) preto, ardido escuro e imaturo; enquanto NIR proporcionou a discriminação das amostras em três principais grupos: (a) sadios, ardido claro e imaturo, (b) ardido escuro e (c) preto. Nas três técnicas a variância entre as amostras levou à discriminação de cafés prioritariamente por suas classes, independentemente das suas condições de torração. Os modelos de classificação para os espectros obtidos por DRIFTS foram desenvolvidos por LDA enquanto que os modelos para ATR-FTIR e NIR foram desenvolvidos por rede Elástica. Porcentagens altas de amostras corretamente classificadas (até 100%) foram obtidas nos três modelos desenvolvidos. As variáveis discriminantes que contribuíram para a correta classificação de amostras nos modelos desenvolvidos por rede Elástica, para os dados de ATR-FTIR e NIR, foram extraídas e proporcionaram a seguinte interpretação dos modelos: (a) café sadio foi diretamente relacionado a altos teores de carboidratos e lipídios e baixos teores de proteína e/ou aminoácidos e cafeína; (b) café ardido claro foi relacionado a altos teores de carboidratos e cafeína; (c) café ardido escuro foi diretamente relacionado a altos teores de ácidos alifáticos e baixos teores de lipídios; (d) café preto foi relacionado a níveis altos proteínas e/ou aminoácidos e baixos níveis de lipídios; e (e) café imaturo foi relacionado a altos níveis de proteínas e/ou aminoácidos e cafeína e baixo conteúdo de lipídios. Misturas de grãos sadios e defeituosos, com %defeitos variando de 0% a 30% em passos de 3%, foram produzidas e analisadas por ATR-FTIR e NIR para um estudo quantitativo. PLSR foi utilizada para o desenvolvimento dos modelos quantitativos que proporcionaram resultados satisfatórios. Valores de RMSEP baixos como 2,6% e valores de R^2 altos como 0.956 no conjunto de validação foram obtidos. De um modo geral, os modelos desenvolvidos com espectros obtidos por NIR apresentaram-se mais robustos e acurados em relação aos modelos de ATR-FTIR.

Palavras-chave: café, grãos defeituosos, FTIR, NIR.

ABSTRACT

A major parameter directly related to coffee quality is the presence of defective beans, which impart negative sensory aspects to the beverage. The defects that contribute the most to the depreciation of the beverage quality are black, sour and immature beans. The conventional method used to assess the quality of roasted coffees is based on sensory evaluation, which, although reliable, is time-consuming and requires trained cupper experts. In view of the aforementioned, the objective of the present study was to evaluate the potential of FTIR and NIR spectroscopy as practical techniques to assess the quality of coffees based on the presence of defective beans. Coffee beans were manually sorted into five classes: black, dark sour, immature, light sour and non-defective. Each of the coffee classes was roasted at three temperatures (220 °C, 235 °C and 250 °C) and to three roasting degrees (light, medium and dark) obtaining nine roasting conditions. Roasted coffee samples were ground, sieved and analyzed by DRIFTS, ATR-FTIR and NIR for a classification study. Results from PCA indicated that based on DRIFTS spectra, coffee samples could be discriminated into four major groups: (a) non-defective, (b) black, (c) dark sour and (d) light sour, with immature beans scattered among the sour samples. ATR-FTIR provided the discrimination of the coffee samples, although not clearly, into two groups: (a) non-defective and light sour and (b) black, dark sour and immature, and NIR provided the discrimination into three major groups: (a) non-defective, light sour and immature, (b) dark sour, and (c) black. At all cases the variance among the samples led to the discrimination of the coffees primarily by their classes, regardless of roasting degree. Classification models for DRIFTS spectra were developed by LDA while classification models for ATR-FTIR and NIR were developed by Elastic net. High percentages of correct classification, up to 100%, were achieved with each of the techniques employed. The discriminating variables that contributed to the correct classification of the samples from the Elastic net models, for ATR-FTIR and NIR data, were extracted and provided the following interpretation of the models: (a) non-defective coffee was directly related to high levels of carbohydrates and lipids and lower levels of proteins and/or amino acids and caffeine; (b) light sour coffee was related to high levels of carbohydrates and caffeine; (c) dark sour coffee was directly associated with high levels of aliphatic acids and low levels of lipids; (d) black coffee was related to high levels of proteins and/or amino acids and low levels of lipids; and (e) immature coffee was related to high levels of proteins and/or amino acids and caffeine and low levels of lipids. In a second part of this study, blends of defective in admixture with non-defective coffee, with %defects ranging from 0% to 30% in steps of 3%, were produced and analyzed by ATR-FTIR and NIR for a quantification assay. PLSR was used to construct the models that provided satisfactory results. RMSEP values as low as 2.6% and R^2 values as high as 0.956 in the validation set were achieved. Overall, NIR overcame ATR-FTIR in terms of robustness and accuracy.

Key-words: coffee, defective beans, FTIR, NIR.

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LIST OF ABBREVIATIONS

*L	Luminosity
ATR	Attenuated total reflectance
DRIFTS	Diffuse reflectance Fourier transform spectroscopy
FTIR	Fourier transform infrared
FT-NIR	Fourier transform near infrared
HPLC	High-performance liquid chromatography
IR	Infrared
LASSO	Least absolute shrinkage and selection operator
LDA	Linear discriminant analysis
MIR	Mid-infrared
MSC	Multiplicative scatter correction
NIR	Near-infrared
PC	Principal component
PCA	Principal component analysis
PLSR	Partial least squares regression
RMSEC	Root mean square errors of calibration
RMSECV	Root mean square errors of cross validation
RMSEP	Root mean square errors of prediction
SPME-CG-MS	Solid phase micro extraction gas chromatography-mass spectrometry

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1. INTRODUCTION

The term 'quality' is officially defined by the International Organization for Standardization (ISO) as "the extent to which a group of intrinsic features (physical, sensorial, behavioral, temporal, ergonomic, functional, etc.) satisfies the requirements, where requirement means need or expectation which may be explicit, generally implicit or binding" (ISO, 2000). Thus, product quality can assume different meanings for consumers, producers and regulating organizations. In the case of coffee, quality may result from factors like the production system, the aspect and chemical composition of the green or roasted beans, and to the final beverage characteristics (RIBEIRO et al., 2011).

The quality of the raw bean is determinant in the coffee commercialization process and price quotations, and can be assessed by many physical and sensory parameters. Although time-consuming and dependent on trained cupper experts (FERIA-MORALES, 2002; PETRACCO et al., 2005), the sensory analysis or 'cupping' is an important and reliable method for this purpose. Each producing country weighs sensory characteristics in a different way, and this parameter must be considered as specific to each commercial origin (BEE et al., 2005). In Brazil, for example, coffees are classified based on their cup quality in seven categories: strictly soft, soft, softish, hard, rioysh, rio and rio zona (BRASIL, 2010). On the other hand, Kenya, Colombia and Central American countries have their own cup quality classification (BEE et al., 2005). It is important to mention that the sensory parameters of the raw coffee are ideally evaluated when roasting and grinding are conducted under controlled conditions. The Specialty Coffee Association of America, that maintains standards for the classification of specialty coffees in an international level, recommends that, to most accurately assess the quality of coffees, the beans must be roasted to a light to light-medium degree of roast, thus the cupper can clearly perceive the flavors and fragrances of the coffee. In addition, the samples should be ground immediately prior to cupping, no more than 15 minutes before infusion with water (SCAA, 2009).

In spite of being reliable for the classification of raw beans, most of times the 'cupping' is not suitable for the classification and inspection of roasted coffees from the market. This happens because there is no previous control of the roasting conditions, and low-grade coffees are generally roasted to a dark roasting degree to mask

unpleasant flavors and/or aromas. Besides the exposed drawback, in general, consumer demands related to food quality have resulted in an enormous increase in food standards, moving to zero-defects (TRIENEKENS and ZUURBIER, 2008). As a result, future technologies for the assessment of food quality will require sensitivity, miniaturization of instrumentation for portable use and simple sample preparation steps (CHO and KANG, 2011), contrary to the current sensory-based method used to assess the quality of coffees. Thus, there is a need to develop fast and reliable methods to assess the quality of roasted coffees.

A good example of such rapid, non-destructive and accurate fingerprinting techniques is infrared spectroscopy (PETRACCO et al., 2005, RODRIGUEZ-SAONA and ALLENDORF, 2011). In particular, much attention has been given to Fourier transform infrared (FTIR) spectroscopy, which detects fundamental molecular vibrations as a result of molecular absorption of mid-infrared radiation, and NIR spectroscopy, which measures broad overtone and combination bands of fundamental molecular vibrations (LARKIN, 2011). Many studies have shown that these techniques in association with multivariate statistics can be successfully applied to the analysis of crude, roasted and ground coffee and the coffee beverage. FTIR has been applied to the discrimination of arabica and robusta varieties (KEMSLEY et al., 1995), detection of adulterants (BRIANDET et al., 1996, REIS et al., 2013), discrimination between decaffeinated and regular coffees (RIBEIRO et al., 2010), evaluation of roasting degree (LYMAN et al., 2003, WANG and LIM, 2012, WANG et al., 2011) and geographical authentication (WANG et al., 2009). NIR spectroscopy has been applied to the discrimination and quantification of arabica and robusta blends (ESTEBAN-DÍEZ et al., 2004a, PIZARRO et al., 2007a, DOWNEY et al., 1997, DOWNEY and BOUSSION, 1996), quantification of caffeine, theobromine and theophylline (HUCK et al., 2005), evaluation of roasting degree (ALESSANDRINI et al., 2008) and prediction of sensory properties (ESTEBAN-DIEZ et al., 2004b, RIBEIRO et al., 2011).

In the present study, infrared spectroscopy is applied to assess the quality of roasted coffees. Specifically, the proposed methodology is based on the presence of defective beans. Defective beans are directly related to coffee quality, imparting negative sensory aspects to the beverage. Volatile substances from defective beans are often perceived at extremely low levels, masking the pleasant aroma of the non-defective beans (BEE et al., 2005). The defects that contribute the most to the depreciation of the beverage quality are black beans, associated with a heavy and ashy

flavor; sour beans, related to sour, acetic, and oniony tastes; and immature beans, that impart astringency and bitterness to the beverage (BEE et al., 2005, CLARKE, 1987b). The negative impact of such beans has led the scientific community to devote much effort to the characterization of defects from a chemical, physical and morphological point of view (BEE et al., 2005, VASCONCELOS et al., 2007, OLIVEIRA et al., 2006, FRANCA et al., 2005b, FRANCA et al., 2005a, RAMALAKSHMI et al., 2007).

Recently, CRAIG et al. (2011, 2012b) applied FTIR, using different measurement techniques, for the classification of defective and non-defective crude coffees. These measurement techniques included attenuated total reflectance (ATR), diffuse reflectance Fourier transform spectroscopy (DRIFTS) and transmittance measurements using KBr discs. In sequence, SANTOS et al. (2012) applied NIR to quantify defective and non-defective crude beans, from arabica and robusta coffees, and from different geographical origins. When it turns to roasted coffee, MANCHA AGRESTI et al. (2008) observed that, based on their volatile profiles, immature and black coffee could be discriminated from non-defective and sour coffee beans, while MENDONÇA et al. (2009a) did not find statistical difference among the electrospray ionization-mass spectra of defective and non-defective arabica coffees.

In view of the aforementioned, the objective of the present study was to evaluate the potential of FTIR and NIR spectroscopy for the classification and quantification of defective (black, immature, light and dark sour) and non-defective roasted coffee blends. The specific objectives that characterize the main steps of this study are:

- to evaluate the feasibility of employing DRIFTS for the discrimination between defective and non-defective roasted and ground coffees;
- to evaluate the feasibility of employing ATR-FTIR for the discrimination and quantification of defective and non-defective roasted and ground coffees;
- to evaluate the feasibility of employing NIR for the discrimination and quantification between defective and non-defective roasted and ground coffees;
- to develop classification models based on Elastic net for variable selection;

- to develop quantitative models based on partial least squares regression (PLSR) to predict the percentage of defective coffees in admixture with non-defective one.

2. LITERATURE REVIEW

2.1. Coffee: From fruits to roasted coffee

The coffee plant is an evergreen shrub or small tree from the *Rubinaceae* family with different species. The most economically important species are *Coffea arabica* L. (arabica coffee), that may become 4-6 m tall, and *Coffea canephora* Pierre ex Froehn (robusta coffee), that may grow 8-12 m. In cultivation both species are pruned to manageable heights of less than 2 m and less than 3 m in mechanically harvested plantations in Brazil (SIVETZ, 1979, ANZUETO et al., 2005). Although all species within the genus Coffea are of tropical African origin (BRIDSON and VERDCOURT, 1988), coffee cultivation is now widespread in tropical and subtropical regions, with the bulk of arabica coffee concentrated in Latin America and robusta coffee predominant in South-East Asia and Africa (ANZUETO et al., 2005).

Brazil is the leading coffee producer, with 36% of the world coffee production and 30% of the global exportations. In 2012, were produced and exported 50.5 and 33.5 million bags (60 kg), respectively (ABIC, 2013). The arabica coffee is the oldest known specie and is cultivated in mountainous regions at optimal altitudes from 1000 to 2000 m and optimal temperature from 15 to 24 °C. It is more susceptible to diseases, pests and frosts. The arabica specie produces those coffees most appreciated by discerning coffee drinkers (BANKS et al., 1999, CLARKE and MACRAE, 1985), and represents 60.3% of the world coffee supply (ICO, 2013).

Robusta coffee, on the other hand, grows at relatively low altitudes; tolerates higher temperatures and heavier rainfall, and demands higher soil humus content than arabica. Usually robusta is processed via dry processing and is mostly used to constitute coffee blends, soluble and instant coffees (BANKS et al., 1999). The demand for robusta has been especially high in recent years, since it provides a less costly alternative to Arabica. Nowadays this specie represents approximately 39.7% of the coffee produced worldwide, and Vietnam is the leading producer (ICO, 2013).

The coffee fruit is a drupe, usually called a berry or cherry, containing two seeds (coffee beans) embedded in a freshly pericarp and a sweet tasting mucilage layer. Sometimes the fruit contains only one round seed called “peaberry”. Arabica fruits are 12-18 mm and those of robusta 6-16 mm long. The coffee seeds are plano-

convex in shape, grooved on the flat side, and consist mostly of endosperm with a small embryo at the base of the seed. Those seeds are enveloped in a silverskin and and a fibrous endocarp (parchment). Robusta beans are usually smaller, rounder and present a tighter centre cut than those of arabica (ANZUETO et al., 2005).

2.1.1. Green coffee processing

Harvesting of the coffee fruit should only start after a careful examination of the level of maturation, when most of the fruits are ripe, with a minimum presence of unripe fruits. It can be accomplished in different ways: by stripping onto the ground (not recommended), or onto sheets, by selective hand picking or by mechanical means. It is widely believed that quality coffee can only be obtained if selective hand picking is used, in order to guarantee that only ripe coffee fruits will be harvested. This is certainly valid for small plantations, but becomes a long-standing myth on modern medium-sized to large estates. Top-quality coffee can be produced regardless of the harvesting technique. When unwanted fruits are picked, which is unavoidable, quality must be maintained by post-harvest separation techniques, so that high quality coffee may still be produced from the remaining ripe fruits (BEE et al., 2005).

After harvesting the first step of the coffee processing is the separation. Basically, fruits pass through a washer-separator where stones and impurities are eliminated and fruits are separated by density: on one side the lighter fruits (the dry and over-ripe) and on the other side the heavier ones (immature and ripe fruits) (BEE et al., 2005). Then the fruits will be processed by one of the three methods presented in Figure 1 to the removal of the pulp and to obtain intermediate products that will further provide the coffee beans found in the market. The wet processed coffee is most commonly regarded as a superior coffee with more enjoyable aroma (VINCENT, 1987). However, when dry process is well conducted, it is possible to produce good coffee with ‘body’ and pleasant ‘aroma’ (BEE et al., 2005). As an example, in Brazil and Ethiopia the dry process is predominant and many of the most prized coffees in the world are produced (BANKS et al., 1999).

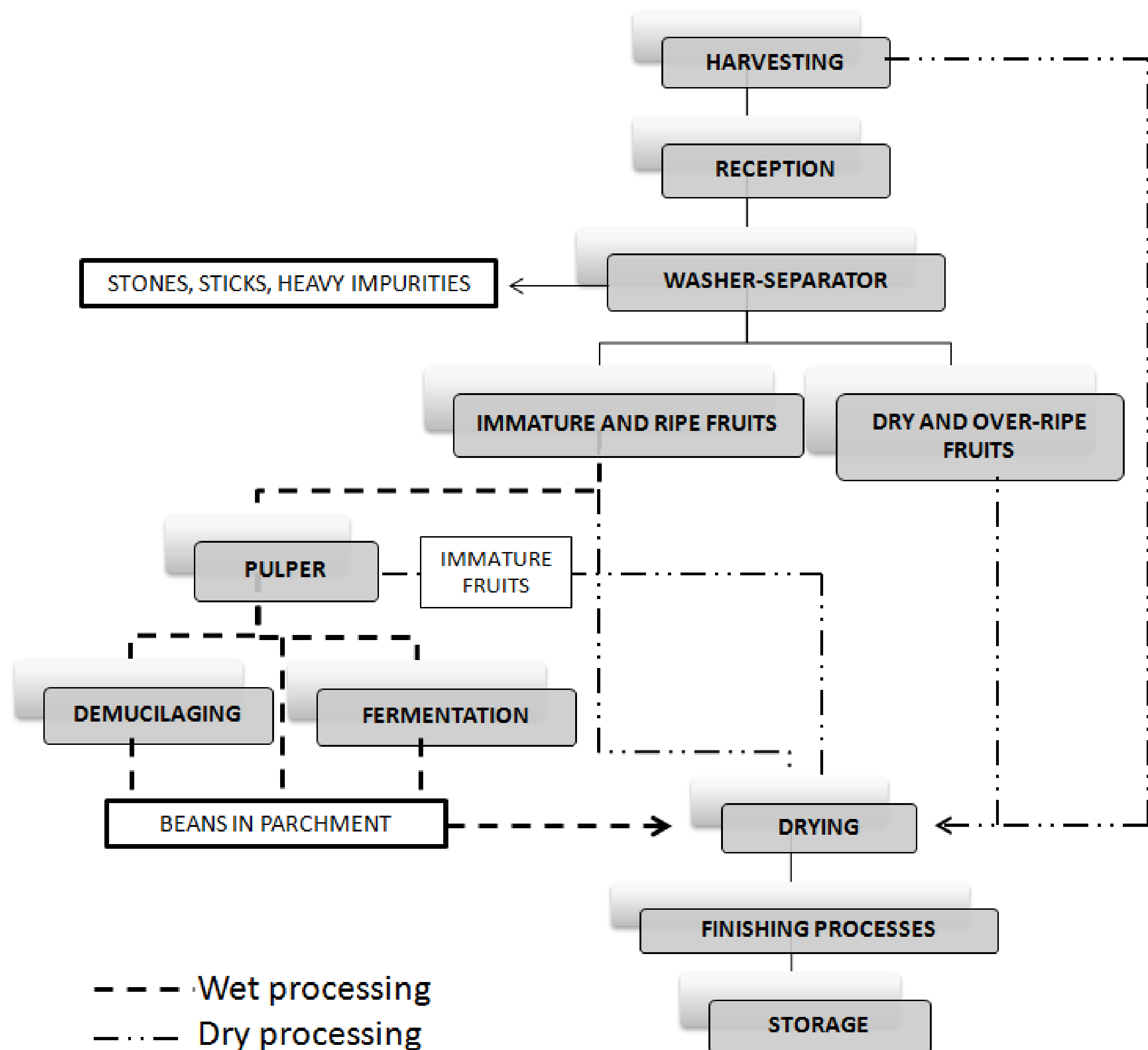

Figure 1. Flow chart of raw bean processing. Adapted from BORÉM (2008)

The dry process is practiced where the climate is considerably warm and dry following harvest and where the copious quantities of water required for the wet process may not be available. Most of robusta coffees and most of the Brazilian crop are handled this way (CLARKE and MACRAE, 1985). In this process, the whole fruits are dried on large patios under the sun and/or in mechanical driers. Because of the long time required to dry the beans (from 2 to 4 weeks), the cost of labour and the chance for proliferation of different microorganisms on the fruit skin, artificial drying has also been used instead of or in addition to natural drying (VINCENT, 1987).

The wet process was developed mainly in the equatorial regions where there is continuous rainfall during the harvest period, and hence the production of natural coffees often results in low quality coffees. Currently, the wet method may be

conducted in three ways. The traditional wet process consists in the removal of the pulp by a pulper, followed by the removal of the mucilage from the parchment by biological fermentation. The pulped natural process produces beans known in Brazil as *cereja descascado*. In this process, the husk and part of the mucilage are mechanically removed by a pulper, and the beans in parchment are dried in patios or artificial dryers. Fermentation for the removal of mucilage is not used, thus, this process consumes less water and energy than wet process. The quality of pulped coffee, when well processed, has been shown to be excellent, with the advantage of producing coffee with greater body than the wet processed coffee (BEE et al., 2005, BORÉM, 2008). In a study comparing different processing techniques, it was found that pulped natural coffee beans presented more positive attributes of quality, less defects and lower microbial count (SANTOS et al., 2009). The third processing consists in removing the husk and the mucilage mechanically, resulting in the demucilaged coffee (BORÉM, 2008).

The operations carried out subsequently to dry, wet or pulped natural processing aim to prepare the green beans for consumption and exportation. An artificial re-drying step is performed to ensure that the moisture level of the beans is lower than 11%. This is especially important to provide stability during storage and to enable husk and parchment to be removed more easily. The cleaning step is performed to remove impurities, such as metal pieces and foreign bodies. It can be carried out by the use of a hopper with screens to remove large and medium-sized impurities, followed by a magnetic separator to remove metal pieces, and cleaner-separator which combines sifting and pneumatic dust removal (VINCENT, 1987).

At this point, dry processed beans still have husks or outer coverings of the fruits, and the wet processed beans still have the dried parchment surrounding the bean. The process called hulling is then accomplished to remove these outer layers, and may be accompanied by a polishing step if the silverskin removal is required. After the outer coverings have been removed, it is advantageous in terms of marketing purposes, to size-grade the green coffee beans. Size graders use sieves of different sizes and shapes to separate the beans according to such attributes. The densimetric sorting separation is purposed to remove defects associated with less dense beans, such as malformed, insect-damaged, fermented beans, etc. Densimetric sorting is usually done by upward current of air or gravimetric table (VINCENT, 1987, BEE et al., 2005).

Electronic sorting machines are usually employed in cooperatives of producers and industries to actually separate defective and non-defective beans prior to other processing steps such as storage and roasting. In these machines, beans pass one by one through electronic eyes that assess the color of each one and control a mechanical ejector that removes the defective beans as required (see Figure 2). These sorters use monochromatic or bichromatic light and can be adjusted to eliminate either more or fewer defective beans (VINCENT, 1987). However, studies indicate that color separation is not absolutely effective for defective beans that have similar color to non-defective ones such as immature and sour (FRANCA et al., 2005b, FARAH et al., 2006, VASCONCELOS et al., 2007). A good instance of such a rapid and non-destructive technique that could separate these beans more efficiently is based in infrared spectroscopy. In particular, the studies by CRAIG et al. (2011) and CRAIG (2012b) demonstrated that FTIR in combination with multivariate statistics presents potential to be used for the classification of defective and non-defective coffee bean.

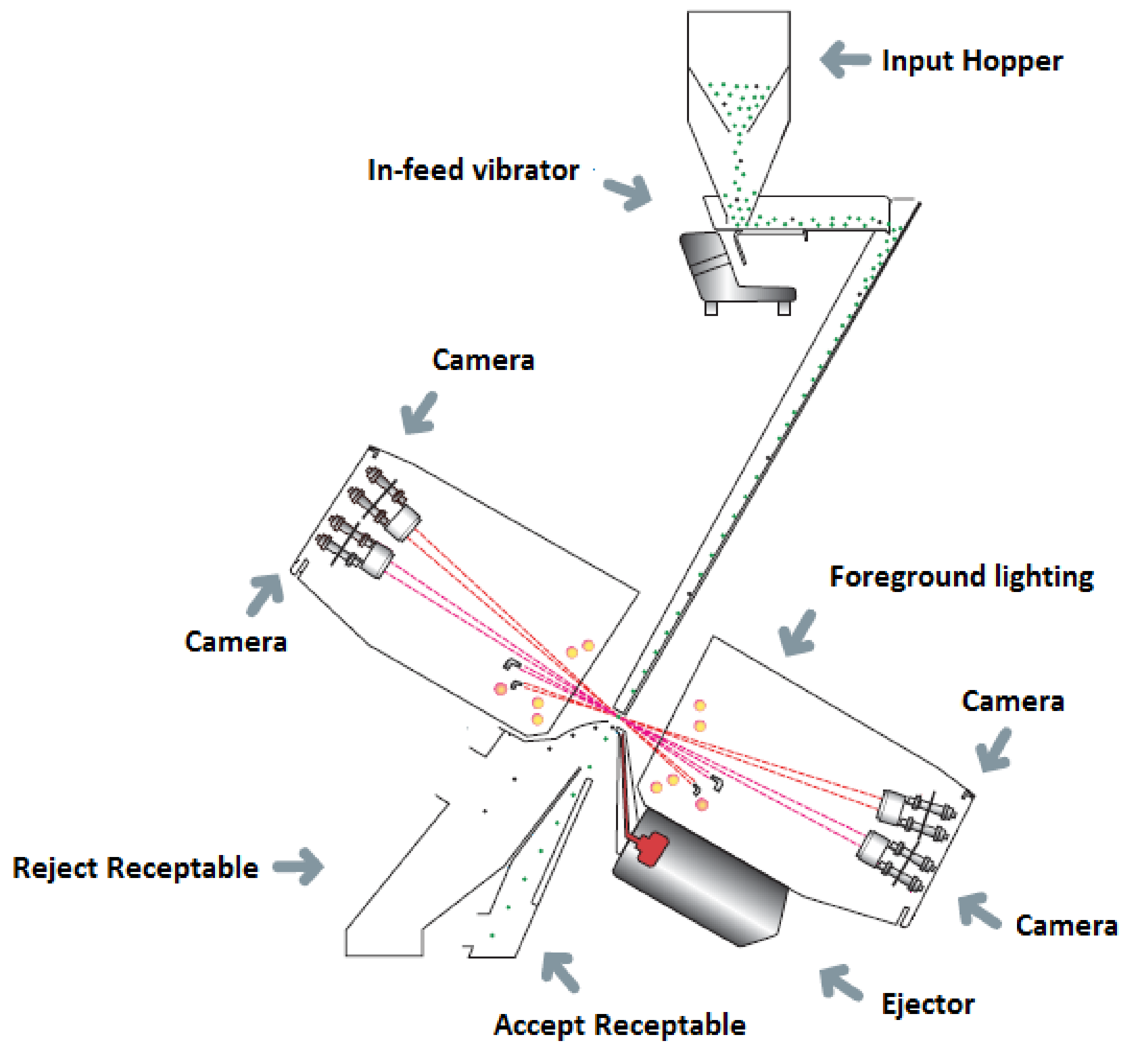

Figure 2. Schematic layout of an electronic sorting machine (Source: http://www.buhlergroup.com)

Finally, beans are stored in 60 kg bags. As already mentioned, the moisture of the beans must not exceed 11% for suitable storage. Therefore, the relative humidity of the storage area should be carefully controlled. When the relative humidity of the air is over 74% (corresponding to an equilibrium moisture content in the beans of about 13% w/w), certain innocuous moulds develop. A relatively humidity of over 85% is sufficient to the multiplication of yeasts and bacteria (VINCENT, 1987).

2.1.2. Raw bean classification

The classification of the coffee beans, which includes physical and sensorial analysis, is a crucial phase in the commercialization process, influencing in price quotations as well as national regulations governing importation into consuming countries. Unfortunately, the existence of a variety of classification systems means that each country adopts a different classification, requiring equivalency norms for use at international level. Overall, the classification is based on the evaluation of all parameters related to coffee bean described in Table 1 (BEE et al., 2005).

Table 1. Parameters used in commercial classification

Parameter	Description
Designation	Species: *C. arabica*/ *C. robusta*
	Processing: natural (dry), pulped natural and washed (wet)
	Geographical origin
	Crop year
Classification by bean size	Size and shape of the beans
Classification by type (number of defects)	Defective beans and foreign matter
Density	Specific mass of the beans
Humidity	Moisture content
Color and appearance	Coloration and uniformity of the beans
Roast	Roast regularity, smell, etc
Cup quality	Characteristic aroma and flavors

Adapted from BEE et al. (2005) and CLARKE et al. (1987b)

Some considerations regarding the parameters listed in Table 1 are: (a) arabica coffee fetches a higher price than robusta. (b) The size or screen of the beans is measured by the dimension of the holes in the screen that holds them back. The separation and classification of the beans by size is important to guarantee an adequate and uniform roast. (c) In coffee that has been correctly dried, the humidity level should be 11±0.5% for raw coffees. (d) The drying processing system can be

recognized by the bean color and appearance of the silverskin. Washed coffee is translucent, shiny and green-bluish. A green-bluish color in washed coffee indicates freshness and high quality, while a yellowish color is a sign of old and low quality coffee. Natural coffee has a semi-opaque color and a yellowish or even brown skin. Pulped natural coffee has an intermediate aspect. (e) Observing the uniformity of a roasted coffee sample helps to identify defects that were not observed previously (BEE et al., 2005).

With regard to the classification by type, most producing countries adopt their own classification system to describe the presence of defective beans, which will be explored in details in section 2.2. At the international level the 'New York Coffee and Sugar Exchange' introduced the concept of the black bean equivalent according to which all defects are accounted for in terms of equivalence to black beans, as indicated in Tables 2 and 3 (BEE et al., 2005, FRANCA et al., 2005b). These defects are visually identified and manually sorted in a 300 g sample by a professional trained for green coffee classification.

Table 2. Summary description of the type classification system

Type no.	Maximum allowable number of defects per 300 g sample
NY2	6
NY3	13
NY4	30
NY5	60
NY6	120
NY7	240
NY8	450

Source: FRANCA et al. (2005b)

Table 3. Equivalence ratings according to the type classification system

Defect type and quantity	Equivalency (defects)
1 black bean	1
2 sour beans	1
5 immature beans	1
2/5 insect damaged beans	1
1 small stone	1
1 large stone	5
1 small twig	1
1 large twig	5

Source: FRANCA et al. (2005b)

In terms of cup quality, each producing country weighs organoleptic defects in a different way, which may lead to discrepancies in sensorial evaluation between producing and consuming countries. Brazilian coffees, as an example, are classified into cup quality according to the ranking shown in Table 4. Rioysh, rio and rio zona beans are considered defects associated with irregular beans in cup taste or with *off-taste*. These beans present normal appearance but medicinal and phenolic flavor of iodine. These defects are caused by overripe fruits contaminated by microorganisms on branch, or dried under contact with patio soil contaminated by microorganisms and/or trichloroanisole (BEE et al., 2005).

Table 4. Cup quality classification

Cup quality	Description
Strictly soft	The same as soft, but more accentuated
Soft	Pleasant, mild and sweetish flavor and taste
Softish	Mildly sweetish and soft taste, without harshness or astringency
Hard	Sour, astringent and harsh taste, but without strange taste
Rioysh	Mild flavor of iodoform
Rio	Typical and accentuated flavor of iodoform
Rio zona	Very strong aroma and taste, similar to iodoform or carbolic acid

Source: BRASIL (2010)

Besides the aforementioned classifications, coffee certification has gained importance in the international commercialization of the raw beans. Studies have shown that the demand for certified and verified coffee will continue to grow strongly in the foreseeable future (ZAMBOLIM, 2007; ICO, 2013). These certifications may assure not only the quality of the bean, but it may also assure that a coffee was produced under good agricultural practices and management, safe and healthy working conditions, no child labor and protection of the environment. Some examples of certifications applied to coffees are Fairtrade, UTZ Kapeh and EureGAP (ZAMBOLIM, 2007).

2.1.3. Roasting and grinding

Coffee roasting consists of applying considerable heat to the beans, which must be kept in motion to ensure an uniform roast, until their color reaches the desired shade of brown and their aroma is fully developed. At this stage the coffee must be rapidly cooled by air current with or without the aid of water spray or “quench” (BONNLANDER et al., 2005, CLARKE and MACRAE, 1985). The principles of roasting vary from a mechanical, with a wide variety of rotating drums, a thermal, including contact, radiation and convective radiation heat transfer, and an operational point of view (BONNLANDER et al., 2005). Conventional and newer developments on roasting techniques were described by CLARKE (1987b) and CLARKE and VITZTHUM (2008).

The roasted beans are characterized by the process to which they have been subjected. The roasting degree expresses the external color, the flavor developed, the dry mass loss that has occurred and the chemical changes in the beans, and is divided into: ‘light’, ‘medium’ and ‘dark’ roasts. Depending on the gas temperature applied to the beans, that can vary from 220 °C to 260 °C, roasted coffees may additionally be described as having been ‘fast’ roasted (roasting time of a few minutes or even less) or ‘conventionally’ roasted (time in order of 12-15 min) with an intermediate time of 5-8 min (CLARKE and VITZTHUM, 2008, CLARKE, 1987a). The actual dry mass loss observed at different degrees of roasted is shown in Table 5.

Table 5. Approximate percentage of dry mass loss for different roasting degrees

Degree of roast	Dry mass loss (%)
Light	1-5
Medium	5-8
Dark	8-12
Very dark	>12

Source: CLARKE (1987a)

The main transformations occurring in the bean with increasing temperature from a macroscopic standpoint are given in Table 6. The color changes and the formation of micro fissures in the bean can be observed in Figure 3.

Table 6. Macroscopic changes during roasting

Temperature within the bean (°C)	Effect
20-130	Liquid-vapour transition of water. Color fades
130-140	First endothermic maximum. Yellow coloring and swelling with beginning of non-enzymatic browning. Roast gases are formed and start to evaporate
140-160	Complex series of endothermic and exotermic peaks. Color changes to light brown. Large increase in bean volume and micropores. Rests of silverskin are removed. Little fissures at the surface occur. Aroma formation starts
160-190	Roasting reactions move towards the inner dry structure of the bean
190-220	Micro fissures inside the beans. Smoke escapes. Large volumes of carbon dioxide escape and leave the bean porous. Typical flavor of roasted coffee appears.

Source: BONNLANDER et al. (2005)

Figure 3. Stereo microscope section of a bean: (a) green; (b) toasted to 70 °C; (c) roasted, showing the porous structure. Source: BONNLANDER et al. (2005)

Following the above mentioned reactions, numerous chemical changes also occur in the bean during roasting. Among them, sucrose is partly dehydrated and hydrolized to reducing sugars, giving rise to many volatile and non-volatile compounds, water and carbon dioxide, and the rest being pyrolysed (caramelized). The Mailllard reaction, which generates melanoidins, has a low activation energy and is favored in the presence of reactive nitrogen compounds. Polysaccharides, except the highly insoluble cellulose, are partly solubilized generating anhydrides and polymers, such as melanoidins. The lipid fraction remains almost the same during roasting, with a slightly increase in the levels of *trans* fatty acids, and a decrease in the levels of linoleic acids, cafestol, and tocopherols. Regarding nitrogen compounds, caffeine remains stable, 50% of the trigonelline is decomposed to nicotinic acids and other compounds, free amino acids are pyrolysed or react to form Maillard products, and proteins are denatured. The mineral content does not change. Most of the chlorogenic acids content is hydrolysed, isomerated and, to a smaller extent, lactonized. The compounds formed are melanoidins, free quinic acid, quinides, phenols and volatile compounds. Finally, carboxylic acids are formed during the first stages of roasting due to carbohydrate hydrolysis, but are degraded after a longer exposition to heat. Thus, light roasted coffees contain higher quantities of carboxylic acids than dark roasted (SIVETZ, 1979, LYMAN et al., 2003, BONNLANDER et al., 2005, CLARKE and MACRAE, 1985).

Table 7 summarizes the chemical composition of green and roasted arabica and robusta coffees, where the changes that occur as a result of roasting process are evidenced.

Table 7. Summary of composition data (%dry weight) for green and roasted arabica and robusta coffee beans

Component	Arabica		Robusta	
	Green	Roasted	Green	Roasted
Minerals	3-4.2	3.5-4.5	4-4.5	4.6-5
Caffeine	0.9-1.2	~1	1.6-2.4	~2
Trigoneline	1-1.2	0.5-1	0.6-0.75	0.3-0.6
Lipids	12-18	14.5-20	9-13	11-16
Chlorogenic acids	5.5-8	1.2-2.3	7-10	3.9-4.6
Aliphatic acids	1.5-2	1-1.5	1.5-2	1-1.5
Oligosaccharides	6-8	0-3.5	5-7	0-3.5
Total polysaccharides	50-55	24-39	37-47	-
Amino acids	2	0	2	0
Proteins	11-13	13-15	11-13	13-15
Melanoidins	-	16-17	-	16-17

Source: CLARKE and MACRAE (1985)

Roasted beans require a cutting action to provide a ground coffee with particles of suitable size and shape for subsequent brewing, and this siza is best obtained by means of cutting rolls. Nevertheless, various impact-type grinders have been used for producing finer grind coffee, and the flaking rolls have been used to produce particles of a flaked shape which may provide benefits on brewing (CLARKE, 1987a). Due to the porous and brittle structure of the dark coffee, the highest the degree of roast, the easier to obtain finer particles (BANKS et al., 1999). Coffee grinds are qualitatively classified into 'coarse', 'medium' or 'fine'. There is no national or international consensus of agreement as to the average particle size that constitutes each of these classes but there are recommendations for the various grinders in terms of screen sizes. The traditional method of assessing the degree of grind is from sieving analyses using a number of different screen or sieve mesh sizes in a specific procedure (CLARKE, 1987a).

2.1.4. Quality assurance of the roasted coffee

As discussed so far, many physical and sensory parameters are used to classify the raw bean, and such classification is determinant in the coffee commercialization process and price quotations. On the other hand, there is a lack of methodologies to assess the quality of roasted coffees from the market.

At the international level, ISO has standards for the determination of bulk density of the whole beans (ISO, 2011a) and caffeine content (ISO 2009, ISO 2011b). But in terms of quality, each country adopts its own system. In Brazil, for example, roasted coffees from the market are officially inspected by means of moisture content, which should not be higher than 5%, and by means of the presence of impurities, fruit parts (e.g. hulls, husks) and foreign matter, that should not exceed 1%. Individually, the presence of foreign matter should not exceed 0.1% (BRASIL, 2010). The current method used to identify the presence of those impurities and foreign matter is, however, based on the microscope analysis of coffee samples. Since the method relies on the visual examination of small amounts of sample, it is subject to lack of reproducibility. Microbiologic and mycotoxin analyses may also be conducted. In addition, the cup test was initially proposed to evaluate the global quality of the beverage. The sensory characteristics to be evaluated would be the fragrance, aroma, acidity, bitterness, astringency, taste, residual taste, influence of defective beans, body and beverage category, which is divided in strictly soft, soft, softish, hard, rioysh, rio and rio zona. A coffee with global score lower than 4 would be disqualified and forbidden to be sold (BRASIL, 2010). This regulation was later revoked and the sensory analysis is currently not being applied for the inspection of coffees from the market (BRASIL, 2013).

The traditional cup test is in most of times not suitable for the classification and inspection of roasted coffees from the market. This happens because that is no previous control of the roasting conditions, and low-grade coffees are generally roasted to a dark roasting degree to mask unpleasant flavors and/or aromas. Besides that, sensory-based methods are time-consuming and require trained cupper experts (FERIA-MORALES, 2002, PETRACCO et al., 2005), which represent a barrier to the implementation of such methodologies for routine analysis and inspection purposes. As a consequence of the aforementioned drawbacks, regulatory organizations and industry strive to correlate sensory data collections and experimental data in order to

calibrate instrumental screening methodologies (FERIA-MORALES, 2002, PETRACCO et al., 2005). A good example of such a rapid, non-destructive fingerprinting technique is near-infrared spectroscopy that, in previous studies, has accomplished the prediction of sensory parameters in roasted coffee (PETRACCO et al., 2005, RIBEIRO et al., 2011, ESTEBAN-DIEZ et al., 2004b).

2.2. Coffee: Defective beans

The type and number of defective beans influence significantly the quality of the coffee beverage. Table 8 shows a summary of the main defects visually identified, their origin and their effect on the brew flavor and/or roasting process. It is important to note that other defects not listed in the Table may also occur.

Table 8. Main defects visually identified among coffee beans, their origin and their effect in the brew flavor and/or roasting process

Group	**Defect**	**Brew flavor/roasting**	**Origin**
Foreign matter	Stones, sticks, clod	Effect mainly economic	H/P
Fruit parts	Bean parchment, dried cherry, husk fragment	Non-specific downgrading, lack of flavor	P
Irregularity/integrity of bean shape	Shell, bean fragment, broken bean, insect damaged bean, etc.	Uneven roast, bitterness, less acidity	F/P
Irregular in color and surface texture	Black, black-green, sour, immature, mouldy bean, etc.	Slow roast, harshy/strange flavors, less acidity, astringency, metallic flavor	F/H/P/S

F, Field damaged beans: genetic problems, environmental conditions and attacks by pests and diseases.

H, Harvested-damaged beans: inadequate crop management

P, Process-damaged beans: imperfect processing operations (pulping, washing, drying, cleaning, hulling, etc.)

S, Storage damaged beans: deficient storage

Adapted from BEE et al. (2005) and MIYA et al. (1973)

Among these defects, those that affect the beverage quality the most are the black, sour and immature (OLIVEIRA et al., 2008). Black beans derive from dead

beans within the cherry on the tree, over-ripe cherry fallen on the ground or attacked by fungi and other pests. Sour beans are associated with 'overfermentation' during the wet process or with beans that have been in adverse conditions, becoming fermented by bacteria or xenophilic moulds, with the embryo dead. Immature beans derive from unripe coffee beans. Black-green beans occur due to the fermentation of unripe coffee beans (BEE et al., 2005, CLARKE, 1987b).

Only a few studies have attempted to discriminate defective and non-defective beans. Prior to roasting, these beans can be discriminated by several physical and chemical parameters (MENDONCA et al., 2009b, VASCONCELOS et al., 2007, FRANCA et al., 2005b, SANTOS et al., 2012). After roasting, solid phase micro extraction gas chromatography-mass spectrometry (SPME-CG-MS) has shown success in achieving this purpose. TOCI and FARAH (2008) reported volatile compounds that could be used as potential defective coffee markers. MANCHA AGRESTI et al. (2008) showed that roasted defective and non-defective coffees could be separated into two distinct groups based on their volatile profiles: immature/black beans and non-defective/sour coffees. In spite of the positive results reported in the previously mentioned studies, the drawbacks of the technique employed must be considered. First, the volatile composition of coffee beans may vary according to many parameters such as soil composition, climate, agricultural practices, and most importantly, roasting conditions, making the achievement of reproducible results arduous. In addition, the SPME-CG-MS technique is costly, time consuming, and not suitable for routine analysis. Sequentially, MENDONÇA et al. (2009a) employed electrospray-ionization mass spectrometry for the same purpose, but no discrimination among defective and non-defective coffees was observed after roasting.

2.2.1. Physical attributes

The size and shape of the beans vary with species and quality. Arabica coffee beans are bigger than robusta and non-defective is bigger than defective in both species. Thus, the size of the beans is measured by the dimension of the holes in the screen or sieve and used as a means of commercialization (MENDONÇA et al., 2009b, FRANCA & OLIVEIRA, 2008). After roasting, the separation of defective and

non-defective beans by size is efficient among arabica coffee beans. For robusta coffee only sour and black beans can be discriminated (MENDONÇA et al., 2009b).

A comparison between the densities of green defective and non-defective beans have shown that there is no significant difference between them except for robusta black beans, which have a lower density than the others. During roasting, there is an increase in the volume and lost in the mass, which results in a decrease in the density of the bean. Because arabica black beans are smaller, their volume increases less than others resulting in higher density after roasting (MENDONÇA et al., 2009b, FRANCA et al., 2005b, FRANCA et al., 2005a). However, MENDONÇA et al. (2009bb) did not find a significant difference among robusta black beans.

Green beans contain 10-13% of moisture content, but no significant difference was found among the moisture content levels of non-defective and defective beans (CLARKE and MACRAE, 1985, RAMALAKSHMI et al., 2007). During roasting under the same conditions, the moisture loss is lower among defective than among non-defective beans, indicating that defective beans roast at a lower level (VASCONCELOS et al., 2007). This observation is explained by the fact that defective beans contain less sucrose than non-defective ones.

Color is an important attribute of green coffee beans because it varies considerably with species, presence of defect, origin, processing conditions and age of the bean. Therefore, the labour-intensive manual sorting is employed in the commercial classification of sample lots by type, and the electronic color sorting is employed in cooperatives of producers and industries to actually separate defective and non-defective beans prior to other processing steps (CLARKE, 1987b). Immature beans are identified by their greenish or metallic-green silverskin color with the ventral closed. Sour beans can have a wide range of colors: light to dark brown-reddish, dark brown or yellowish green endosperm, sometimes with a waxy appearance or a brown silverskin color. Black beans have the endosperm totally black, and black-green beans have a dark-green to black-green silverskin color (BEE et al., 2005, COELHO, 2000). Nevertheless, in the study by TEIXEIRA et al. (1971), black-green beans were visually classified as sour after the removal of the silverskin.

2.2.2. Chemical attributes

In actual amounts, sucrose is the major free sugar present in green coffee, varying with species, variety, stage of maturity, processing and storage conditions (CLARKE and MACRAE, 1985). The presence of immature and fermented beans is directly related to the sucrose level in a given sample. Sucrose levels are expected to increase with coffee maturation and, since this is the main free sugar available, sucrose levels decrease if fermentation occurs (MAZZAFERA, 1999). Before roasting, MAZZAFERA (1999) found that immature-black and immature Brazilian beans present one-thirty and one-fifth of the sucrose level of a normal bean. This was also observed for Vietnamise robusta coffee, where black beans contained 0.9% sucrose and non-defective beans contained 4% (BEE et al., 2005). VASCONCELOS et al. (2007) and RAMALAKSHMI et al. (2007) found higher levels of sucrose in non-defective compared to defective beans. After roasting, only traces of sucrose were detected by VASCONCELOS et al. (2007) in light roasted coffee and no sucrose was detected in medium and dark roasting degrees. Polysaccharides are present in green coffee in amounts of 40-50% d.b., with mannans, galactans, cellulose and araban being the most important (CLARKE and MACRAE, 1985). Other polysaccharides such as starch or pectin are present at only low levels in mature coffee beans (BEE et al., 2005). After medium roasting, approximately 75% of the original polysaccharides remained (CLARKE and MACRAE, 1985). VASCONCELOS et al. (2007) have observed higher levels of total carbohydrates before and after roasting in non-defective than in defective coffee, but such result was determined by difference.

The green coffee lipids are composed of coffee oil substantially present in the endosperm and a small amount in the wax, located in outer layers of the beans. The oil contains triglycerides and a considerable amount of other lipid components. The lipids loss during roasting is minimal (CLARKE and MACRAE, 1985). Arabica coffee contains higher level of oil than robusta, and non-defective beans contain higher levels than defective ones (MAZZAFERA, 1999, OLIVEIRA et al., 2006, VASCONCELOS et al., 2007). Nevertheless, OLIVEIRA et al. (2006) did not find a significant difference in the fatty acid profile of both defective and non-defective coffee, before or after roasting.

Crude protein content is calculated from total nitrogen content. Thus, it must be corrected for caffeine and, ideally, trigonelline nitrogen. If such corrections are

made, the protein content in green coffee is close to 10% with little quantitative and qualitative differences between species (BEE et al., 2005, CLARKE and MACRAE, 1985). MAZZAFERA (1999) reported higher levels for black beans in comparison to immature and immature-black. Nevertheless, this result did not correlate with free amino acid content, which was higher for immature beans than black and immature-black coffees. COELHO et al. (2000) also observed higher levels of protein in black coffee. VASCONCELOS et al. (2007) and OLIVEIRA et al. (2006) did not find a significant difference in the protein contents of defective and non-defective coffees, before or after roasting. RAMALAKSHMI et al. (2007), on the other hand, reported slightly lower protein contents for low quality coffees.

Trigonelline is a nitrogenous base with a pyridine ring that is decomposed during roasting in the order of 50-80%, giving rise to volatile compounds of sensory significance (CLARKE and MACRAE, 1985). Half of the volatile compounds generated during its thermal degradation consist of pyridines and pyrroles, both detected in roasted coffee (MANCHA AGRESTI et al., 2008). Nicotinic acid, which also exhibits a pyridine ring, is especially important from a sensory and a nutritional point of view, since it is an essential vitamin (CLARKE and MACRAE, 1985). Prior to roasting, FRANCA et al. (2005b) reported trigonelline levels of approximately 1% in non-defective, immature and sour coffee beans and lower values (~0.8%) for black beans. FRANCA et al. (2005a) did not find significant differences in trigonelline levels between defective and non-defective coffee beans. FARAH et al. (2006) reported a strong negative correlation between trigonelline levels and poor quality and Rio-off flavor. After roasting, no significant correlation between trigonelline levels and cup quality was observed. Slightly higher levels of trigonelline were observed for black/sour beans, which was associated to the fact that such beans attained lighter roasting degrees in comparison to other classes (FRANCA et al., 2005b, FRANCA et al., 2005a).

Among the amines present in green coffee, studies indicate that putrescine is the prevailing amine, regardless of variety, quality or growth and processing conditions. OLIVEIRA et al. (2005) found higher putrescine levels for the lowest quality coffee; however, because the samples did not come from the same crop, one could argue that these differences could also be attributed to other factors such as growth and processing conditions. On the other hand, VASCONCELOS et al. (2007) reported higher levels of amines in high quality coffee. In both studies histamine was detected

only in defective beans or low quality coffee, indicating that this amine could be a potential marker for the detection of defective beans in green coffee. After roasting, the levels of amines decreased dramatically. In light roasted coffee, only traces of serotonin were found while, in the medium and dark roasted coffees, no amines were found.

Caffeine is an alkaloid, with a methylated dioxypurine structure relatively stable to roasting. Although caffeine sublimation point is at 178 ° C, the pressure build-up within the bean and the poor rate of diffusion of vapor through its outer layers leads to low losses of caffeine during roasting unless severe roasting conditions are employed (CLARKE and MACRAE, 1985). MAZZAFERA et al. (1999) did not find significant difference in the caffeine levels of defective and non-defective coffee. Using a UV-based methodology, FARAH et al. (2006) found that the concentrations of caffeine and trigonelline were higher in high quality coffees in comparison to bad quality ones. The same observation was made by FRANCA (2005a) and RAMALAKSHMI et al. (2007). Nonetheless, FRANCA et al. (2005b) found that non-defective beans exhibited lower caffeine levels than defective ones.

The types of acids found in coffee are aliphatic carboxylic acids but also phosphoric acid, some alicyclic and heterocyclic acids, and chlorogenic acids that will be discussed separately. The presence of acids in the beverage contributes to sensory parameters such as acidity and astringency, and may add flavor effect through aroma and taste in the case of undissociated molecules. Aliphatic acids that may add desirable flavors to the beverage are pyruvic, 2-methylbutyric, 2-methylvaleric, 2-ethylbutyric and levulinic. It has been observed that coffees stored for a long time are slightly more acid than those made from corresponding new crop (WOODMAN, 1985). Studies have demonstrated that prior to roasting defective present higher acidity than non-defective beans, with highest values for sour beans. Acidity decreases after roasting without difference among defective and non-defective beans. It must be considered that such acidity may also be influenced by the presence of chlorogenic acids (VASCONCELOS et al., 2007, FRANCA et al., 2005a, RAMALAKSHMI et al., 2007).

Chlorogenic acids (CGAs) are phenolic compounds esterified to quinic acid. Through roasting process, CGAs form numerous isomers that exert diverse beneficial effects to health, including caffeoylquinic acids (CQA), dicaffeoylquinics (diCQA) feruloylquinic acids (FQA), and coumaroylquinic acids (CoQA). However, the

excessive roasting may lead to the destruction of these compounds (CLARKE and MACRAE, 1985). According to WHITE (1995), the CQA content of green coffee beans can be used as an indicator of quality and maturity, in a way that the relationship diCQA/CQA is higher in immature beans in comparison to non-defective beans. FARAH et al. (2006) observed that prior to roasting the low quality immature and black-green beans had higher levels of CGA, and 5-CQA and 5-FQA had a high relationship with poor beverage quality. Light roasted and medium roasted low quality coffees were correlated with high levels of 3-CQA, 4-CQA, 5-CQA, and 5-CQA, 4-FQA, and 5-FQA, respectively. 93% of the original CGA content was lost during dark roast, and no relationship between CQA and coffee quality was found. Furthermore, it was found a positive relationship between CGA content, especially 5-CQA, and color intensity of the beans. Indeed, CLARKE and MACRAE (1985) have previously reported that the greater the discoloration in the sequence green, yellow, brown to black, the lower the total CGA content and the lower the CGA:diCQA molar ratio.

There is no evidence that arabica and robusta differ significantly in their levels of mineral content, but studies suggest that dry-processed arabica and robusta coffee have slightly higher levels than wet-processed ones. This is probably due to leaching out of minerals during the fermentation and washing stages of the wet-processed coffee (BEE et al., 2005, CLARKE and MACRAE, 1985). Potassium represent 40% of the total minerals of the coffee beans (BEE et al., 2005). OLIVEIRA et al. (2006) and VASCONCELOS et al. (2007) found higher mineral content in defective than non-defective beans, with black beans exhibiting the highest amount.

Coffee quality is directly related to its aroma and flavor. Numerous volatile compounds are present in green and roasted coffees, varying considerably with several factors such as specie, climatic conditions and soil, storage and roasting conditions. Because of that, the volatile profile of coffee has been successfully used for the discrimination of coffees by specie, geographical origin, quality and presence of adulterants (NUROK et al., 1978, DART and NURSTEN, 1985, COSTA FREITAS et al., 2001, ZAMBONIN et al., 2005, RISTICEVIC, 2008, OLIVEIRA et al., 2009). MANCHA AGRESTI et al. (2008) attempted to discriminate defective and non-defective roasted coffees by their volatile profile using SPME-CG-MS. Although 250 volatile compounds were identified, only five of them were detected in defective but not in non-defective and could be used as defective coffee markers. Sour beans were those with highest number of compounds not detected in non-defective coffee,

followed by black and immature. Results from principal component analysis (PCA) indicated that defective and non-defective beans could be separated into two groups based on their volatile profile, one represented by immature and black beans, and other by non-defective and sour beans. These findings suggested that sour and black beans could be associated with the fermentation of non-defective and immature beans, respectively.

2.2.3. Sensory attributes

Black beans are generally regarded as giving a 'heavy' and 'ashy' flavor to the beverage. Their volatile profile is distinct from non-defective or other defective beans, being considered the worst intrinsic defect. The black-green beans are characterized by astringency and a taste reminiscent of rotten fish. Sour beans are regarded as especially important in downgrading flavor, contributing to sour, oniony and fermented taste and smell. Immature beans contribute to the astringency, due to the presence of tannins, which in low concentrations produce an acceptable gustatory sensation called 'mouthfeel'. It also adds bitterness and metallic taste to the beverage (COELHO, 2000, CLARKE, 1987b, MANCHA AGRESTI et al., 2008).

Studies have demonstrated that the smell and taste of defective beans can be perceived even at low levels. COELHO et al. (2000) evaluated the sensory attributes of Brazilian coffees, previously classified as strictly soft, after an increasing addiction of immature, sour and black beans. Before the addition of defective beans, higher values of positive attributes, such as peanut, nuts, cereal and caramel, were observed. With the inclusion of 5 to 10% of immature beans the attribute astringency was noted. 30% of immature beans were sufficient to increase the attribute chemical, oily and fermented. The defect black was the most detrimental to the quality of the beverage, contributing to the attributes bitter and sour. The transition of the cup quality classification from strictly soft to hard occurred after the addition of 19.49%, 16.36% and 14.26% of immature, sour or black beans, respectively.

PUERTA-QUINTERO (2000) evaluated the impact of the immature beans on the sensory quality of the beverage. It was found that 2.5% of immature in mixture with ripe beans was sufficient to reject 30% of the samples by the cuppers due to unpleasant tastes. Nonetheless, BEE et al. (2005) asserted that, in espresso coffee,

the astringency and metallic tastes of immature beans are perceivable at percentages of immature beans as low as 1%.

2.3. FTIR and NIR spectroscopy

2.3.1. Basic concepts

The electromagnetic radiation covers a wide wavelength range, from low-energy radio waves to high-energy γ-rays, as illustrated in Figure 4. When exposed to radiation, many processes may occur in an atom or molecule. A molecule may undergo rotational, vibrational, electronic or ionization processes, in order to increasing energy. A molecule may scatter light in a Raman process. Nuclear magnetic resonance and electron spin resonance processes involve transitions between nuclear spin and electron spin states, respectively. Nevertheless, an atom may only undergo an electronic transition or ionization because it has no rotational or vibrational degrees of freedom. Spectroscopy is basically an experimental subject concerned with the absorption, emission or scattering of electromagnetic radiation by atoms or molecules (HOLLAS, 2004). At each of the electromagnetic regions, different information on the radiated sample may be obtained.

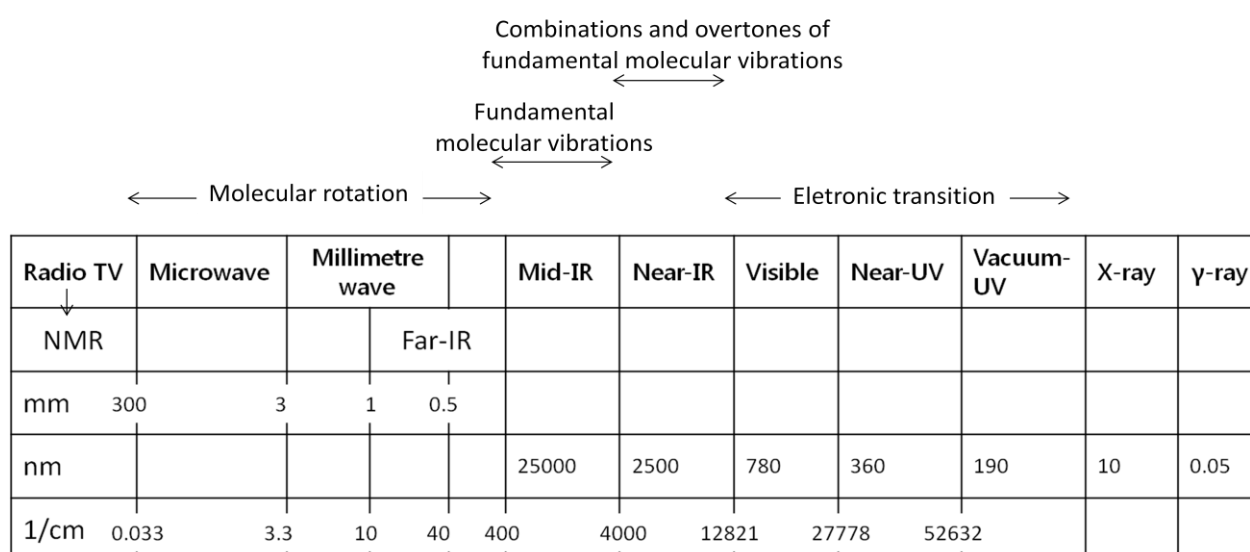

Figure 4. Regions of the electromagnetic spectrum and processes that may occur in an atom or molecule exposed to the radiation. IR = infrared, UV = ultra-violet and NMR = nuclear magnetic resonance.

The regions of the electromagnetic spectrum where molecular vibration will take place are far-infrared, mid-infrared and near-infrared. When a sample is radiated with IR light at each of these regions different chemical bonds absorb this light at different wavelengths, depending on the atoms connected, the surrounding molecules, and the type of vibration the absorbance gives rise to (THYGESEN et al., 2003). The low energetic far-infrared is typically absorbed by heavy molecules such as inorganic and organometallic substances (SMITH, 2002), which limits the application of this radiation to food systems. The other two infrared regions of the spectrum give rise to different techniques: MIR and NIR spectroscopy. Figure 5 presents an energy level diagram showing the states involved in MIR and NIR techniques.

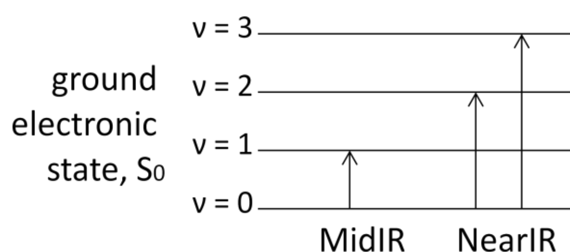

Figure 5. Energy level diagram showing the states involved in infrared absorption.

MIR provides characteristic fundamental vibrations during which the electrical dipole moment changes are employed for the elucidation of molecular structure (LARKIN, 2011). This implies that bonds that connect two identical or nearly identical parts of a molecule, for example C=C bond, tend to be less active than a weakly polarizable bond, such as the OH bond. For this reason, water dominates and interferes in the IR spectrum (THYGESEN et al., 2003). Concerning instrumentation, MIR spectrometers are classified into two groups: dispersive and Fourier-transform (FT). The most significant advantage of FT spectrometers is that radiation from all wavelengths is measured simultaneously by an interferometer, whereas in dispersive spectrometers all wavelengths are measured consecutively. Due to the higher sensitivity and speed, almost all commercially available MIR equipments are based upon an interferometer, which led MIR spectroscopy to also be called Fourier transform infrared (FTIR) spectroscopy (SABLINSKAS, 2005, LARKIN, 2011).

NIR spectroscopy measures the broad overtone and combination bands of some of the fundamental vibrations and is an excellent technique for rapid and accurate quantification analysis, overcoming MIR (LARKIN, 2011). The main disadvantage is that the superposition of many overtone and combination bands causes broad peaks and a very low structural selectivity in comparison to MIR, where many fundamental peaks can be observed in isolated positions (KAROUI and DE BAERDEMAEKER, 2007). Of all the optical spectroscopic techniques, NIR offers the greatest diversity of instrumentation principles, including FT, scanning-grating, diode array, filter, light-emitting diode and acoustic-optical tuneable filter spectrometers. This diversity of instrumentation principles as well as the development of the equipments allowed NIR to be applied in online and at-line modes in food processing facilities (SABLINSKAS, 2005). While FTIR has been prominent and attractive in industrial applications over the past decade or more, NIR spectroscopy has been adopted in the food industry and in agriculture for more than 30 years (GAUGLITZ and VO-DINH, 2006).

2.3.1.1. Measurement Techniques

When interacting with a sample, incident light of intensity I_0 may be partly reflected (I_R) at optical interfaces, partly scattered (I_S), absorbed (I_A), and transmitted (I_T), as shown in Figure 6.

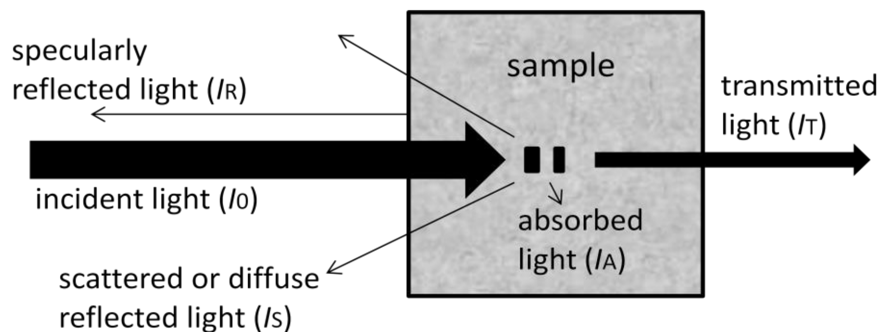

Figure 6. Energy balance of incident light upon interaction with a sample

The energy balance for the incident light may be written as (STEINER, 2005):

$$I_0 = I_A + I_R + I_T + I_S \quad \text{[Eq. 1]}$$

The chemical information about the sample goes into I_A. This value cannot be measured directly, but it can be evaluated knowing I_0 , I_R, I_T and I_S and applying these values to Eq. 1. In the commercial spectrometers only one detector is used to measure a particular couple of intensity values (I_0 and either I_T, I_R, or I_S). Thus, the goal of sample preparation is to bring the remaining intensities to zero (or close to zero) (STEINER, 2005).

Several measuring techniques can be employed to assess I_R, I_T or I_S. The traditional technique used for MIR measurements is based on transmittance, usually requiring considerable sample processing, e.g. the production of KBr pellets for powder analysis. Transmittance requires very small samples, on the order of milligrams, and although this may be an advantage for identification of synthesized molecules, it can pose problems for heterogeneous materials, such as food, in relation to representativeness (KAROUI et al., 2010). In NIR spectroscopy, diffuse transmittance is usually measured in the region of the spectrum between 800 and 1100 nm, where weak absorptions enable useful data to be obtained from samples with 1-2 cm thickness, such as meat, cheese or whole grain (HUANG et al., 2008).

Sample presentation developments have included diffuse reflectance mode (KAROUI et al., 2010), an external reflection measurement technique. Diffuse reflectance can be applied to analyze powders and rough surface solids. The technique relies upon scattering of radiation within the sample. The incident light may result in absorption, in regularl reflection from the sample surface or in multiple diffusely scattered light, which is the part of radiation used in diffuse reflectance measurements. The optical collection accessory is designed to reject the specular reflected radiation and to collect as much of the diffuse reflected light as feasible. Disadvantages are the strong dependence on the refractive index of the sample, the particle size and distribution, packing density, and sample homogeneity that must be carefully controlled (STEINER, 2005, LARKIN, 2011). In MIR, diffuse reflectance is very weak and could only be measured after routine FTIR spectrometers became available, generating the technique called diffuse reflectance Fourier transform spectroscopy (DRIFTS) (STEINER, 2005). In NIR spectroscopy diffuse reflectance is used in the region of 1100-2500 nm of the spectrum where the amount of scattering makes the path length so high that transmittance through 1 cm thickness of most samples is negligible. In this situation, diffuse reflectance is measured because most of the incident radiation is

reflected. This measurement mode is suitable for thicker samples, such as wheat powder and fruits (HUANG et al., 2008).

The introduction of the versatile and powerful reflectance technique called attenuated total reflectance (ATR) is probably the most useful increment in MIR measurement techniques (KAROUI et al., 2010). In this technique, a radiation beam enters a crystal and undergoes total internal reflection. The beam penetrates a fraction of a wavelength beyond the reflecting surface and when a material in close contact with the reflecting surface absorbs radiation, the beam loses energy at the wavelength where the material absorbs (see Figure 7). The depth of penetration is a function of the wavelength, the refractive index of the crystal, and the angle of incident radiation (STUART, 2005). ATR is a nondestructive method that requires little or no sample preparation and allows fast and simple sampling regardless of the state of the food system (liquid, gel, solid, etc.). However, attention must be given to the fact that a good contact between sample and crystal surface is needed, limiting the utility of ATR for thick powders. In addition, when using a zinc selenide crystal, the pH of liquid samples must be between 5 and 9 (KAROUI et al., 2010).

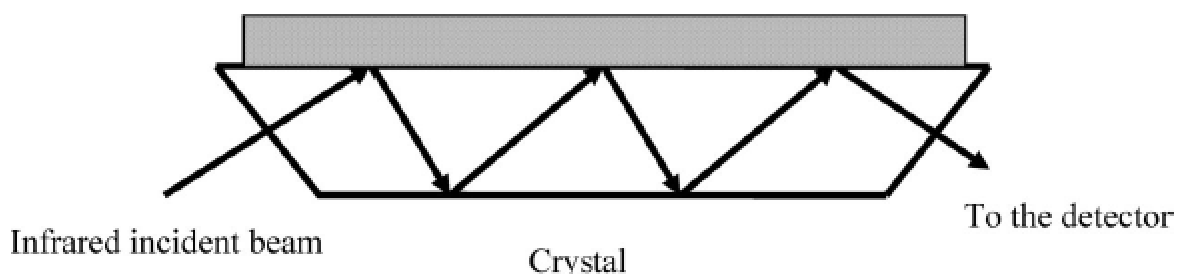

Figure 7. Attenuated total reflectance Fourier transform infrared (FTIR-ATR). Source: KAROUI et al. (2010)

2.3.2. Applications in coffee analysis

A large number of studies have demonstrated the potential of FTIR and NIR in association with chemometrics for the analysis of coffee. The application of such techniques covers the analysis of crude, roasted and ground, soluble and instant coffees.

Studies on FTIR aimed to discriminate arabica and robusta species (KEMSLEY et al., 1995, DOWNEY et al., 1997), detect adulterants such as glucose,

starch or chicory in soluble coffee (BRIANDET et al., 1996) and corn and coffee husks in roasted and ground coffee (REIS et al., 2013). Other studies aimed to classify defective and non-defective crude coffees (CRAIG et al., 2011, CRAIG et al., 2012b), classify decaffeinated and regular coffees (RIBEIRO et al., 2010), evaluate roasting conditions (LYMAN et al., 2003, WANG and LIM, 2012), and discriminate (WANG et al., 2011) and quantify (WANG et al., 2009) coffees from different geographical origins. In particular, the high structural selectivity observed in FTIR, where many fundamental peaks can be observed in isolated positions, makes this technique a powerful tool for assessing the composition of roasted coffees. In the carbonyl region (1680-800 cm^{-1}), for example, it is possible to attribute peaks to different compounds such as vinyl esters/lactones, esters, aldehydes, ketones, and acids, and observe how the composition of such compounds change as the roasting degree increases or decreases (LYMAN et al., 2003, WANG and LIM, 2012, WANG et al., 2011).

Studies on NIR include the discrimination between arabica and robusta species (DOWNEY and BOUSSION, 1996, DOWNEY et al., 1997), the quantification of robusta in admixture with arabica coffee (PIZARRO et al., 2007a), the quantification of defective and non-defective crude beans enabling a fast assessment of coffee grade (SANTOS et al., 2012), the quantification of caffeine, theobromine and theophylline (PIZARRO et al., 2007b, HUCK et al., 2005), the prediction of roasting degree (ALESSANDRINI et al., 2008) and the prediction of sensory characteristics of the beverage (ESTEBAN-DIEZ et al., 2004b, RIBEIRO et al., 2011). Concerning the latter studies, based on NIR spectra, sensory parameters such as body, flavor, bitterness, cleanliness and overall quality could be predicted in espresso and roasted and ground coffee, and associated with regions of the spectra where different compounds may absorb.

From the aforementioned applications it can be noticed that, in general, FTIR has mostly been applied for discrimination, classification and characterization purposes while NIR has been mostly employed in the development of quantitative models.

2.4. Spectra preprocessing

Infrared spectra quite often suffer from the problems of unwanted spectral variations that may have sources such as light scattering from solid samples or cloudy liquids, poor reproducibility due to path length variations, variations in temperature, density or particle size of the sample, and noises such as those from the detector or amplifier (OZAKI et al., 2007). Therefore, the use of mathematical preprocessings to reduce these variations is particularly important in the development of calibration models with minimal errors. However, there is always the danger of applying the wrong type or applying a too severe preprocessing that will remove the valuable information from the spectra. The proper choice of preprocessing methods is difficult to assess prior to model validation, but in general, performing several preprocessing steps is not advisable, and, as a minimum requirement, preprocessing should maintain or decrease the effective model complexity (RINNAN et al., 2009).

The classification of such preprocessings varies considerably in the literature. They are usually divided into four categories: (a) noise reduction (e.g. smoothing), (b) baseline correction (e.g. derivatives, MSC, SNV), (c) resolution enhancement (e.g. difference spectra), and (d) centering and normalization methods (e.g. mean centering, area normalization) (OZAKI et al., 2007). Other authors may classify MSC, SNV and normalizations as scatter correction methods, spectral derivatives as additive and multiplicative effects correction methods (RINNAN et al., 2009), and mean centering as resolution enhancement methods (OZAKI et al., 2007). In this section, only the preprocessings employed in the present study will be explored. A complete description of mathematical preprocessings applied to spectra are available in the literature (LASCH, 2012, RINNAN et al., 2009, WORKMAN JR, 2001).

Simple normalization is basically an adjustment to a data set that equalizes the magnitude of each sample and a common approach to the multiplicative scaling problem. The method attempts to identify some aspect of each sample that should be essentially constant from one sample to the next, and correcting the scaling of all variables based on this characteristic. Such characteristics include the area under the curve or the maximum value (maximum absorbance intensity) observed for all variables for the given sample. The ability of a normalization method to correct multiplicative effects depends on how well it

can separate the scaling effects that are due to properties of interest (e.g., concentration) from the interfering systematic effects. Area normalization is a common approach to correct the multiplicative scaling problem, where each variable is divided by the sum of the absolute value of the area under the curve (WISE, B. M. & GALLAGHER, 2013). Baseline correction is basically a treatment used to subtract a baseline offset from a spectrum. Multiplicative scatter correction (MSC) is a relatively simple processing step that attempts to account for undesirable scaling effects and baseline effects. This correction is achieved by regressing a measured spectrum against a reference or mean spectrum and then correcting the measured spectrum using the slope (and possibly intercept) of this fit. In most applications, the average spectrum of the calibration set is used as the reference spectrum (RINNAN et al., 2009, EIGENVECTOR RESEARCH, 2003).

Derivative transformations are common methods used to remove unimportant baseline signal from samples and reduce instrument effects in which each variable in a sample is subtracted from its immediate neighboring variable. This subtraction removes the signal which is the same between the two variables and leaves only the part of the signal which is different. Thus, derivatives de-emphasize lower frequencies and emphasize higher frequencies, tending to accentuate noise. For this reason, the Savitzky-Golay algorithm is often used to simultaneously smooth the data as it takes the derivative, greatly improving the utility of derivatized data (WISE, B. M. & GALLAGHER, 2013).

A first-order derivative transformation removes linear baseline offsets and results in a curve containing peaks and valleys that correspond to the point of inflection on either side of the log*(1/R)* peak. Bands, peaks and valleys do not follow the log*(1/R)* spectral pattern, and false peaks in the negative direction are generated, making it rather difficult to visually interpret the first derivative spectra. On the other hand, the second-order derivative calculation removes multiplicative baseline offsets resulting in a spectral pattern of absorption peaks pointing down rather than up. The second derivative transformation can be very helpful in spectral interpretation due to the fact that, in this form, band intensity and peak location are maintained with those in the log(I*/R)* spectral pattern, and apparent band resolution enhancement takes place. A few or any false peaks in the negative direction are generated; meanwhile two false

valleys in the positive ordinate scale for every band in a negative direction are generated (JOHN et al., 2007).

Mean-centering is a preprocessing method that requires special attention. It can significantly improve the performance of spectral models and is generally always recommended. The method refers to a procedure in which the average of the calibration spectra (average absorption over the calibration spectra as a function of wavelength or frequency) is calculated and subtracted from the spectra of the individual calibration samples prior to the development of the model. This way, each spectrum of the mean-centered data includes only how that spectrum differs from the average spectra in the original data (ASTM, 2012; WISE, B. M. & GALLAGHER, 2013).

2.5. Multivariate statistics applied to spectral data

Given the large amount of information provided by each single spectrum, multivariate statistics techniques are required for spectral data analysis. Figure 8 summarizes the conventional multivariate techniques used for spectral data analysis. Classification techniques can be divided into unsupervised and supervised. The first aims to classify unknown observations into groups, according to similarity correlations. Unsupervised methods can be used when there is no known information on the dataset evaluated, or as a preliminary exploratory analysis. For example, PCA can be employed to evaluate if the studied samples can be discriminated by their spectral profile. If so, the analyst is encouraged to perform more experiments and get enough data to construct a classification or quantification model. The supervised techniques aim to create classification models based on a training set of data containing observations whose category is known. These models are sequentially used to identify the category of new observations from a validation set on the basis of their explanatory variables or features.

Once the sample classification has been achieved, it can be useful to determine more precisely to what extend samples differ (ROGGO et al., 2007). The multivariate regression techniques consist of modeling a relationship between a desired physical, chemical or biological attribute of an object, which represents the dependent variables, and its spectrum response, or independent variables. This way, a regression model

describes and estimates how the properties and attributes vary when the spectra change. In sequence, the techniques that were employed in the present study will be briefly described.

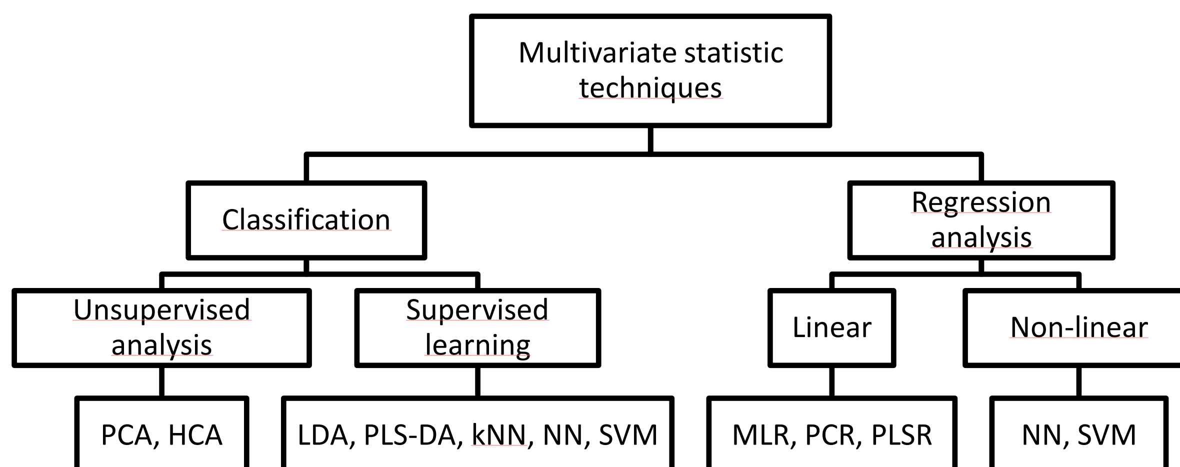

Figure 8. Conventional multivariate statistic techniques used for modeling spectral data. PCA = principal components analysis, HCA = hierarchical clustering analysis, LDA = linear discriminant analysis, PLS-DA = partial least squares discriminant analysis, kNN = *k*-nearest neighbor, NN = neural networks, MLR = multiple linear regression, PCR = principal components regression, PLSR = partial least squares regression, SVM = support vector machines.

2.5.1. Principal Components Analysis

PCA is a general multivariate statistical projection technique for data dimension reduction, and it has been used in various areas, such as exploratory data analysis, pattern recognition, quality monitoring and control (CHEN et al., 2009). The power of PCA is in revealing relationships based on similarity and difference between objects or samples that were not previously suspected. Thereby, PCA allows interpretations in chemical or physicochemical terms that would not ordinarily result. In addition, PCA is frequently used as an intermediate step for other multivariate techniques, e.g. PCs may serve as inputs to linear discrimination analysis (LDA) or multiple regressions (ADAMS, 1995, JOHNSON and WICHERN, 2007) and as a robust tool for outlier removal (LIN et al., 2007, CHEN et al., 2009).

The analysis is concerned with explaining the variance-covariance structure of a set of variables through a few linear combinations of these variables, providing data reduction and interpretation (JOHNSON and WICHERN, 2007). The traditional approach to implementation of PCA involves rotating and transforming the original *p* axes, each representing an original variable, into new axes. This transformation is performed in a way so that the new axes lie along the directions of maximum variance of the data with the condition that the axes are orthogonal, i.e. the new variables are uncorrelated (ADAMS, 1995). Although *p* components are required to reproduce the total system variability, often much of this variability and information can be accounted by a small number of *k* principal components (PCs). This way, the original data set, consisting of *n* measurements on *p* variables, is reduced to a data set consisting of *n* measurements on *k* PCs (JOHNSON and WICHERN, 2007).

2.5.2. Linear Discriminant Analyisis

LDA is a parametric and linear classifier that focuses on finding optimal boundaries between classes. In the same way as PCA, LDA is a feature reduction method. However, while PCA selects a direction that retains maximal structure in a lower direction among the data, LDA selects the direction that achieves a maximum separation among the different classes (SHARAF et al., 1986). The algorithm is based on the assumption that the classes have multivariate normal distributions. For establishing a reliable LDA classifier model, the number of objects required needs to be higher than the number of variables. Hence, for spectral data analysis, variables reduction is usually necessary (WANG & MIZAIFOFF, 2008). A common practice is using PCs obtained from PCA as inputs for LDA.

2.5.3. Partial Least Squares Regression

PLSR is a one step regression technique computed with least squares algorithms. The technique can analyze data with strongly collinear, noisy, and numerous *X*-variables, and also simultaneously model several response variables, *Y,* providing the benefit of giving a simpler overall picture than one separate model for each *Y*-variable (CLARK and CRAMER, 1993, WOLD et al., 2001, SMITH, 2002). The goal of the PLSR is to establish a linear link between two matrices, the spectral data *X*

and the reference values *Y*. This approach involves modeling both *X* and *Y* in order to find out the variables in *X* matrix that will best describe the *Y* matrix. This can be explained by the representation of the spectra in a space of linear combinations of the original variables, i.e. latent variables, which describe best the studied property (ROGGO et al., 2007). A combination of these latent variables is further used as the regression coefficients for predicting *Y*.

When analyzing highly correlated *X*-variables, which is the case of spectral data, a substantial risk for “over-fitting”, i.e., getting a well fitting model with little or no predictive power, must be considered. In addition, spectrometers typically have limited range over which they will respond linearly. For dispersive spectrometers, scattered light may limit the linear response range. Similarly, for FTIR spectrometers, phase errors can limit the linear response range. This nonlinearity in the *X*-Block may limit the transferability of the model between spectrometers as well as the robustness of the model. When spectral regions exhibiting nonlinear response are included in multivariate models, the number of variables needed to model the calibration data will increase (ASTM, 2012). This number must however be carefully chosen based on the predictive significance of each component in order not to include components when they are non-significant (MANTANUS et al., 2009, WOLD et al., 2001). This predictive significance can be reliably accessed by cross-validation. According to ASTM (2012), cross-validations is a standard procedure used in PLSR where one or more sample spectra are removed from the data matrix, their corresponding reference values are removed from the reference value vector, and a model is built on the remaining samples. The model is then used to estimate the value for the samples that were left out. This process is repeated until each sample has been left out once. The error from the cross validation is calculated as:

$$e_{cv} = \hat{y}_{cv_i} - y_i$$

where $\hat{y}_{cv}$ is the vector containing the cross validation estimates. The standard error of cross validation is then calculated as:

$$SECV = \sqrt{\frac{\sum_{i=1}^{n}\left(\hat{y}_{cv_i} - y_i\right)^2}{n}}$$

where n is the number of samples. Finally, the calibration model with the smallest SECV can be selected as the optimum model for the calibration set used (ASTM, 2012).

2.5.4. Sparse learning dimensionally reduction algorithms

One of the primary focuses in multivariate statistics applied to spectral data is finding a succinct and effective representation for original high dimensional data. Due to the typical "$p >> n$" feature in spectral data, where n is the number of observations and p is the number of variables, and the fact that these variables are correlated, statistical analysis and results interpretation of spectral data is still challenging. The conventional algorithms applied for spectroscopy data, e.g., PCA, LDA, are categorized into linear dimensionality reduction algorithms, assuming that samples are drawn from different Gaussians. These algorithms produce a low dimensional subspace and each basis of the subspace is a linear combination of all the original variables used for high dimensional sample representation. Since each of the new variables (PC's, latent variables) is a linear combination of the original ones, it is reasonable to consider each new variable as the response of several variables, representing a problem in terms of variable selection and coefficients shrinkage (ZHOU et al., 2011).

Sparse learning dimensionally reduction algorithms, e.g. least absolute shrinkage and selection operator (LASSO) (TIBSHIRANI, 1996, TIBSHIRANI, 2011) and Elastic net (ZOU and HASTIE, 2005), were developed not only to achieve dimensional reduction, but also to reduce the number of explicitly variables used in a model. In the last years these algorithms have became popular in domains with very large datasets, such as genomics and web analysis (FRIEDMAN et al., 2010, ZHU and HASTIE, 2004), according to ZHOU et al. (2011) because:

- Sparsity can make the data more succinct and simpler, so the calculation of the low dimensional representation and the subsequent processing, e.g. classification and regression, becomes more efficient;
- Sparsity can control the weights of original variables and decrease the variance brought by possible over-fitting with the least increment of the bias; and
- Sparsity provides a good interpretation of a model, revealing an explicit relationship between the objective of the model and the given variables. This is

important for understanding practical problems, especially when the number of variables is larger than the number of samples.

LASSO regression is a popular penalized least squares method that imposes an L_1-penalty on the regression coefficients. The L_1-penalty corresponds to a Laplace prior, which expects many predictors to be close to zero and a small subset to be larger and nonzero. This way, LASSO regression provides both continuous shrinkage and automatic variable selection simultaneously. A successful application of LASSO was reported by ZANON et al. (2011) for the reconstruction of glucose levels from 150 multisensor channels measured with dielectric spectroscopy and optical sensors, in a continuous glucose monitoring (CGM) sensor approach. In comparison to ordinary least squares and PLSR it was observed that LASSO regression provided better generalization performances in predicting "unseen" data from the validation set and selected original variables that were likely to be less sensitive to noise. In another study, DYAR and coworkers (2012) compared PLSR and LASSO regression techniques to determine the elemental composition of igneous and highly-metamorphosed rocks based on the spectra obtained by a remote laser-induced breakdown spectrometer (LIBS). Despite the results of both techniques were comparable in terms of accuracy, the interpretability differed greatly in terms of fundamental understanding. While PLSR generated latent variables projected into the original feature space of the spectra, LASSO required a much smaller number of nonzero correlation coefficients to determine the concentration of each of the rock elements. Thus, LASSO could directly provide an understanding of the underlying physical processes that gave rise to LIBS emissions by determining which coefficients can best represent concentration and which ones were causing matrix effects.

Although LASSO has shown success in many situations, it presents some limitations in the following scenarios: (a) in the $p > n$ case, LASSO selects at most n variables before it saturates due to the nature of the convex optimization problem, and (b) if there is a group of variables among which the pairwise correlations are very high, LASSO tends to select only one variable from the group and does not care which one is selected. The regularization technique called Elastic net was proposed to fix these problems (ZOU and HASTIE, 2005). Elastic net is a version of penalized least squares that combines both Ridge and LASSO regression. Ridge regression, or Tikhonov regularization, shrinks (toward zero) the least square coefficients, while LASSO not only shrinks the coefficients but also provides model selection. Unlike LASSO penalty, the

Ridge penalty (L_2-penalty), drawn from a Gaussian distribution, is ideal if there are many predictors and all have nonzero coefficients. Therefore, in Elastic net the penalty is a compromise between the Ridge-regression penalty ($\alpha = 0$) and the LASSO penalty ($\alpha = 1$) (FRIEDMAN et al., 2010).

FU and coworkers (2011) proposed a multi-component spectral data analysis, a combination of Elastic net for variable selection with PLSR that can be seen as a two-step variable shrinkage. First Elastic net eliminates uninformative variables. Second, the recursive leave-one-group-out strategy shrinks the variables in terms of PLSR in the root-mean-square error of cross-validation (RMSECV) sense. The algorithm was applied to near infrared (NIR) spectroscopy data sets and provided competitive results with full-spectrum PLS regression method. More recently, STEPHEN et al. (2012) applied Elastic net to the discrimination of a single bacterial strain grown on solid media culture with three different chromate levels by surface-enhanced Raman spectroscopy. Elastic net allowed the classification and visualization of discrete points or wavelengths that discriminated environmentally induced cell surface composition.

3. MATERIAL AND METHODS

3.1. Material

Arabica green coffee samples were acquired from a coffee roasting company located in Minas Gerais (MG) State, Brazil (Café Fino Grão, Contagem, MG). The samples consisted of coffee beans harvested by the strip-picking method that were rejected by color sorting machines. Samples of 2kg of whole beans were randomly taken, mixed and their beans were manually sorted (by a professional trained and certified for green coffee classification) into five lots: non-defective, immature, black and sour (separated into light and dark colored). Samples of 25g were taken from each lot and roasted in a convection oven (Model 4201D Nova Ética, São Paulo, Brazil) at 220, 235 and 250 ^{o}C. For each temperature, samples were roasted at three roasting times, resulting in nine different roasting conditions for each lot, and a total of 45 coffee samples. These conditions were established for each specific lot, given that defective coffee beans have been shown to roast to a lesser degree than non-defective coffee beans when submitted to the same processing conditions (MANCHA AGRESTI et al., 2008). Different from industrial coffee roasters, the convection oven used in this study had fixed temperature during the roasting processes, and there was no rotation or motion of the beans.

Thereafter samples were ground in a coffee grinder (Arbel, Brasil).

To assess the roasting degree of each coffee sample, color evaluation was performed in both whole and ground beans 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°. Roasting degrees were defined according to luminosity (L*) measurements similar to commercially available coffee samples (19.0 < L*< 25.0), corresponding to light (23.5 < L*< 25.0), medium (21.0 < L*< 23.5) and dark (19.0 < L*< 21.0) roasts. The weight loss was calculated as the weight difference, in percentage, of each sample before and after roasting, as described in the following equation:

$$Wl = 100\left(\frac{wi - w_f}{wi}\right) \quad \text{[Eq. 2]}$$

where, Wl is weight loss, wi is the initial weight and wf is the final weight.

Samples were then sieved, obtaining fractions with the following particle sizes: (a) 0.84 mm > particle diameter > 0.39 mm, (b) 0.39 mm > particle diameter > 0.25 mm, (c) 0.25 mm > particle diameter > 0.15 mm, and (d) particle diameter < 0.15 mm. In previous experiments it was observed that smaller particle sizes provided more repeatable spectra. Thus, fraction "d" was used for DRIFTS and fraction "c" was used for the ATR-FTIR experiment. Fraction "a" was used in the NIR experiment since the amount of sample required for the readings was higher, and this fraction was available in a larger amount.

For the classification and characterization of defective and non-defective coffees by DRIFTS, FTIR and NIR, pure samples of each of the sample classes at different roasting conditions were analyzed.

For the quantitative analysis of coffee blends by FTIR and NIR, dark and light sour, black, and immature coffees were mixed with non-defective coffee, with %defects ranging from 3% to 30% in steps of 3% (10 blends for each of the four defects). These samples corresponded to those roasted at 235 °C and to a medium roasting degree. In addition, blends of a mixture of the four defects (25% of each defect) with non-defective coffee were also produced. Therefore, the following blends were produced: (a) light sour in admixture with non-defective coffee, (b) dark sour in admixture with non-defective coffee, (c) black in admixture with non-defective coffee, (d) immature in admixture with non-defective coffee and (e) defects (25% of each defect) in admixture with non-defective coffee. These blends were disposed in Falcon tubes and shaken for one minute in tube shaker (Fisatom, Brazil). Furthermore, pure samples of non-defective coffee, representing 0% of defects, were used.

The samples were storage in plastic bags, at room temperature. The same coffee samples were analyzed for the DRIFTS, FTIR and NIR studies, but the NIR analyses were performed some months after roasting. Thus it is possible to consider that, when the samples were analyzed by NIR, their moisture content was higher.

3.2. Methods

3.2.1. DRIFTS measurements and spectral collection

A Shimadzu IRAffinity-1 FTIR Spectrophotometer (Shimadzu, Japan) with a DLATGS (Deuterated Triglycine Sulfate Doped with L-Alanine) detector was used in the measurements that were all performed in a dry controlled atmosphere at room temperature (20 ± 0.5 °C). Diffuse reflectance (DR) measurements were performed in diffuse reflection mode with a Shimadzu sampling accessory (DRS8000A). The ground coffee sample was mixed dried KBr, with a coffee/KBr mass ratio of 10%, and then 23 mg of this mixture was placed inside the sample port. Pure KBr was employed as reference material (background spectrum). Each of the 45 samples was analyzed in triplicate, obtaining a total of 135 readings. All spectra were recorded within a range of 3100–600 cm^{-1} with a 4 cm^{-1} resolution and 20 scans, and submitted to background subtraction.

3.2.2. ATR-FTIR measurements and spectral collection

A Shimadzu IRAffinity-1 FTIR Spectrophotometer (Shimadzu, Japan) with a DLATGS (Deuterated Triglycine Sulphate Doped with L-Alanine) detector was used in the ATR-FTIR measurements that were performed in a dry atmosphere and room temperature (20 ± 0.5 °C). A horizontal ATR sampling accessory (ATR-8200HA) equipped with ZnSe cell was employed. 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. Approximately 2 g of the ground and roasted coffee samples was then placed inside the metal recipient and pressed with a spatula to obtain the best possible contact with the crystal. The empty recipient was used to obtain the background spectrum. The approximate total time required for sample preparation was 5 min. All spectra were recorded within a range of 3100–800 cm^{-1} with a 4 cm^{-1} resolution and 20 scans and submitted to background subtraction. For the classification assay, pure samples of defective and non-defective coffees were read in triplicate, obtaining a total of 135 readings. For the quantitative assay, each of the 10 samples that constituted a blend of

defective and non-defective coffee as well as pure samples of non-defective coffee, representing 0% of defects, were read in five replicates. A total of 55 readings were obtained for each group of defect or the mixture of all defects to be quantified in admixture with non-defective coffee.

3.2.3. NIR measurements and spectral collection

A SpectraStar 2400 Drawer NIR spectrophotometer (Unity Scientific) with an InGaAs detector was used in the measurements. Approximately 3 g of ground coffee samples were placed inside a glass cup, filling the entire empty space, and covered. The atmosphere air was used to obtain the background spectra, which was automatically taken from time to time. The approximate time required for sample preparation and analysis was 2 min. All spectra were recorded within a range of 1200–2400 nm with 1 nm resolution and submitted to background subtraction. For the classification assay, samples of pure defective and non-defective coffees were read in triplicate, obtaining a total of 135 readings. For the quantitative assay, each sample that constituted a blend of defective and non-defective coffee was read in five replicates, and the non-defective pure coffee in eight replicates. A total of 58 readings were obtained for each group of defect or mixture of defects to be quantified in admixture with non-defective coffee.

3.2.4. Data analysis

Preprocessings were applied to raw data prior to statistical analysis to compensate any changes in experimental conditions and enhance the results. These pre-treatments included: baseline correction, area and maximum value normalization, MSC and 1st and 2nd derivatives Savitzky-Golay. Prior to the statistical analysis all datasets were mean centered. The softwares Matlab (The MathWorks, Co., Natick, MA) and the computational package PLS_Toolbox (Eigenvector Research, Inc.) were employed for the pre-treatments calculation.

3.2.4.1. DRIFTS data analysis

PCA is an exploratory multivariate analysis technique that projects the data matrix to a lower dimensional space spanned by the eigenvectors. The loading vectors corresponding to the *k* largest eigenvalues are retained to optimally capture the variance of the data and to minimize the effect of random noise (JACKSON & MUDHOLKAR, 1979). PCA was used to provide an explanation of the data variability.

Sequentially, LDA was applied to develop classifier models. LDA is a method that focuses on finding optimal boundaries between classes. As PCA, LDA is a feature reduction technique, however, while PCA selects a direction that retains maximal structure in a lower direction among the data, LDA selects the direction that achieve a maximum separation among the different classes (SHARAF et al., 1986). For establishing a reliable LDA classifier model, the number of objects required needs to be higher than the number of variables. Hence, for spectral data analysis, variable reduction is usually necessary (WANG & MIZAIKOFF, 2008). Thus, different combinations of wavenumbers that would be associated with key coffee compounds were evaluated. The wavenumbers that provided the best prediction ability of the coffee classes were considered. Model validation was performed using 25% of the samples as the evaluation set. Recognition ability was calculated as the percentage of members of the calibration set that were correctly classified, and prediction ability was calculated as the percentage of members of the validation set that were correctly classified. The software XLStat was used for the statistical analysis of the data obtained by DRIFTS.

3.2.4.2. FTIR and NIR data analyses

3.2.4.2.1. Removal of outliers and exploratory analysis

Besides being an interesting tool to provide an explanation of the data variability, PCA was also used to detect outliers prior to the classification analysis. The fitness between data and the PCA model can be calculated using the residual matrix and *Q* statistics that measures the distance of a sample from the new space of the PCA model (JACKSON & MUDHOLKAR, 1979). The Hotelling's *T*2 statistics indicates how far the estimated sample by the PCA model is from the multivariate mean of the data, thus this statistics provides an indication of variability within the normal subspace

(WISE, 1991). The combination of *Q* and *T*2 tests are used to detect remaining abnormal observations (Lin et al., 2007). Given the significance level for the *Q* and *T*2 statistics, in this case, 99%, observations with *Q* and/or *T*2 values over the threshold were classified as outliers (LIN et al., 2007). After the elimination of the outlier observations from the model, the procedure was continually repeated until no outliers were identified. The software Matlab (The MathWorks, Co., Natick, MA) and the computational package PLS_Toolbox (Eigenvector Research, Inc.) were employed.

3.2.4.2.2. Classification and characterization of pure samples of defective and non-defective coffees

Elastic net (ZOU and HASTIE, 2005) was used to classify pure samples of coffee (non-defective, dark and light sour, black and immature) and to select the variables or wavenumbers/wavelengths that reveal an explicit relationship with the different classes of samples. This algorithm was applied using the glmnet package for the R software that fits generalized linear models via penalized maximum likelihood. Samples were randomly separated into training (75%) and validation (25%) data sets. The regularization parameter lambda causes coefficient shrinkage, minimizing the residual sum of squares. In order to obtain the lambda value that gives a minimum cross-validated error, leave-one-out cross-validation was performed. In sequence, multinomial logistic models were fitted with the training data set at α values ranging from 0 to 1, in steps of 0.25. The α parameter controls the mixing between Ridge and Lasso regression. Ridge regression ($\alpha = 0$) imposes a L_2-penalty to the model inducing coefficient shrinkage, while Lasso regression ($\alpha = 1$) imposes a L_1-penalty which expects many predictors to be close to zero and a small subset to be nonzero, providing automatic variable selection. Elastic net ($0 < \alpha < 1$) provides both coefficient shrinkage and variable selection. The developed models were used to predict the classification of observations from the calibration and new observations from the validation data set. The α-value that provided the higher predictability based on the lowest classification errors was considered as the best fit model. The discriminant wavenumbers from the best fit model and their respective coefficient estimates were then extracted.

3.2.4.2.3. Quantification of coffee blends

PLSR was the technique of choice for the quantification of coffee blends, which was carried out using Matlab software (The MathWorks, Co., Natick, MA) with the PLS_Toolbox computational package (Eigenvector Research, Inc.). Samples were separated into calibration (75%) and validation (25%) sets. The optimal number of latent variables employed in the models was automatically chosen by leave-one-out cross-validation based on the minimum value of RMSECV. The combination of *Q* and *T*2 tests, with 99% of significance level, was used to detect the remaining abnormal observations in the calibration set. Observations with *Q* and/or *T*2 values over the threshold were classified as outliers and removed from the model. The evaluation of the goodness of the models was based on the following parameters: the correlation coefficient (R^2) that should be as close to 1 as possible, and the root mean square errors for both the calibration (RMSEC) and prediction (RMSEP) sets, that must be as small as possible (HUCK et al., 2005). The latter parameters were calculated as follows:

$$RMSEC = \sqrt{\frac{\sum_{i=1}^{I_C}(y_i - \hat{y}_i)^2}{I_C - v}} \quad \text{[Eq. 3]}$$

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{I_P}(y_i - \hat{y}_i)^2}{I_P}} \quad \text{[Eq. 4]}$$

where y_i *and* $\hat{y}_i$ correspond to the actual and predicted adulteration levels of sample *i*, and I_C and I_P are the total number of samples in the calibration and prediction (validation) sets, respectively. *v* is the number of degrees of freedom, or the number of latent variables used in the model more 1 for mean centered data.

4. RESULTS AND DISCUSSION

4.1. Sample preparation

In this study, only defects associated with beans irregular in visual appearance (color and surface texture) were considered. But it is worth noting that other types of defects may also occur, such as defects associated with foreign matter, coffee fruit parts and defects associated with beans irregular in cup taste after proper roasting and brewing, i.e. ‘stinker’ and ‘rio’ beans.

Figure 9 shows green coffee beans manually sorted into five lots: non-defective, immature, black and sour (separated into light and dark colored). The separation of sour beans into light and dark was performed to obtain more homogeneous lots. It must be mentioned that some black green beans may be found together with the dark sour lot. Indeed, in an experiment reported by TEIXEIRA et al. (1971) it was observed that after the removal of the silverskin from black green beans, these beans were classified, based on their appearance, as sour.

Figure 9. Manually sorted defective and non-defective coffee beans

The values of luminosity of defective and non-defective whole and ground coffee beans, respectively, are shown in Figure 10 and 11. The colorimetric results for green beans were already published by CRAIG et al. (2012b). Prior to roasting, black and dark sour, either whole or ground beans, presented lower luminosity values than non-defective, immature and light sour ones. Luminosity values were higher for ground beans than for wholes as a consequence of the fact that the bean surface is darker than its core. The results obtained for both whole and ground beans are in agreement with that obtained by FRANCA et al. (2005b) and MENDONÇA et al. (2009b). After the beans were roasted to a medium roasting degree, the values of luminosity decreased, as expected. It was observed that the interior of the beans was darker than its surface for non-defective and immature, while similar values were observed for light sour. Dark sour and black coffee still exhibited a darker surface than its core, indicating that these beans were roasted to a lighter extent than others, which is agreement with the observations by FRANCA et al. (2005b).

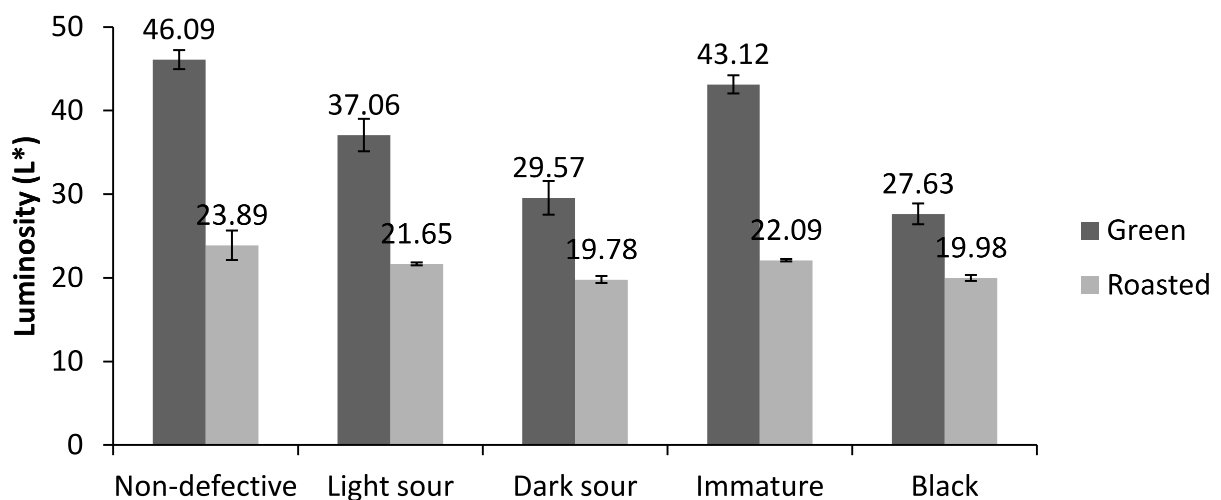

Figure 10. Average L* values of defective and non-defective whole coffee beans before and after a medium roasting

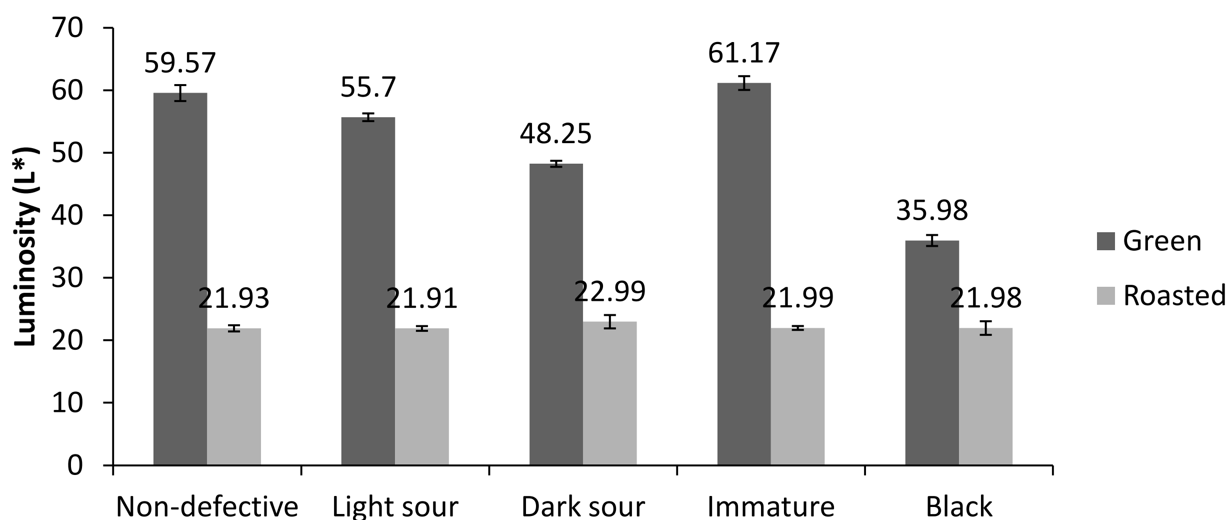

Figure 11. Average luminosity L* values of defective and non-defective coffees before and after a medium roasting

Table 9 summarizes both the colorimetric and weight loss results obtained from the roasting tests performed at 250 °C, 235 °C and 220 °C. Roasting times were adjusted in order to obtain coffees with light, medium and dark roasting degrees. These degrees were defined on the basis of the L* measurements of the coffees after grinding. It was established, based on previous colorimetric analysis of commercial ground coffees, that values of L* for light roasting degree would range from 23.5 to 25.0, from 21.0 to 23.5 for medium roasting degree and from 19.0 to 21.0 for dark roasted coffee.

Table 9. Roasting results based on color measurement (L*) and percentage of weight loss (wet weight)

	250 °C								
	Light			Medium			Dark		
	Time	L*	%WL	Time	L*	%WL	Time	L*	%WL
Non-defective	7”10”’	25.4	13.47	7”30”’	21.74	14.07	8”	19.72	15.73
Light sour	8”	24.74	13.81	8”30”’	21.22	15.33	9”	19.71	16.55
Dark sour	9”	24.55	13.78	9”30”’	21.92	15.03	10”	19.92	16.02
Black	6”45”’	24.97	11.69	8”	21.97	14.43	8”30”’	20.68	14.45
Immature	8”30”’	25.15	14.23	9”	21.98	16.13	10”30”’	18.84	17.09
	235 °C								
	Light			Medium			Dark		
	Time	L*	%WL	Time	L*	%WL	Time	L*	%WL
Non-defective	9”	23.75	13.69	10”	21.93	14.23	11”	19.38	15.89
Light sour	10”	23.56	14.45	10”30”’	21.91	15.28	11”	19.87	15.74
Dark sour	12”5”’	23.97	13.65	13”	22.99	14.62	15”	20.17	16.21
Black	8”40”’	23.75	12.53	10”	21.98	13.33	12”	20.58	15.55
Immature	13”	23.19	15.51	15”	21.99	16.04	15”30”’	19.97	16.56
	220 °C								
	Light			Medium			Dark		
	Time	L*	%WL	Time	L*	%WL	Time	L*	%WL
Non-defective	13”	23.49	14.08	14”	22.24	14.54	17”	19.66	15.4
Light sour	13”	25.15	14.19	16”	22.15	14.95	19”	20.55	16.18
Dark sour	27”	23.76	14.54	30”	2.2	14.99	34”	20.37	15.96
Black	14”	24.55	12.9	17”	21.95	13.73	20”	20.68	15.21
Immature	29”	23.28	15.61	30”	22.5	15.83	35”	21.02	16.89

Carbohydrates are quite important for the coffee roasting process. During roasting, the cell-wall matrix of the beans is opened, resulting in the solubilization of polysaccharides upon extraction. The hydrolysis of these polysaccharides results in the release of oligosaccharides and monosaccharides that will be converted into Maillard and pyrolysis products (OOSTERVELD et al., 2003). Carbohydrate content is directly associated with the bean development and fermentation processes. Previous studies suggested that non-defective beans contain more carbohydrates, especially sucrose, than sour, black (RAMALAKSHMI et al., 2007, VASCONCELOS et al., 2007) and immature beans, which presents the lowest content (MAZZAFERA, 1999, VASCONCELOS et al., 2007). Thus, it was expected that non-defective and immature beans would take a shorter and a longer time to be roasted, respectively. According to the literature, non-defective beans roast slightly faster than defective beans (VASCONCELOS et al., 2007, FRANCA et al., 2005b).

Considering the time that each coffee class took to achieve the established value of L*, results shown in Table 9 revealed that, indeed, under most roasting conditions, non-defective coffee achieved the established value of luminosity faster than other classes while immature beans were slower. Black beans, which also present low sucrose content because of the fermentation processes, achieved the established values of luminosity faster than non-defective beans at 250 °C and 235 °C. However, this result is possibly related to the fact that black beans exhibited a lower luminosity value than other beans even before roasting (CRAIG et al., 2012b). Looking at the percentages of weight loss at each roasting condition, it was observed that, because of the longer and the shorter times of exposition to heat, immature and black coffees lost more and less weight than other classes, respectively. The roasting processes were, however, conducted in laboratory scale using a convective oven with fixed temperature and no motion of the beans. In this system, the heat transfer is not as efficient and homogeneous (BONNLANDER et al., 2005). Thus, it is reasonable to consider that the roasting results obtained in this study could be changed if an industrial coffee roaster was used.

4.2. DRIFTS

4.2.1. Observations on DRIFTS spectra

The results from this experiment were already published and can be found at Annex C. Average spectra obtained for defective and non-defective roasted coffee samples are shown in Figure 12. A comparative evaluation of these spectra indicates that they are quite similar, although variations in band intensity are perceived, with absorbance values being higher for non-defective and light sour beans and lower for black beans.

The two sharp bands at 2920 and 2850 cm^{-1} have been previously identified in Arabica and Robusta roasted coffee samples (KEMSLEY et al., 1995) and also on Arabica green coffee samples (CRAIG et al., 2011ab). Studies of FTIR analysis of caffeine on soft drinks have reported two sharp bands at 2882 and 2829 cm^{-1}, with the latter being due to the asymmetric stretching of C–H bonds of methyl ($-CH_3$) group in the caffeine molecule (PARADKAR & IRUDAYARAJ, 2002). The sharp band at 1740

cm^{-1} was also reported on previous FTIR studies on roasted coffee, in association to carbonyl (C=O) vibration of the ester group in triglycerides (KEMSLEY et al., 1995) or to aliphatic esters (LYMAN et al., 2003), indicating that this band could be associated to lipids. The combination of absorptions at 1740 cm^{-1} (C=O stretch) and at 2830-2695 cm^{-1} (H–C=O stretch) with a weak shoulder-type peak at 2725-2740 cm^{-1} could be interpreted as a presence of aldehydes (MILLER et al., 2003), which are volatile compounds found aplenty in roasted coffee, as a result of the thermal degradation of unsaturated fatty acids, such as linoleic acid (OLIVEIRA et al., 2006). The wavenumber 1659 cm^{-1} has been identified by GARRIGUES et al. (2000) as due to the presence of carbonyl groups in caffeine in their FTIR analysis of trichloromethane extracts of roasted coffee. However, in this study, this band appears rather modestly in the spectra for roasted and ground coffee. Thus, it can be assumed that several other compounds in roasted coffee also absorb in that range of wavenumbers.

Several bands can be viewed in the range of 1700 to 600 cm^{-1}. The wavenumber range of 1400 to 900 cm^{-1} is characterized by vibrations of several types of bonds, including C–H, C–O, C–N and P–O (SABLINKAS et al., 2005; WANG et al., 2009). Other studies on FTIR analysis of roasted coffees (KEMSLEY et al., 1995; BRIANDET et al., 1996) have reported that carbohydrates exhibit several absorption bands in this region, so it is expected that this class of compounds will contribute to several of the observed bands. According to KEMSLEY et al. (1995), BRIANDET et al. (1996), and LYMAN et al. (2003), chlorogenic acids also present absorption in the region of 1450 to 1000 cm^{-1}. Chlorogenic acids represent a 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 et al., 2008). Axial C–O deformation of the quinic acid occurs in the range of 1085 to 1050 cm^{-1}, and 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 et al., 2005) and therefore the bands located in the range of 1450 to 1050 cm^{-1} could be partially due to chlorogenic acids. Another substance that can be associated to peaks in the 1600 to 1300 cm^{-1} range is trigonelline, a pyridine derivative that has been reported to present four bands in this range, due to axial deformation of C=C e C=N bonds (SILVERSTEIN et al., 2005).

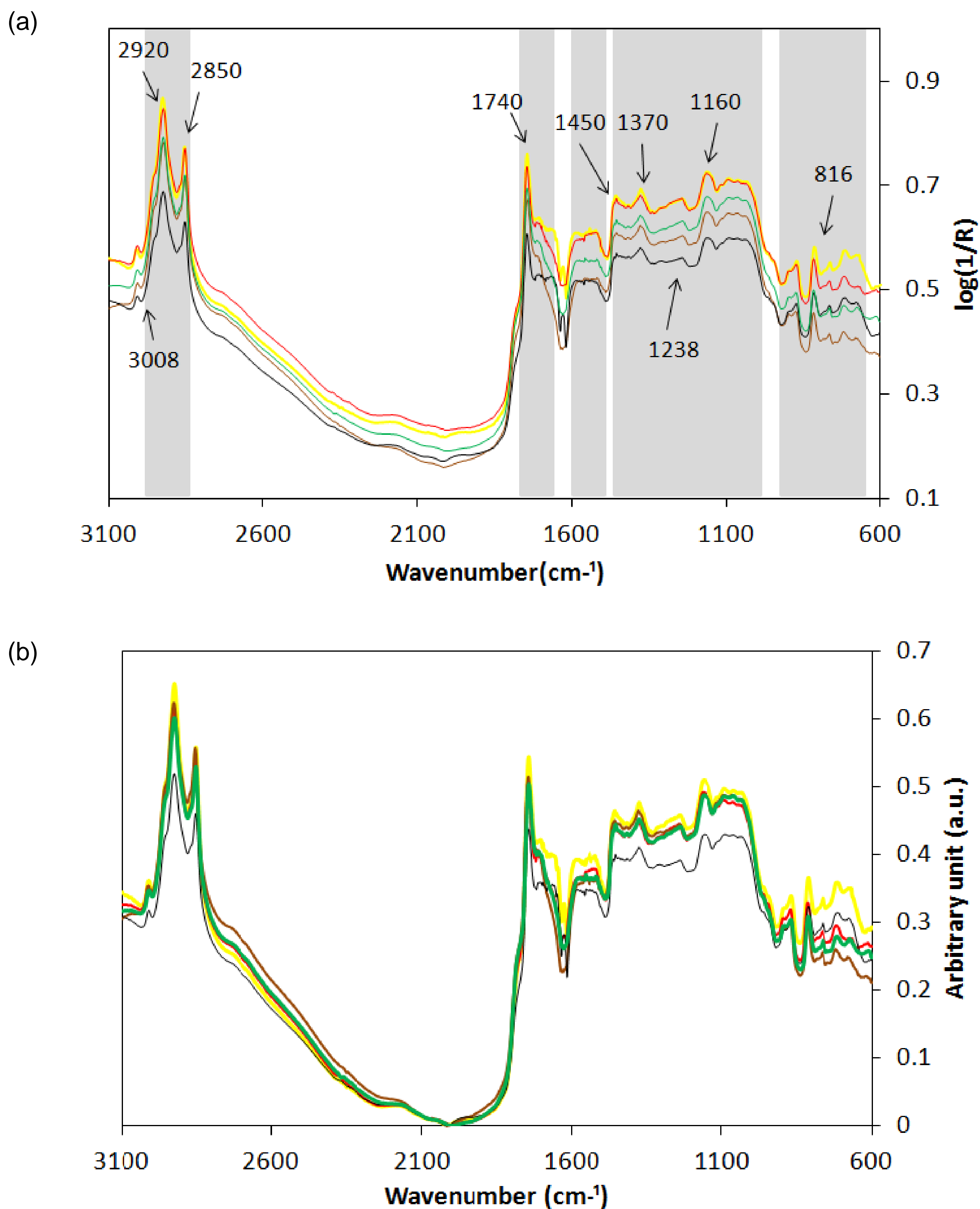

Figure 12. Mean average spectra obtained by ATR-FTIR for defective and non-defective roasted coffees. (a) original spectra and spectra submitted to (b) baseline correction and area normalization and (c) 1st derivative Savitzky-Golay. — light sour, — dark sour, — black, — non-defective, — immature (continued).

4.2.2. Exploratory and classification analyses

The scatter plots obtained by PCA analysis are displayed in Figure 2. A clear separation between categories can be observed, with four distinct major groups: non-defective, black, dark and light sour, with some outlier points. The few outlier samples from each group that were present in other classes (for example, a few non-defective and black beans in the light sour group) correspond to samples subjected to extreme roasting conditions (light roast/lower temperature and dark roast/higher temperature). Regardless of the employed spectra processing technique, immature beans are somewhat scattered between light and dark sour defects. Clustering of immature and sour defects was also observed in the analysis of green coffees by ESI (+)-MS profiles (MENDONÇA et al., 2008) or DRIFTS (CRAIG et al. 2011a), whereas MANCHA AGRESTI et al. (2008) reported grouping of immature and black roasted coffee beans according to their volatile profiles.

A clear separation between non-defective and defective coffee beans can be observed in all the plots displayed in Figure 13. Evaluation of the loadings plots obtained after PCA analysis of raw and processed spectra (not shown) indicated that the spectral ranges that presented the highest influence on PC1 and PC2 values in association with the non-defective coffees were the following: 1700-1500 cm^{-1} and 970 to 600 cm^{-1} in general representing the regions in which non-defective coffees presented higher absorbance intensity in comparison to all defective categories. Loadings obtained for first derivatives could not be associated to specific regions in the spectra.

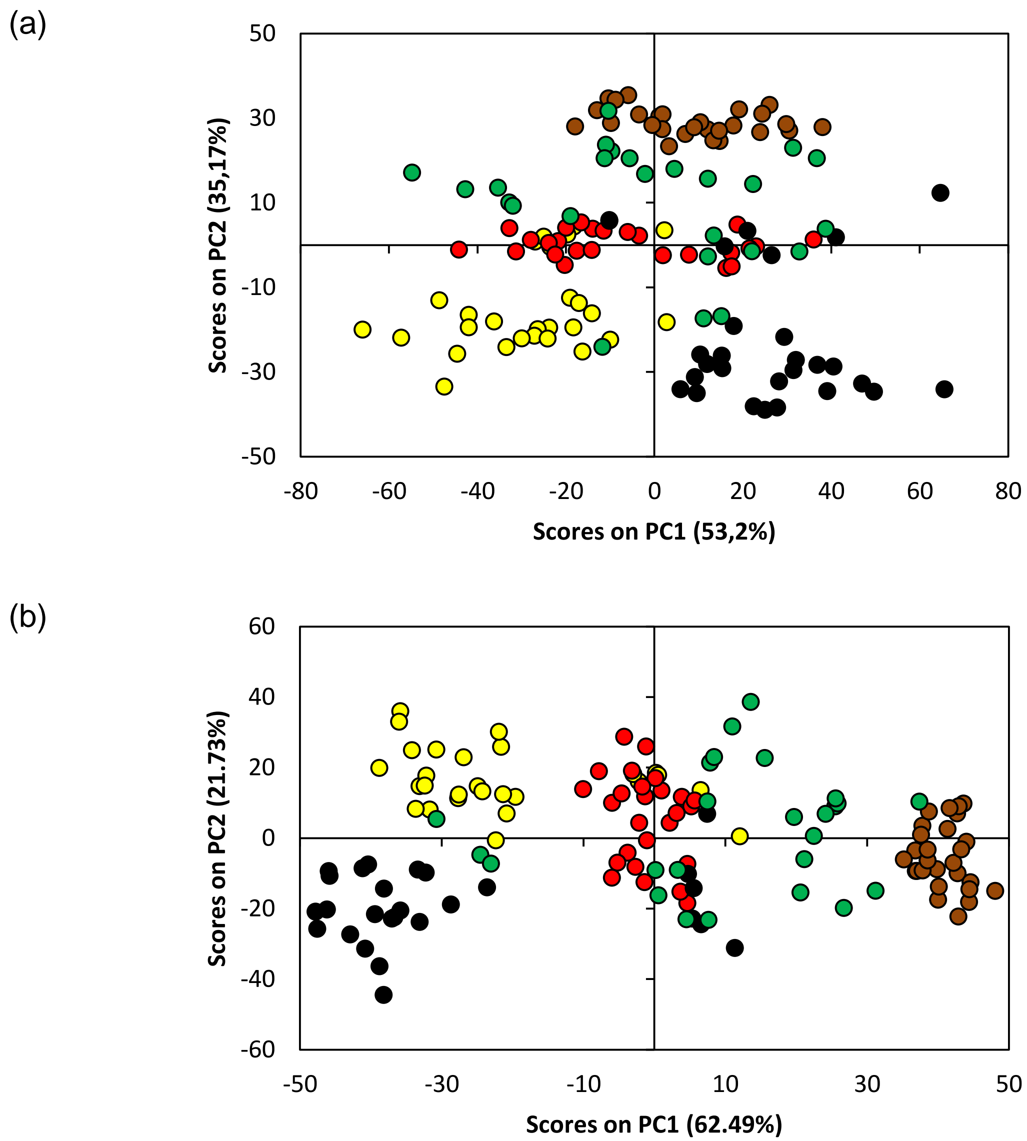

Figure 13. PCA scores scatter plots of ATR-FTIR spectra (a) original; (b) after baseline correction and area normalization; and (c) after 1st derivative Savitzky-Golay. ● non-defective, ● immature, ● black, ● light sour, ● dark sour.

(c)

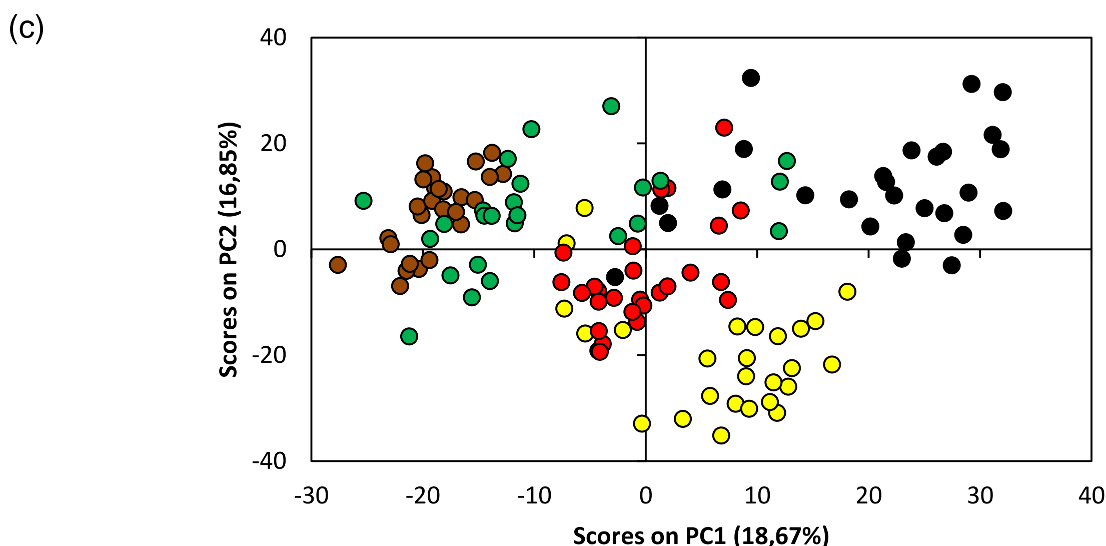

Figure 13. PCA scores scatter plots of ATR-FTIR spectra (a) original; (b) after baseline correction and area normalization; and (c) after 1st derivative Savitzky-Golay. ● non-defective, ● immature, ● black, ● light sour, ● dark sour (continued).

Results from PCA indicate that the obtained spectra could provide enough information to develop classification models for non-defective and each specific class of defective coffees. Thus, LDA was applied with the purpose of obtaining classification models for assigning categories to samples. LDA models were constructed employing different combination of variables (wavenumbers). It was observed that model recognition ability varied significantly with the number of variables, with the best correlations being provided by eight-variable models. In general the models were satisfactory (average recognition and prediction abilities above 75%) as long as the selected wavenumbers presented high loading values. Therefore, the following wavenumbers, which have been previously reported in other FTIR studies on coffee, were selected for the final models: 2924, 2852, 1743, 1541, 1377, 1076, 910 and 816 cm^{-1}. These wavenumbers can be associated with caffeine, carboxylic acids, lipids, chlorogenic acids, trigonelline and carbohydrates.

For all the developed models, the first three discriminant functions were enough to provide sample classification. The total sample variance for the models based on original spectra, normalized spectra and first derivative spectra were, respectively, 95.2, 95.3 and 97.6%. The calculated values of each discriminant function at the group centroids are displayed in Table 10. For example, considering the model based on the original spectra, it can be observed that non-defective coffee was

the only sample class who exhibited positive values for DF1, DF2 and DF3, while black coffee, negative values. The corresponding values obtained for correct classification rates for each specific model and group are shown in Table 11. Recognition and prediction abilities were quite similar for all the developed models.

Table 10. Calculated values of the first three discriminant functions at each sample group centroid

Model	Non-defective	Immature	Dark sour	Light sour	Black
Original					
DF1	4.695	-3.409	-1.918	1.614	-1.115
DF2	0.577	-0.04	2.88	-0.489	-2.975
DF3	0.454	2.121	-1.493	0.35	-1.437
Normalized spectra					
DF1	-3.21	3.274	2.847	-1.266	-0.945
DF2	-1.691	0.506	-1.588	0.828	2.513
DF3	-0.507	3.274	1.549	-0.531	1.283
First derivative					
DF1	2.402	-0.711	0.094	0.376	-2.078
DF2	0.885	-0.696	-2.073	0.625	1.28
DF3	0.423	-0.41	0.388	-0.992	0.496

DF1, DF2 and DF3 represent the first, second and third discriminant functions, respectively.

Table 11. Correct classification rates (%) for the LDA models

Model	Non-defective	Immature	Dark sour	Light sour	Black	Total
Original						
Calibration	83.3	87	100	78.3	89.6	95.5
Validation	100	100	100	100	100	100
Normalized spectra						
Calibration	84	90	100	100	78.3	89.6
Validation	100	80	100	100	100	95
First derivative						
Calibration	82.6	75	77.3	70	87	78.6
Validation	75	100	100	66.7	75	800

Classification rates were evaluated as the percent ratio between the number of samples correctly classified in a specific group and the total number of samples of that group.

The data was further re-evaluated in order to develop a more generic classification model, i.e., only one discrimination function that would provide discrimination between non-defective and defective beans, without separating the defects into specific groups. The classification functions and the respective correct classification rates are shown in Table 12. Respective average values of recognition and prediction abilities were 97.3 and 100%, for the model based on original spectra, 96.4 and 100%, for the model based on normalized spectra, and 94.6 and 95%, for the model based on first derivatives. Such results confirm that DRIFTS provides satisfactory discrimination between defective and non-defective roasted coffees.

Table 12. Model equations and correct classification rates (%) based on generic discrimination between defective and non-defective coffees

Model	Non-defective	Defective	Total
Original: $DF = -6.7 + 63.3X_{2924} - 69.9X_{2852} + 66.9X_{1743} - 21.9X_{1541} - 60.7X_{1377} - 111X_{1076} + 49.3X_{910} + 19.7X_{816}$			
Calibration	87.5	100	97.3
Validation	100	100	100
Normalized: $DF = -251.2 + 175.4X_{2924} + 93.6X_{2852} - 36X_{1743} + 18.9X_{1541} - 58.8X_{1377} + 86.6X_{1076} - 29.4b_{910} + 3b_{816}$			
Calibration	84	100	96.4
Validation	100	100	100
First derivative: $DF = -6.2 - 109X_{2924} - 815.8X_{2852} - 433.5X_{1743} - 615.2X_{1541} - 715.4X_{1377} + 2560.3X_{1076} + 859.2X_{910} - 486.3X_{816}$			
Calibration	88	96.6	94.6
Validation	94	100	95

DF represents the discriminant function. X corresponds to the absorbance (log1/R) value at the corresponded wavenumber. Classification rates were evaluated as the percent ratio between the number of samples correctly classified in a specific group and the total number of samples of that group.

In spite of the interesting results achieved, DRIFTS possesses some drawbacks from a practical point of view. First, the sample preparation requires more steps than ATR-FTIR or NIR. The sample must be diluted and well mixed with KBr that must be dry to avoid humidity interference. Then, the sample (~23 mg) is placed in a sample port and read. The particle size and size distribution, sample packing, and dilution must be carefully controlled for quantitative analysis (LARKIN, 2011). Furthermore, because of the small amount of sample analyzed, quantitative analyses are rather complicated. Thus, in this study, a quantitative assay was not attempted by DRIFTS.

4.3. ATR-FTIR

4.3.1. Observations on ATR-FTIR spectra

Average original spectra obtained for defective and non-defective roasted coffee samples are shown in Figure 14a. A comparative evaluation of these spectra indicates that they are quite similar. A considerable difference in their baseline is observed, with absorbance values (log(1/R)) being higher for light sour and non-defective coffees and lower for immature, dark sour and black beans. After the application of baseline correction (see Figure 14b) and 1st derivative (see Figure 14c), the spectra of defective and non-defective coffees exhibited a high similarity, being hardly visually differentiated.

Major peaks were observed at 2920 cm^{-1}, 1747 cm-^{1} and at 1400-900 cm^{-1}. These bands have been previously identified in arabica and robusta roasted coffee samples (KEMSLEY et al., 1995, DOWNEY et al., 1997) and also on arabica green coffee samples (CRAIG et al., 2012b, CRAIG et al., 2012a). The first region is associated with symmetric and asymmetric stretching of CH bonds in CH_2 and CH_3 groups (SILVERSTEIN et al., 2005). The region associated with CH_2 groups is highly related to the presence of lipids (WANG and LIM, 2012, REIS et al., 2013), while the CH_3 region has great importance in the identification of caffeine (PARADKAR and IRUDAYARAJ, 2002). Thus, the sharp bands at 2920 and 2850 cm^{-1} observed in the spectra presented for coffee in Figure 14 can be attributed to both caffeine and lipids. The sharp band at 1747 cm^{-1} is assigned to C=O stretch from aliphatic esters groups, thus it is mostly related to the presence of lipids. Variations in the lipid composition can subtly affect the spectral shape in and around this feature. The regions around 1747 cm^{-1} are also related to C=O stretch, but from different functional groups including aldehydes, ketons and esters. Such compounds add different aromas to the coffee, making this region of the spectrum an important region from a sensory point of view (KEMSLEY et al., 1995, KAROUI et al., 2010, LYMAN et al., 2003, WANG et al., 2009). The third region, from 1400-900 is commonly called *fingerprint* region because of the large amount of characteristic bands from single bonds or strictly specific functional groups. Among these bounds, C-H, C-O, C-N and P-O groups are included

(SABLINSKAS, 2005, SILVERSTEIN et al., 2005). In particular, carbohydrates exhibit large features in this region (KEMSLEY et al., 1995, JOHN et al., 2007).

With regard to the 1st derivative spectra (see Figure 14c), bands, peaks and valleys do not follow the log(1/R) spectral pattern observed in Figures 14a and 14b. A first-order derivative of the log(1/R) results in a curve containing peaks and valleys that correspond to the point of inflection on either side of the log(1/R) peak (JOHN et al., 2007). Thus, the actual highest point of each of the positive peaks observed in Figures 14a and 14b becomes 0 after 1st derivative transformation, and instead, two peaks, one positive and one negative, will occur before and after the actual highest point of the peaks in the original spectra (Figure 14c).

(a)

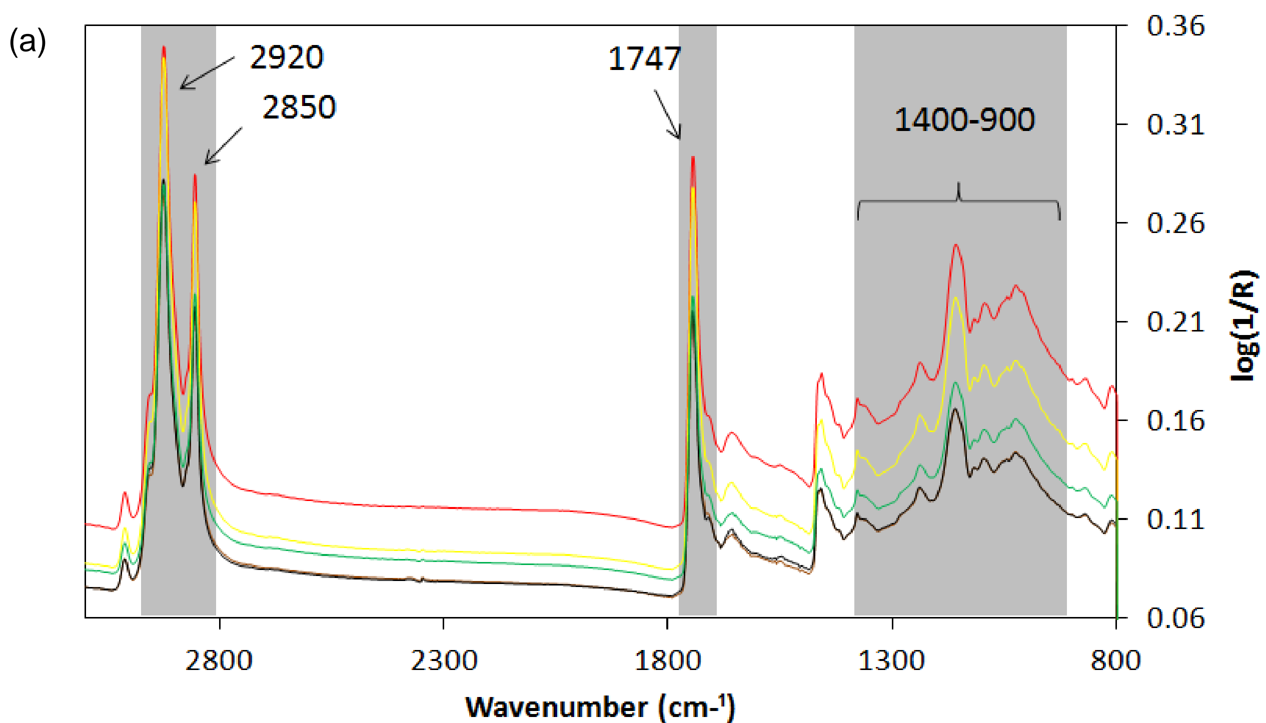

Figure 14. Mean average spectra obtained by ATR-FTIR for defective and non-defective roasted coffees. (a) original spectra and spectra submitted to (b) baseline correction and area normalization and (c) 1st derivative Savitzky-Golay. — light sour, — dark sour, — black, — non-defective, — immature.

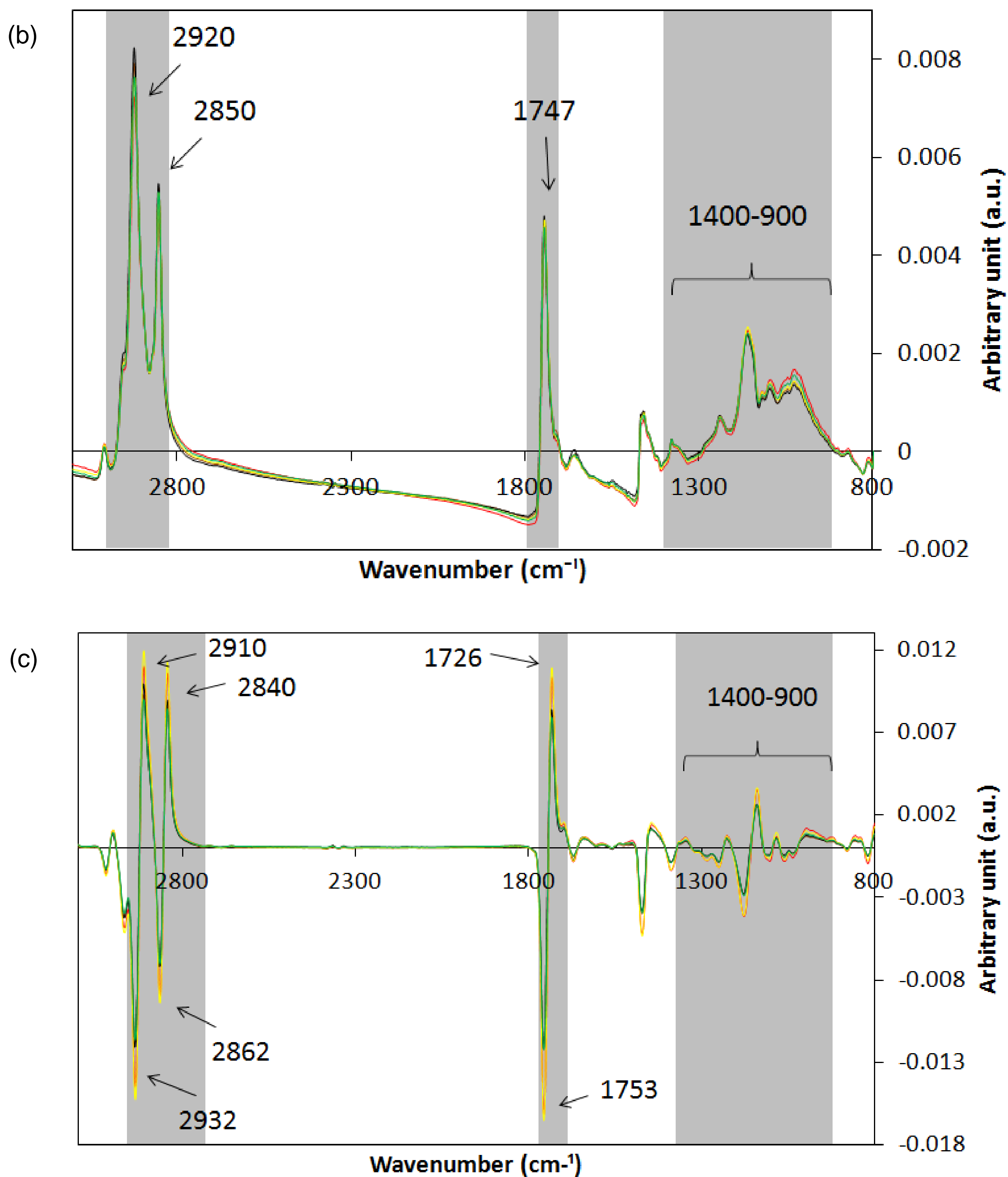

Figure 14. Mean average spectra obtained by ATR-FTIR for defective and non-defective roasted coffees. (a) original spectra and spectra submitted to (b) baseline correction and area normalization and (c) 1st derivative Savitzky-Golay. — light sour, — dark sour, — black, — non-defective, — immature (continued).

4.3.2. Outlier removal and exploratory analysis

PCA analysis was applied to the original and pretreated ATR-FTIR dataset to detect outliers or abnormal observations, and to provide an explanation of the variability within the data. Using PCA, the original high-dimensional spectral dataset containing chemical information of defective and non-defective coffees was projected to a low-dimensional space where the first PCs explained most of the variance between the samples. The fitness between data and model was calculated based on *Q* statistics that measures the distance of a sample from the new space of the PCA model (JACKSON and MUDHOLKAR, 1979). The Hotelling's *T*2 statistics indicates how far the estimated sample is from the multivariate mean of the data. It provides an indication of the variability within the normal subspace (WISE, 1991). Samples with *Q* or *T2* statistics values over the threshold of 99% were classified as outliers and removed from the data set. The procedure for outlier removal was performed and repeated until no outliers were identified. In the ATR-FTIR datasets corresponding to original, baseline corrected and area normalized, and 1st derivative transformed spectra, 0, 4 and 5 outliers were detected and removed, respectively. Figure 15 shows the outliers removed from the first PCA models developed.

(a)

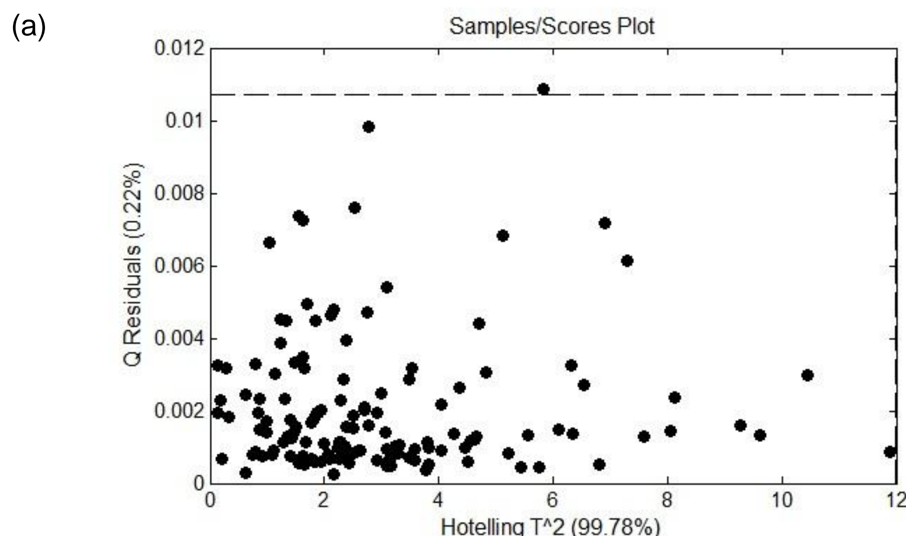

Figure 15. Plot of Q residuals vs. Hotelling's *T*2 statistic for outlier removal in the ATR-FTIR datasets corresponding to (a) original spectra, spectra submitted to (b) baseline correction and area normalization and (c) 1st derivative.

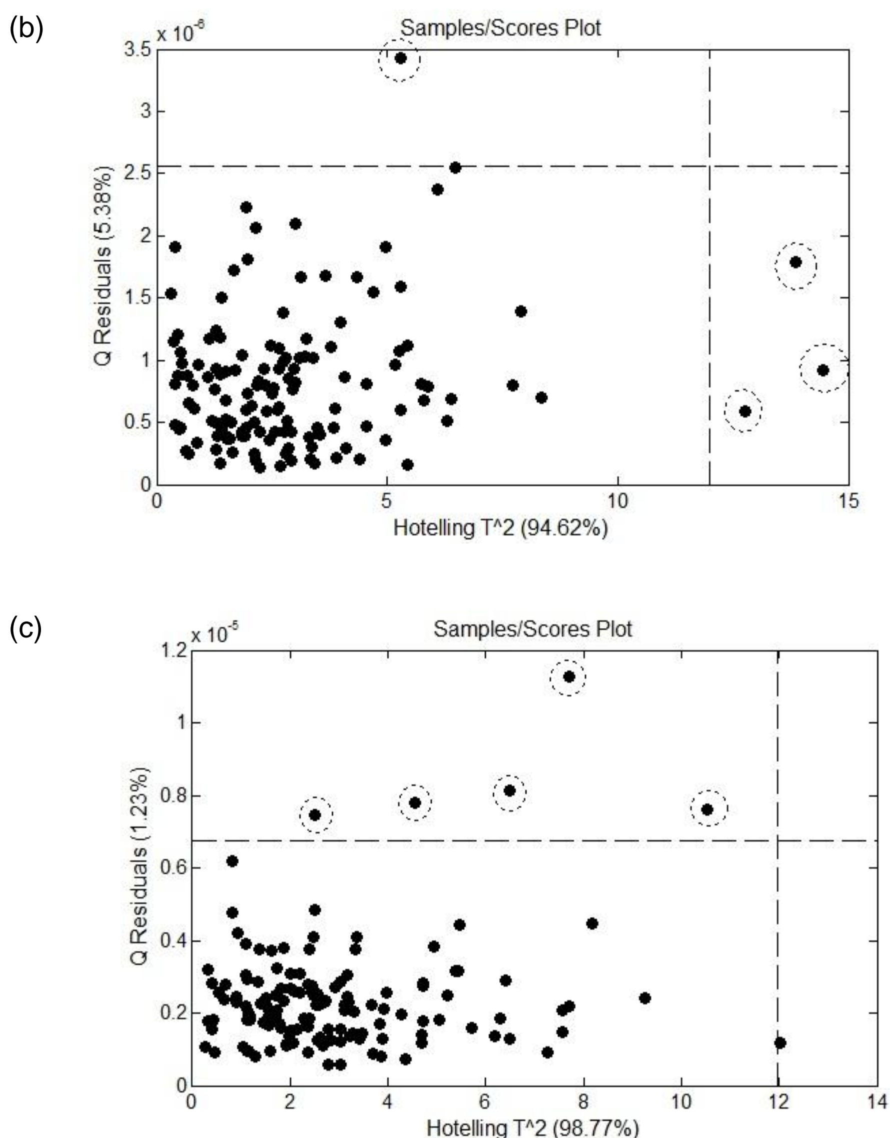

Figure 15. Plot of Q residuals vs. Hotelling's *T*2 statistic for outlier removal in the ATR-FTIR datasets corresponding to (a) original spectra, spectra submitted to (b) baseline correction and area normalization and (c) 1st derivative (continued).

The resulting scatter plots obtained by the PCA analysis of ATR-FTIR spectra are displayed in Figure 16. At all of the Figures, there is a trend, but still not clear, on the separation of non-defective and light sour from black, dark sour and immature coffees. In Figure 16a this trend is observed through PC1, which explained 94.37% of the variability amongst the samples. In Figure 14b this trend appears through PC3 that explained only 3.31% of the variability amongst the samples, although this separation is not well evidenced. The application of 1st derivative transformation slightly improved the separation of non-defective and light sour coffees from the remaining classes through PC1 (86.30) and PC3 (0.73%) (see Figure 16c). But overall, PCA analysis of ATR-FTIR

spectra did not show an evident discrimination of defective and non-defective beans, and neither a discrimination of the samples by roasting condition.

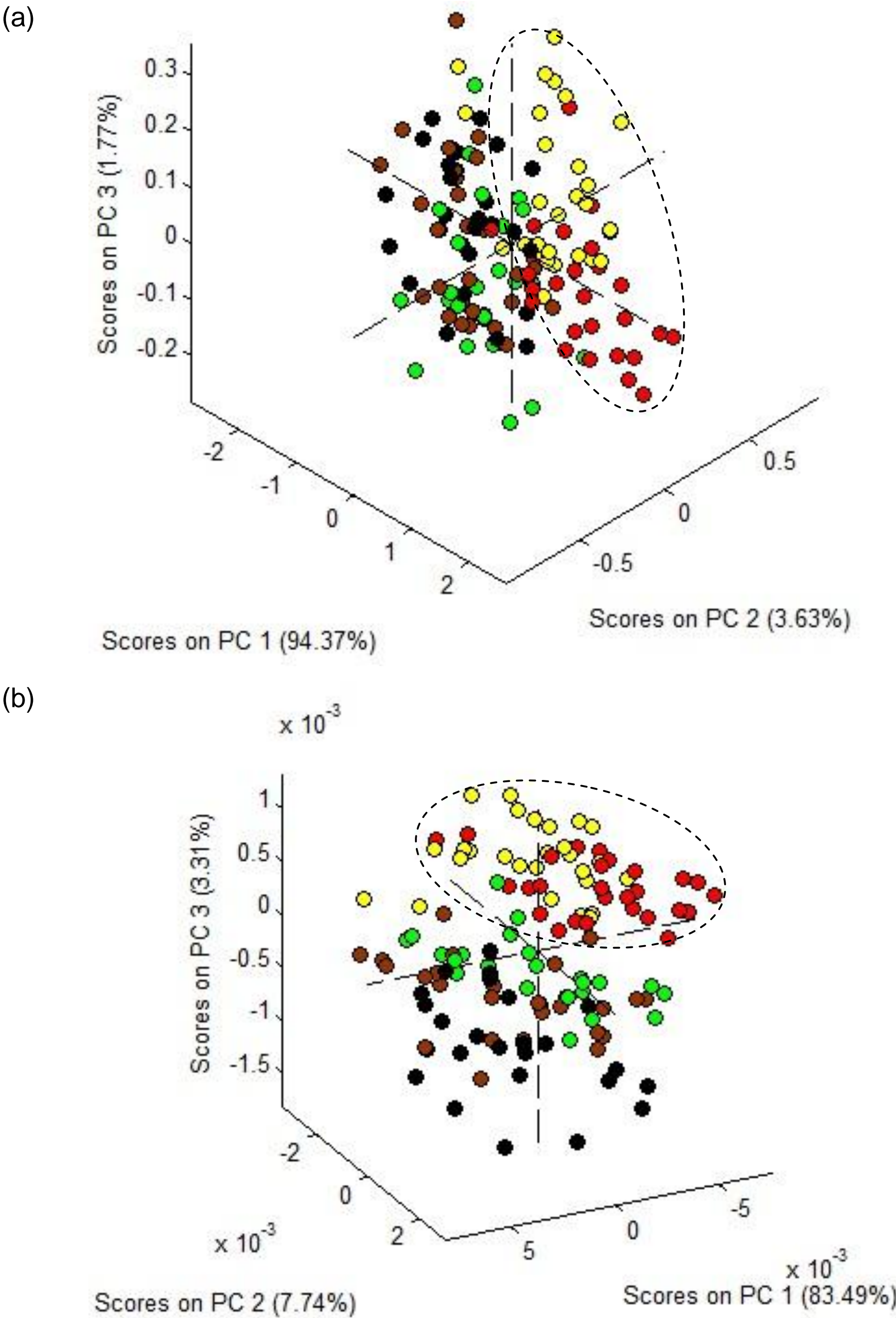

Figure 16. PCA scores scatter plots of ATR-FTIR spectra (a) original; (b) after baseline correction and area normalization; and (c) after 1st derivative Savitzky-Golay. ● non-defective, ● immature, ● black, ● light sour, ● dark sour.

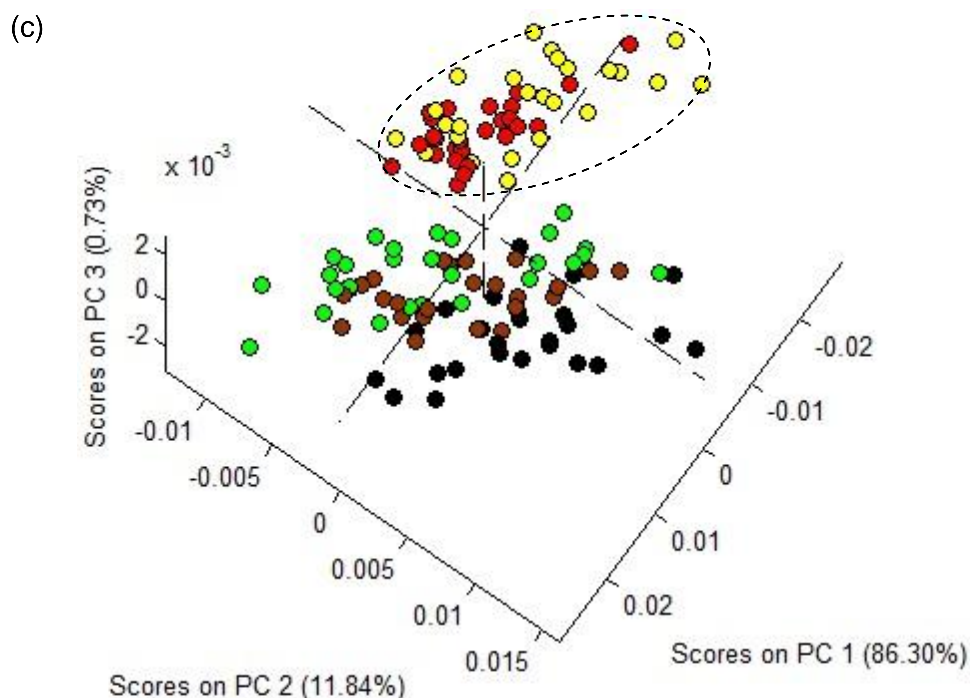

Figure 16. PCA scores scatter plots of ATR-FTIR spectra (a) original; (b) after baseline correction and area normalization; and (c) after 1st derivative Savitzky-Golay. ● non-defective, ● immature, ● black, ● light sour, ● dark sour (continued).

4.3.3. Classification and variable selection by Elastic net: chemical assignments of selected ATR-FTIR absorption bands

Table 13 summarizes the results obtained by the multinomial logistic models constructed via Elastic net to classify the ATR-FTIR spectra. As expected, the application of preprocessings to the data increased the accuracy of the models in a way that models constructed with the original spectral exhibited worse results in comparison to models constructed with preprocessed spectra. In general, models constructed with a lower number of nonzero variables provided better results. Excellent statistical classification of defective and non-defective coffees was achieved at α levels ranging from 0.25 to 1. In particular, perfect classification was obtained with baseline corrected and normalized spectra at α level of 0.25.

These results indicated that an accurate classification can be achieved from relatively small regions of the spectrum, by means of imposing penalties in the model to reduce the number of explicit variables. LASSO regression (α = 1) imposes a L_1-penalty that corresponds to a Laplace prior distribution, which expects many predictors to be

close to zero and a small subset to be nonzero, providing automatic variable selection (ZANON et al., 2011). Although LASSO has shown success in many situations, it presents some limitations if there is a group of variables among which the pairwise correlations are very high. In this scenario LASSO tends to select only one variable from the group and does not care which one is selected. The regularization technique called Elastic net was proposed to fix these problems (ZOU and HASTIE, 2005). Elastic net is a version of penalized least squares that combines both Ridge (α = 0) and LASSO regression, providing shrinkage and model selection at the same time (FRIEDMAN et al., 2010). Considering the classification results shown in Table 13 and the exposed discussion, the variable selection for ATR-FTIR results was based on Elastic net, with the coefficient estimates plotted at α level of 0.75.

Table 13. Percentage of correct classification obtained by Elastic net models based on ATR-FTIR spectra: comparing treatments and penalties

Treatment	**α**	**Nonzero variables**	**Correct Classification (%)**		
			Cal	CV	Val
Original spectra	0	676	0.63	0.54	0.71
	0.25	318	0.88	0.88	0.93
	0.5	239	0.89	0.89	1
	0.75	151	0.9	0.9	0.93
	1	39	0.96	0.96	0.99
Baseline correction + area normalization	0	676	0.91	0.91	0.87
	0.25	291	1	1	0.97
	0.5	175	1	1	0.97
	0.75	99	1	1	0.97
	1	35	1	1	0.97
1st derivative	0	676	1		0.97
	0.25	254	1		0.95
	0.5	112	1	1	0.96
	0.75	73	1		0.95
	1	33	1		0.94

Cal = calibration; CV = cross-validation; Val = validation.

The reason why Elastic net was chosen as the technique for variable selection is because it provides a good interpretation of a model, revealing an explicit relationship between the objective of the model and the given variables. In this situation, it allows the visualization of discriminating wavenumbers and how these wavelengths contributed to the correct classification of each coffee class. For example, a positive coefficient estimate indicates that a specific coffee class exhibited higher intensity at that range of the spectrum, which may be associated with a higher concentration of a specific compound in comparison to other coffee classes.

Figures 17a and 17b show the Elastic net coefficient estimates for original spectra and spectra submitted to baseline correction and area normalization, respectively. Regardless of variations in the absolute coefficient estimate values, the same regions of the spectra were selected for the classification of defective and non-defective coffees. It is also important to mention that the region between 2800 and 1800 cm^{-1} was not included in the development of the classification models for the following reasons: in terms of coffee, there is no compound with chemical importance absorbing in this region; the absorption of carbon dioxide in the region around 2330 cm^{-1} causes considerable noise effect; and there is a slight shift in the baseline of the spectra even after the application of baseline correction.

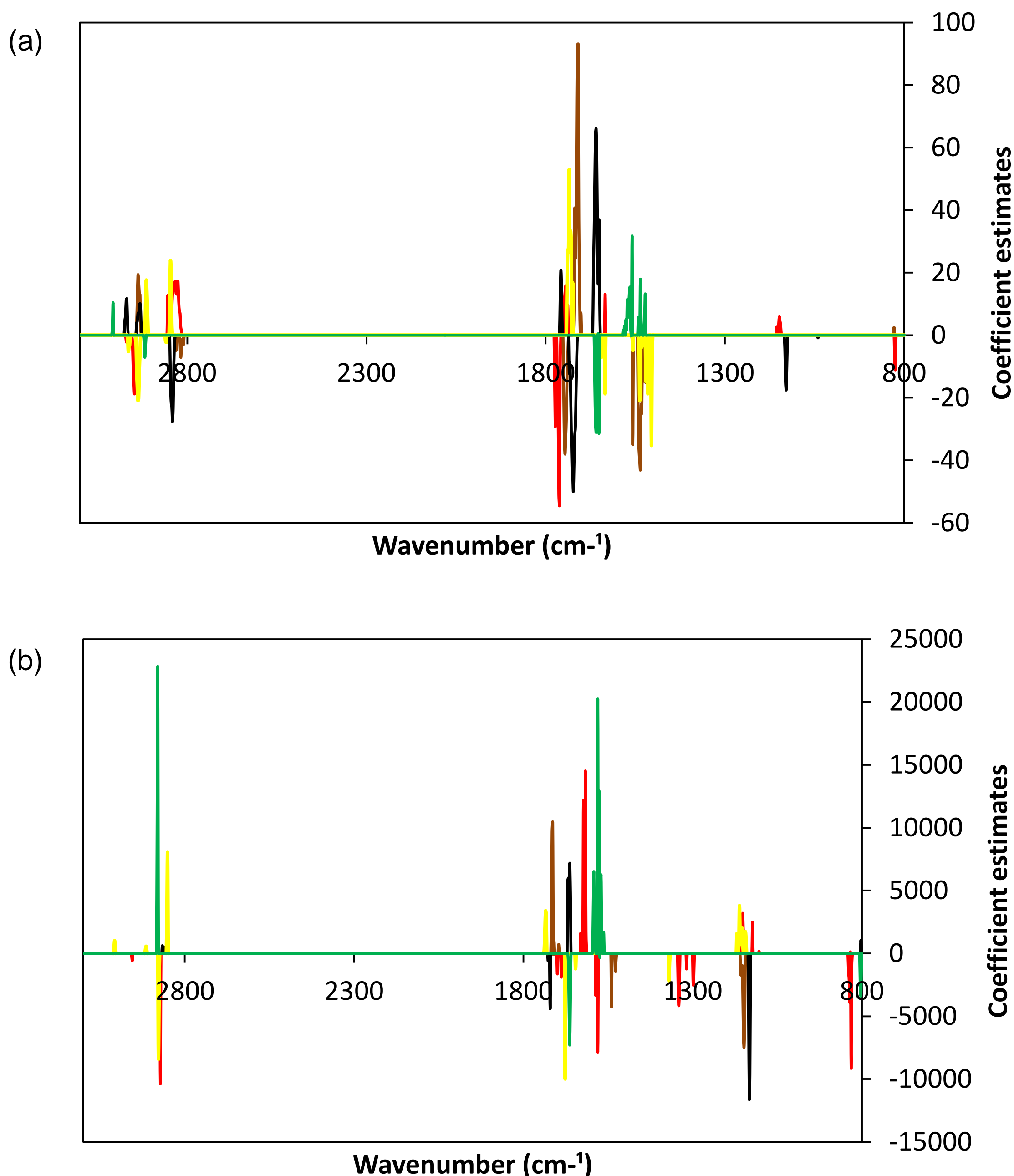

Figure 17. Elastic net coefficient estimates at α = 0.75 for ATR-FTIR (a) original spectra and (b) spectra submitted to baseline correction and area normalization. A peak indicates that correct classification of spectra is associated with the corresponding spectral region. A positive peak indicates higher intensity than other classes; a negative peak indicates lower intensity. —non-defective, —light sour, —dark sour, —black and —immature.

It is rather difficult to interpret a first derivative spectra because bands, peaks and valleys do not follow the log(1/R) spectral pattern. As discussed previously, a first-

order derivative of the log(1/R) results in a curve containing peaks and valleys that correspond to the point of inflection on either side of the log(1/R) peak (JOHN et al., 2007). For this reason, the 1st derivative spectra obtained by ATR-FTIR were disregarded for the Elastic net variable selection.

The following discussion was conducted examining each of the nonzero coefficient estimates obtained by Elastic net and, based on the literature, conducting a tentative assignment of these coefficients to chemical compounds that may absorb in the selected region of the ATR-FTIR spectra. Table 14 summarizes the chemical assignment of the selected spectral regions obtained for the original and the spectra submitted to baseline correction and area normalization. To make the interpretation of these results more clear and simple, only the sign of the coefficients will be taken into account, while the absolute values will be disregarded.

Correct classification of non-defective coffee was associated with 12 spectral regions. The positive region 6 and the positive regions 21, 22, 23, 26 and 29 could be related to higher contents of carbohydrates, especially polysaccharides, and lipids in non-defective coffee, respectively. This finding was expected considering that studies in the literature have reported higher levels of these compounds in non-defective coffee than in defective ones (OLIVEIRA et al., 2006, VASCONCELOS et al., 2007, RAMALAKSHMI et al., 2007, FRANCA et al., 2005b, MAZZAFERA, 1999). The selected regions 10, 14, 15, 16 and 28 exhibited negative coefficients and could be related to lower levels of amino acids, proteins and caffeine in non-defective coffees. This result is in agreement with the studies by MAZZAFERA et al. (1999), FRANCA et al. (2005b) and VASCONCELOS et al. (2007), where slightly lower levels of protein and caffeine were observed in non-defective coffee. According to WANG et al. (2011) and WANG and LIM (2012), roasting coffees to a medium and dark degrees increases the presence of compounds such as aldehydes, that also contributed to the positive region 21, and decreases the content of caffeine. Thus, results from this study suggests that, under the same roasting conditions, non-defective coffee attain a higher extent of roasting in comparison to defective ones. The negative region 9 could not be attributed to any specific compound, but according to SILVERSTEIN et al. (2005), symmetrical C-H bending vibrations of methyl groups occurs near 1375 cm^{-1}. Thus it is possible that compounds with methyl groups, such as caffeine, would contribute in this region.

Ten positive regions were associated with correct classification of light sour coffee. Among them, regions 4, 5 and 6, and regions 22, 25 and 26 suggest that light

sour coffee contains high content of polysaccharides and lipids, respectively. The positive regions 12, 13, 14 and 27 can be related to high levels of protein and most likely caffeine. The negative region 2 was attributed to the absorption of phenolic compounds, and can be related to a low content of chlorogenic acid or pyridine. There is no evidence that light sour coffee presents lower levels of such compounds, but these negative coefficients could be attributed to a higher degradation of chlorogenic acids during roasting. Regions 11 and 28 suggest that light sour coffee is associated with low levels of protein and caffeine, which contradicts the positive coefficients at regions 12-14 and 27. And finally regions 17, 18 and 24 may be related to low level of aromatic acids and vynil esters or lactones in light sour coffee. In the study by WANG and LIM (2012) using ATR-FTIR it was observed that the absorbance values of unsaturated ester/lactones were lowered from the start of first crack to the start of second crack and then stabilized. These findings suggest that light sour as well as non-defective coffee, attained a higher extent of roasting than other coffee classes. The negative regions 7 could not be assigned to any potential vibration that would occur in a coffee sample. Region 8 has not been reported in the literature to any coffee compound, but according to SILVERSTEIN et al. (2005) the OH in-plane bending vibrations in alcohols and phenols occurs between 1330 and 1420 cm^{-1}. Thus, this region could possibly be related to chlorogenic acids.

In the case of dark sour, correct classification was associated with a strong absorption in region 2, where phenolic compounds possibly absorb. This finding could result from the low degree of roasting attained by dark sour coffee. The negative coefficients observed at regions 6 and 10 were related to polysaccharides and amino acids, respectively, and 22 and 25, related to lipids. Dark sour coffee also presented positive coefficients at the characteristic regions of aromatic and aliphatic acids absorption (17-20). The noticeably higher acidity of sour beans in comparison to other coffee classes, which occurs because of bean fermentation, was previously demonstrated in the literature for green coffee. However, it is worth noting that such acidity could also be influenced by the presence of chlorogenic acids (FRANCA et al., 2005a, RAMALAKSHMI et al., 2007, VASCONCELOS et al., 2007), or by a lighter extent of roast.

Correct classification of black coffee was related to three regions with positive coefficients. Region 1 was attributed to phenolic compounds and pyridine, specifically with chlorogenic acids and trigonelline. According to MAZZAFERA (1999) and FRANCA

et al. (2005b) significant lower levels of chlorogenic acids and trigonelline were found in raw black coffee than in other coffee classes. Nevertheless, black coffee attains a lighter roasting extent, resulting in a lower degradation of these compounds. Results from FRANCA et al. (2005b) indicated that, after roasting, black coffee exhibited significant higher level of chlorogenic acids than other coffee classes, corroborating the results found in this work. The positive region 16 was attributed to protein absorption. In the studies by OLIVEIRA et al. (2006) and VASCONCELOS et al. (2007), prior to roasting proteins were found at higher levels in black beans, but no significant difference among defects was found among roasted coffees. According to FABIAN et al. (1994), beyond protein absorption, caffeine could also contribute to region 16. Although there is no evidence that, prior to roasting, caffeine levels among defects vary significantly (FRANCA et al., 2005b, MAZZAFERA, 1999), the fact that black beans roast to a lesser extent would lead to a higher content of caffeine after roasting (FRANCA et al., 2005b), which explains the positive coefficient at region 27. The negative coefficients related to polysaccharides at regions 3 and 5 were somewhat expected considering that black beans can be originated from fermentation processes. Regions 20 and 21 were assigned to aliphatic acids or aldehydes and ketones, compounds that are mainly formed during roasting (LYMAN et al., 2003, WANG et al., 2011), confirming that black coffee roasted to a lesser extent. Concerning the regions 21 and 26, assigned to lipids, according to the literature, black coffee contains lower level of lipids than non-defective (OLIVEIRA et al., 2006) and possibly light sour coffees, but slightly higher lipid content among defective beans (OLIVEIRA et al., 2006, MAZZAFERA, 1999).

The correct classification of immature coffee was associated with higher absorption intensities at regions 10 and 11, attributed to amino acids and proteins, and 28, attributed to caffeine. In terms of amino acids, MAZZAFERA (1999) reported higher levels for immature coffee, although the same was not observed for proteins. There is no evidence that immature coffee presents higher levels of proteins (MAZZAFERA, 1999, FRANCA et al., 2005b, FRANCA et al., 2005a), which dominated the selected region 11. According to the literature, immature coffee contains higher content of caffeine in the endosperm (MAZZAFERA et al., 1991) or either in the whole fruit (SUZUKI and WALLER, 1984) in comparison to non-defective coffee. However, no significant difference among defects was found by MAZZAFERA (1999) and FRANCA et al. (2005b). Negative coefficients for immature coffee were observed at regions 1, 16

and 29, related to phenolic compounds, protein and lipids, respectively. In spite of the high content of chlorogenic acids in green immature beans (MAZZAFERA, 1999, FRANCA et al., 2005a, FARAH et al., 2005), most of this content is expected to be degraded during roasting (FRANCA et al., 2005b). The presence of positive and negative regions associated with proteins reveals some inconsistency in these assignments. Finally the negative coefficients of immature coffee attributed to lipids were in agreement with the study by OLIVEIRA et al. (2006).

Table 14. Tentative chemical assignment of significant ATR-FTIR bands selected by Elastic net (α = 0.75) for the classification of defective and non-defective coffees

Region selected	Gereral region ranges	ND	LS	DS	BL	IM	Vibration modes	Compounds	References
1	800-801				+	-	Out-of-plane CH bend, adjacent CH wag	Phenolic compounds, pyridine	1
2	823-835		-	+			out-of-plane CH bend, adjacent CH wag	Phenolic compounds, pyridine	1
3	1039				-		C-O str	Celulose	2
4	1099		+				C-O str	Carbohydrates	2, 3
5	1118-1132		+		-		C-O str	Carbohydrates	3, 4
6	1138-1165	+	+	-			C-O str	Polysaccharydes, celulose	4
7	1292-1294		-						
8	1334-1338		-						
9	1363-1365	-					sym CH bend in CH_3		1
10	1502-1558	-		-		+	sym NH_3 bend	Amino acids	1, 2, 5
11	1560-1589		-			+	NH bend Amide II	Protein	1, 2
12	1610-1612		+				NH_2, NH bend Amide II, lactam	Caffeine, protein	1, 5
13	1618-1625		+				NH_2, NH bend Amide II, lactam	Caffeine, protein	1, 5
14	1633	-	+				NH_2, NH bend Amide II, lactam	Caffeine, protein	1, 5
15	1641-1645	-					NH_2, NH bend Amide II, lactam	Caffeine, protein	1, 5
16	1649-1674	-			+	-	Amide I	Protein	2
17	1683-1691		-	+			C=O str in aryl conjugated acids	Aromatic acids, CGA	6, 7
18	1695		-				C=O str in aryl conjugated acids	Aromatic acids, CGA	6, 7
19	1701			+			C=O str in aryl conjugated acids	Aromatic acids, CGA	6, 7
20	1705-1720			+	-		C=O str	Ketones, aliphatic acids	1, 6, 8
21	1722-1735	+			-		C=O str	Aliphatic aldehyde, lipids	5, 8
22	1737-1749	+	+	-			C=O str from esters	Lipids	2, 6
23	1753-1759	+					C=O str from esters	Lipids	2, 6
24	1760-1774		-				C=O str adjacent to C-O- group	Vynil esters, lactones	6

25	2810-2833		+	-			sym CH str in CH_2	Lipids	1
26	2835-2848	+	+		-		sym CH str in CH_3	Lipids	1
27	2850-2862		+		+		sym CH str in CH_3	Caffeine	1, 9
28	2868-2877	-	-			+	sym CH str in CH_3	Caffeine	1, 9
29	2908-2920	+				-	asym CH_2 str	Lipids	1, 5, 10

LS = light sour, DS = dark sour, BL = black, ND = non-defective, IM = immature.

sym = symmetric, asym = asymmetric, str = stretching

CGA = chlorogenic acids

List of references:

1. SILVERSTEIN et al. (2005)
2. KAROUI et al. (2010)
3. BRIANDET et al. (1996)
4. KEMSLEY et al. (1995)
5. WANG and LIM (2012)
6. LYMAN et al. (2003)
7. FABIÁN et al. (1994)
8. WANG et al. (2011)
9. YANG et al. (2005)
10. REIS et al. (2013)

4.4. NIR

4.4.1. Observations on NIR spectra

Figure 18a shows the original mean average spectra of defective and non-defective coffees. The spectra exhibit a similar shape to the typical NIR spectra of coffee reported in the literature (ESTEBAN-DIEZ et al., 2004b, RIBEIRO et al., 2011). Overall, spectra of defective and non-defective coffees exhibited notable similarity, being visually differentiated only by a slight variance in the absorbance (log(1/R) intensity.

The shape of the spectra was particularly dominated by broad water absorption bands between 1440-1460 nm (first overtone of O–H stretching) and 1930-1950 nm (combination band of O–H stretching and O–H deformation). Thereby, in these regions the typical absorption bands of coffee components were very weak in comparison with the water bands. This feature was also observed after spectra were submitted to MSC (Figure 18b) and baseline correction (Figure 18c). Other few regions of the spectra that could be identified were extensively reported in the literature as characteristic absorption regions of specific compounds. For example, the two well defined peaks between 1715-1760 nm and 2300-2350 nm were assigned to lipids and the peak around 2100 nm was previously assigned to carbohydrates (JOHN et al., 2007, RIBEIRO et al., 2011). Nevertheless, these peaks are composite of numerous individual bands that cannot be visibly resolved in log*(1/R)* form due to the high overlap of overtones and combination bands. Thus, a discussion on the main differences between spectra of defective and non-defective coffees will be conducted in section 4.4.3 supported by statistical analysis.

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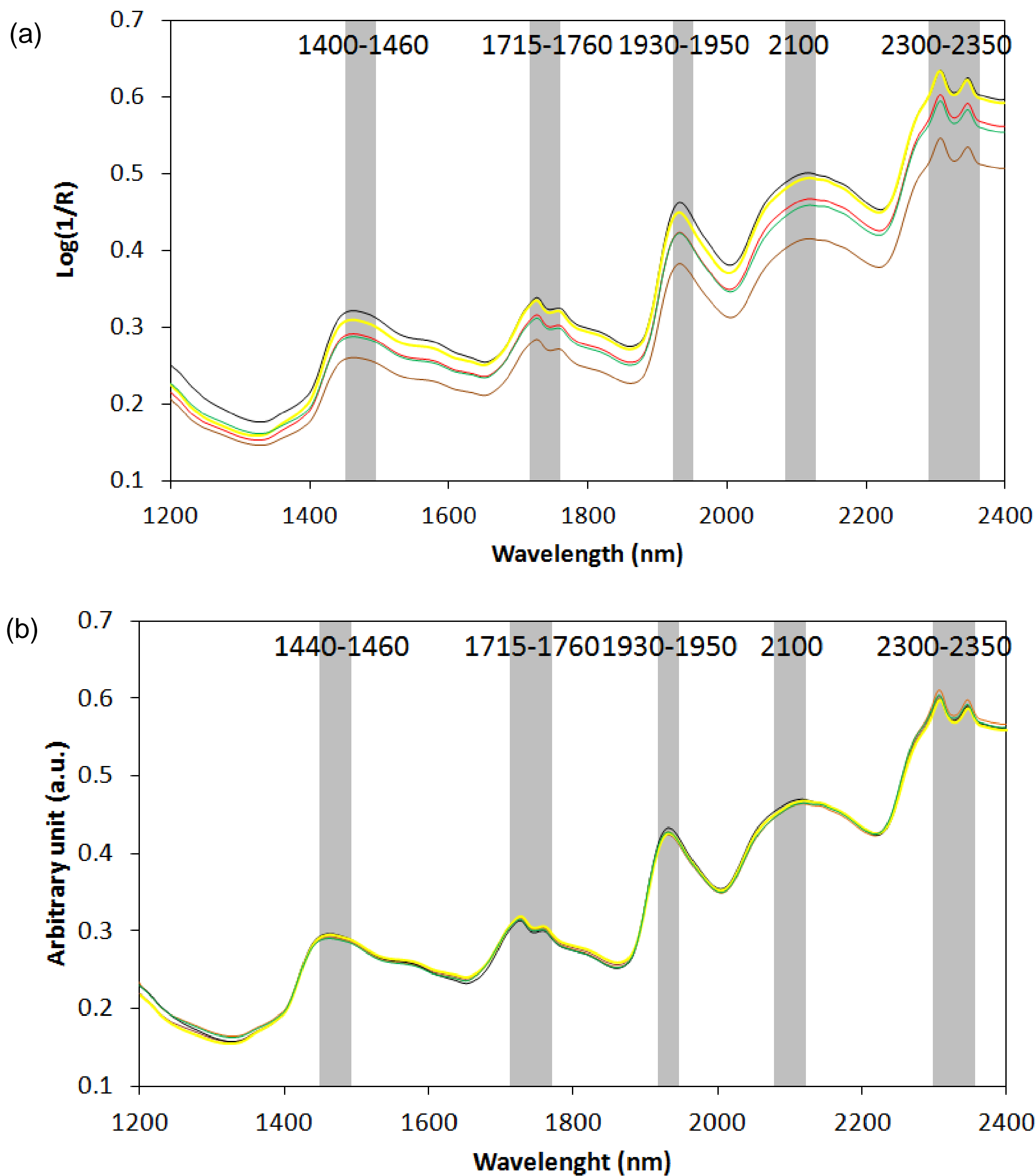

Figure 18. Mean average spectra obtained by NIR for defective and non-defective roasted coffees. (a) original spectra and spectra submitted to (b) MSC normalization and (c) baseline correction. — light sour, — dark sour, — black, — non-defective, — immature.

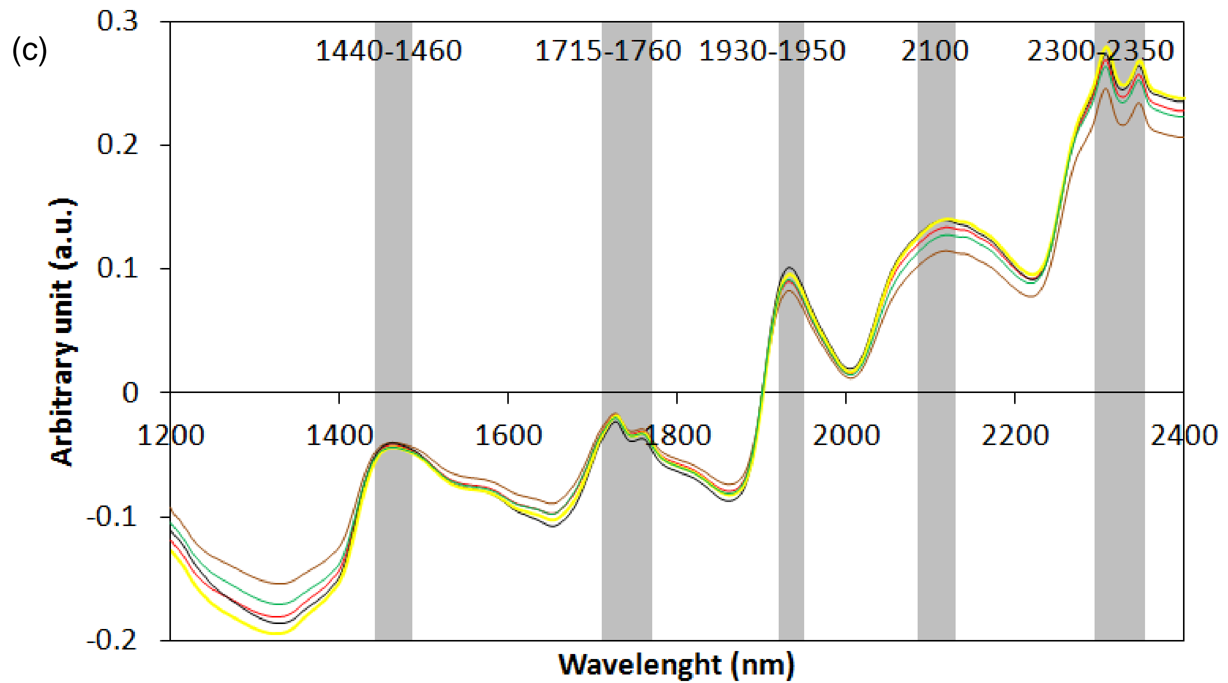

Figure 18. Mean average spectra obtained by NIR for defective and non-defective roasted coffees. (a) original spectra and spectra submitted to (b) MSC normalization and (c) baseline correction. — light sour, — dark sour, — black, — non-defective, — immature (continued).

4.4.2. Outliers removal and exploratory analysis

PCA analysis was applied to original and pretreated NIR datasets to detect outliers or abnormal observations, and to provide an explanation of the variability within the data. Samples with *Q* or *T2* statistics values over the threshold of 99% were classified as outliers and removed from the data set. The procedure for outlier removal was performed and repeated until no outliers were identified. A total of 6 outliers were detected and removed from each of the NIR datasets, corresponding to original, baseline corrected and MSC corrected spectra. Figure 19 shows the outliers removed from the first PCA model developed.

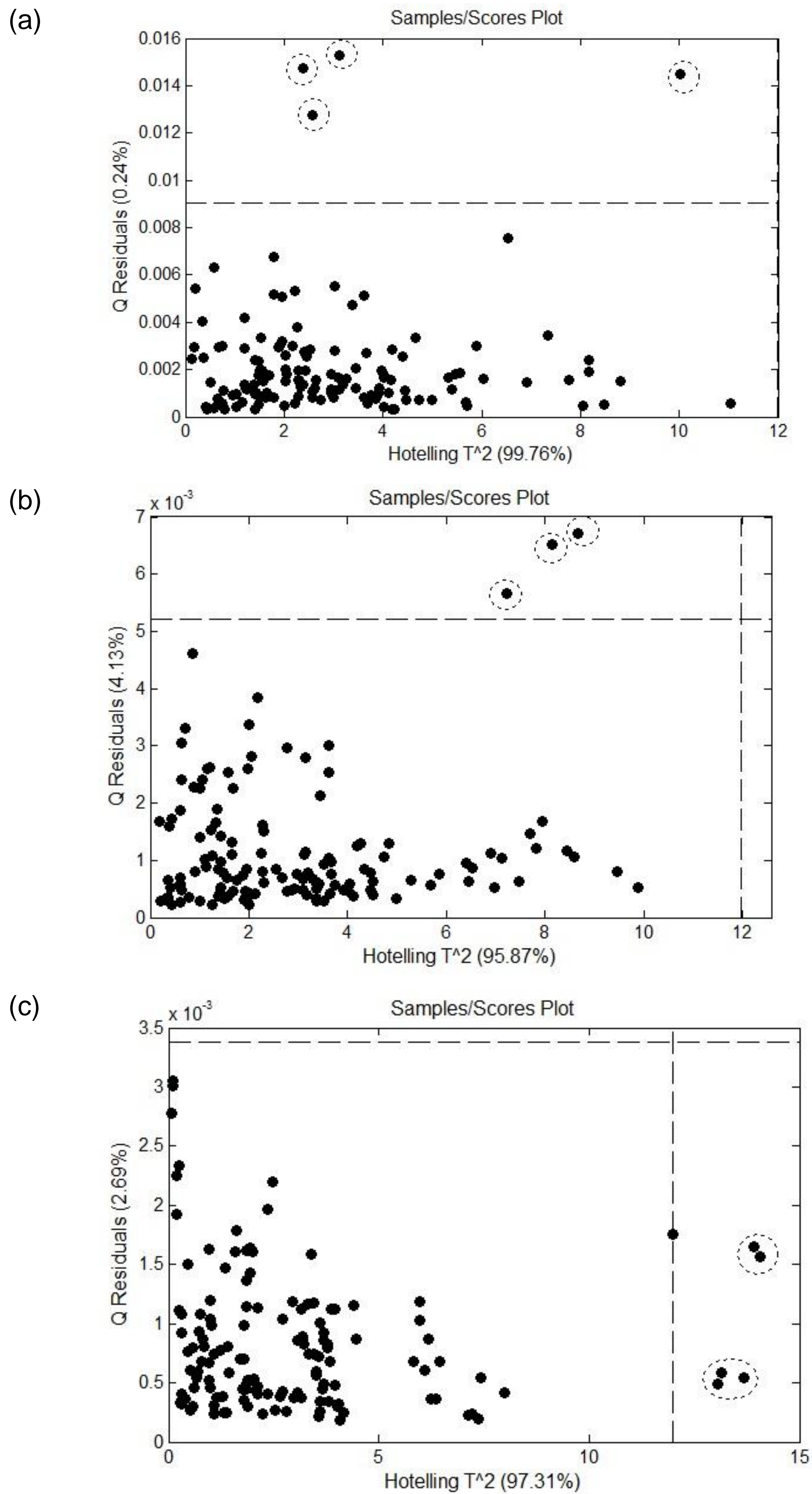

Figure 19. Plot of Q residuals vs. Hotelling's *T*2 statistic for outlier removal in the NIR datasets corresponding to (a) original spectra, spectra submitted to (b) baseline correction and (c) MSC.

The resulting scatter plots obtained by the PCA analysis for NIR spectra are shown in Figure 20. A clear separation between black coffee and other coffee classes can be observed at all plots. In Figure 20a the 1st and the 2nd PC's, which explained 95.64 and 3.89% of the variance amongst the samples, respectively, contributed to the separation of dark sour from non-defective and light sour coffees. In the baseline corrected data (Figure 20b) only the 1st PC, which explained 92.69% of the variance amongst the samples, contributed to the separation of dark sour from non-defective and light sour coffees. In Figure 20c the same separation was observed through 1st and 4th PC's that responded for 80.82% and 1.49% of the variance amongst the samples, respectively.

Looking at Figure 20a, immature coffee was overlapped among all coffee classes. After baseline correction, black coffee was completely discriminated from other classes by the 3rd PC, but immature was still overlapping with dark sour, non-defective and light sour coffee. The application of MSC to the spectral data contributed for a well defined discrimination of black coffee through PC2 and dark sour through PC1, while non-defective, light sour and immature were clustered together. Overall, samples were essentially discriminated by sample class, while the roasting conditions (temperature and roasting degree) did not explain the variance of the first three PC's.

Reports on the classification of defective and non-defective roasted coffees are scarce in the literature. CRAIG et al. (2012a) observed clustering of immature and sour coffee based on DRIFTS whereas MANCHA AGRESTI et al. (2008) reported grouping of immature and black coffee according to their volatile profiles.

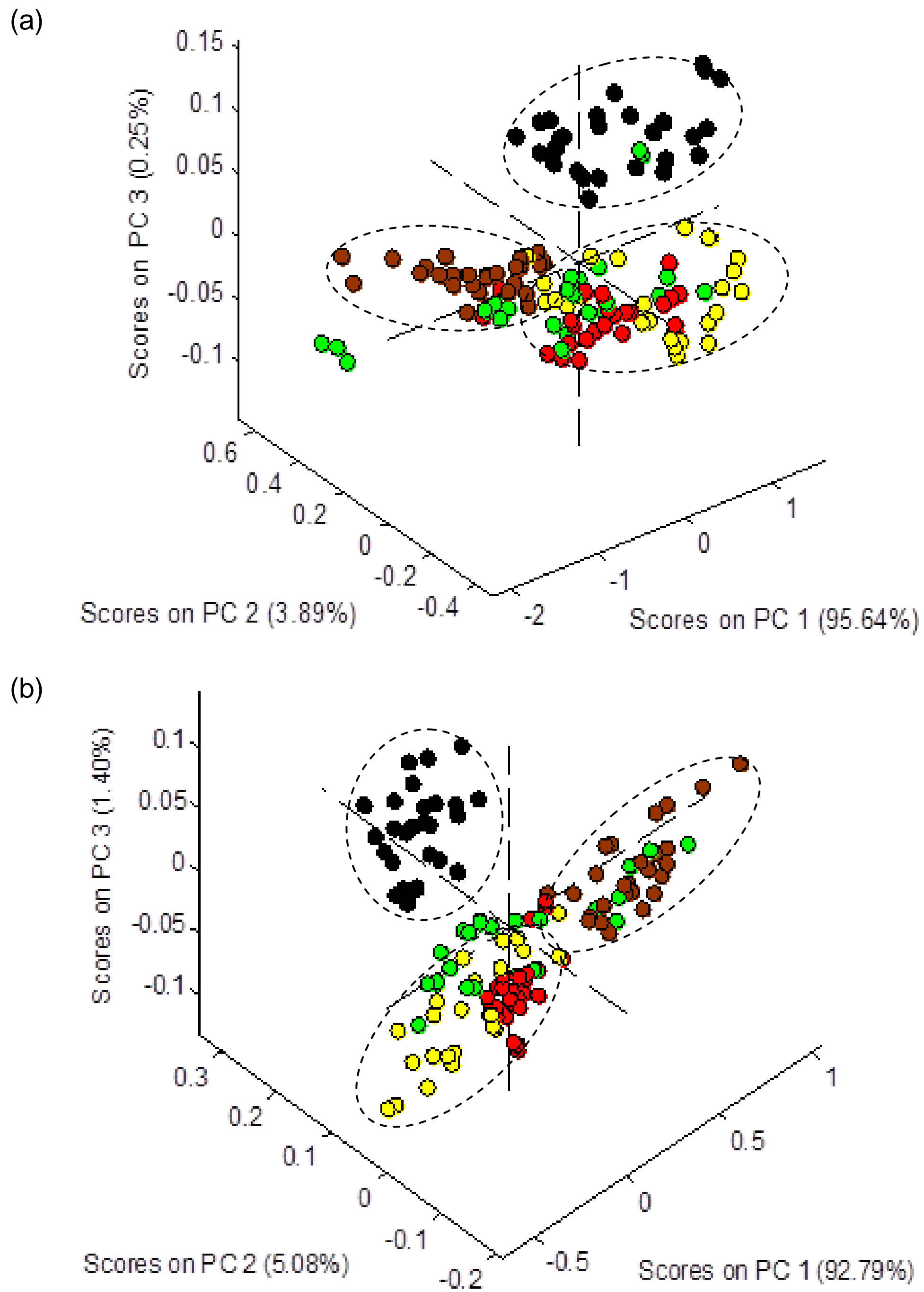

Figure 20. PCA scores scatter plots of NIR spectra (a) original (b) after baseline correction; and (c) after MSC. ● non-defective, ● immature, ● black, ● light sour, ● dark sour.

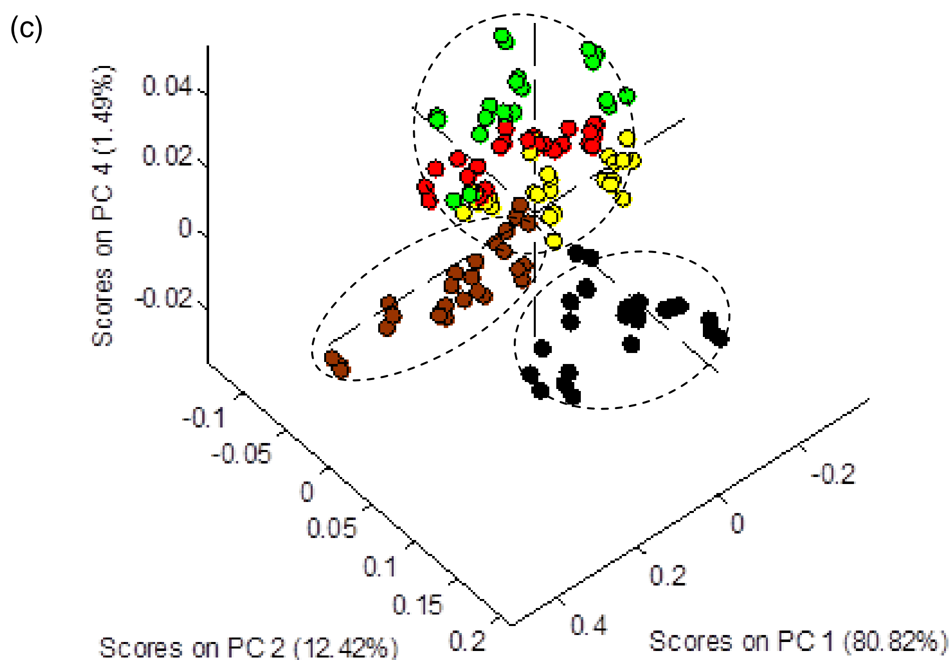

Figure 20. PCA scores scatter plots of NIR spectra (a) original (b) after baseline correction; and (c) after MSC. ● non-defective, ● immature, ● black, ● light sour, ● dark sour (continued).

4.4.3. Classification and variable selection by Elastic net: chemical assignments of selected NIR absorption bands

Table 15 summarizes the results obtained by the generalized linear models constructed for the classification of defective and non-defective coffees based on NIR spectra. As was observed in the ATR-FTIR classification results (section 4.3.3), the application of pre-treatments to the data increased the accuracy of the NIR models in a way that models constructed with the original spectra exhibited worse results in comparison to models constructed with pretreated spectra. In addition, models constructed with a reduced number of nonzero variables provided better results. Excellent statistical classification of defective and non-defective coffees was achieved at α levels ranging from 0.25 to 1. In particular, perfect classification was obtained for baseline corrected spectra at α levels ranging from 0.5 to 1. Taking into account the results presented in Table 15 and the discussion regarding the choice of the α level exposed in section 4.2.3, the variable selection for NIR spectra was based on Elastic net, with the coefficient estimates plotted at the α level of 0.75.

Table 15. Percentage of correct classification obtained by Elastic net models based on NIR spectra: comparing treatments and penalties

Treatment	α	Nonzero variables	Correct Classification (%)		
			Cal	CV	Val
Original spectra	0	1200	0.84	0.84	0.7
	0.25	681	0.92	0.91	0.85
	0.5	519	0.95	0.92	0.85
	0.75	307	0.95	0.95	0.88
	1	27	0.95	0.95	0.88
Baseline correction	0	1200	0.84	0.84	0.82
	0.25	545	0.99	0.99	0.97
	0.5	316	1	1	1
	0.75	188	1	1	1
	1	36	1	1	1
MSC	0	1200	0.89	0.89	0.94
	0.25	446	1	1	0.94
	0.5	271	1	1	0.94
	0.75	174	1	1	0.94
	1	39	1	1	0.88

Cal = calibration; CV = cross-validation; Val = validation

Figures 21a, 21b and 21c show the Elastic net coefficient estimates for non-treated spectra and spectra submitted to baseline correction and MSC, respectively. Visually higher coefficient estimates were found in the regions around 1400-1500, 1670-1900 and 2200-2230 nm. The nonzero variables and their respective coefficient values associated with the correct classification of defective and non-defective coffees varied considerably depending on the pretreatment applied to the spectral data. However, some features were found to be characteristic for each specific coffee class. For example, black coffee exhibited negative coefficient estimates in the region around 1685 nm and immature coffee exhibited positive coefficient estimates in the region around 2245 nm.

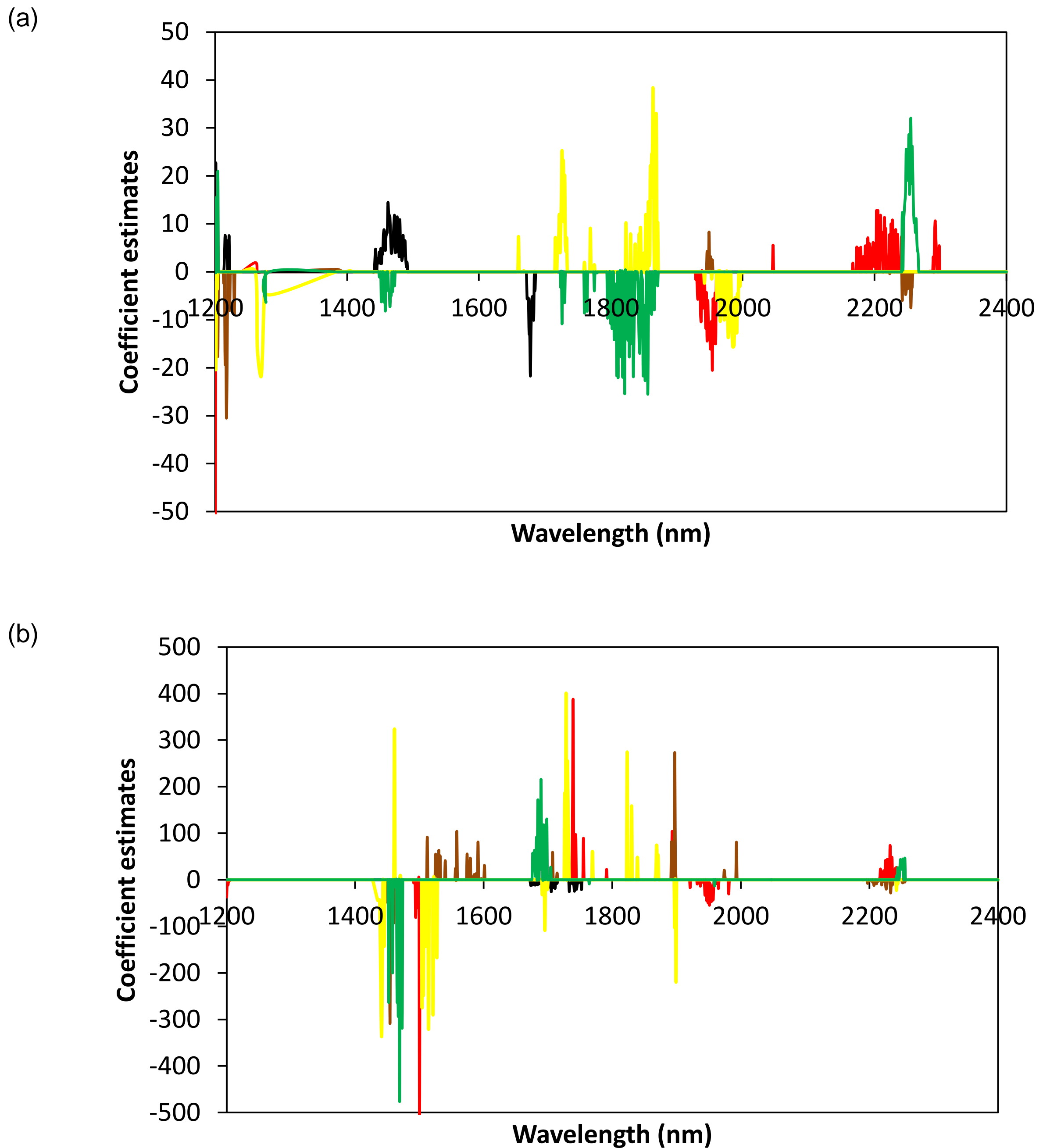

Figure 21. Elastic net coefficient estimates at α = 0.75 for NIR (a) non-treated spectra and NIR spectra submitted to (b) baseline correction and (c) MSC. A peak indicates that correct classification of spectra is associated with the corresponding spectral region. A positive peak indicates higher intensity than other classes; a negative peak indicates lower intensity. —non-defective, —light sour, —dark sour, —black and —immature.

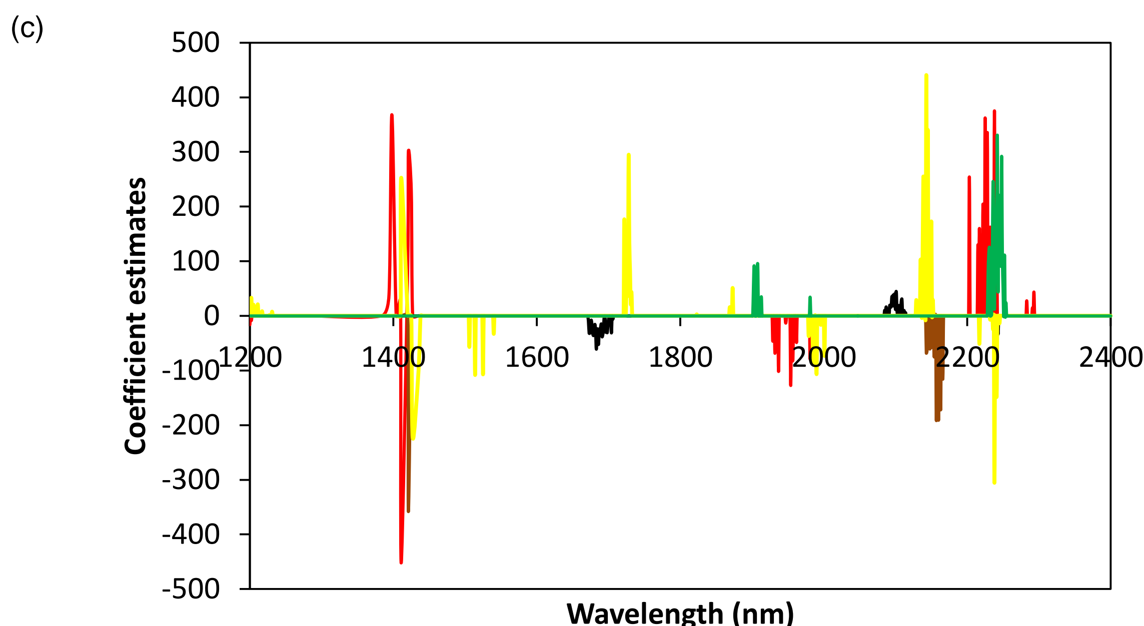

Figure 21. Elastic net coefficient estimates at α = 0.75 for NIR (a) non-treated spectra and NIR spectra submitted to (b) baseline correction and (c) MSC. A peak indicates that correct classification of spectra is associated with the corresponding spectral region. A positive peak indicates higher intensity than other classes; a negative peak indicates lower intensity. —non-defective, —light sour, —dark sour, —black and —immature (continued).

The interpretation of the Elastic net coefficient estimates graphs for NIR spectra was achieved in the same way as the ATR-FTIR discussion reported previously. A tentative assignment of each of the nonzero coefficient regions to chemical compounds that may absorb in the selected regions, based in the literature, was conducted. Table 17 summarizes the chemical assignment of the selected spectral regions obtained by non-treated spectra as well as spectra submitted to baseline correction and MSC. Only the sign of the coefficients will be taken into account, while the absolute values of the coefficients will be disregarded. In general different regions of the spectrum were selected by the Elastic net classification models. The selection of regions 7 and 21 suggests that coffee samples may contain more or less moisture; although no significant differences in the water content between defective and non-defective roasted coffees was reported in the literature (OLIVEIRA et al., 2006, VASCONCELOS et al., 2007). Considering that samples roasted at light, medium and dark roasts were included

in the model, this variation could be assigned to differences due to roasting degree, or absoption of humidity by the samples during the storage.

Correct classification of non-defective coffee was associated with 13 spectral regions. Regions 4, 14 and 15, and possibly region 24, exhibited positive coefficients, and could be associated with lipids. The positive regions 16 and 18 were previously related in the literature to carbohydrates, although amino acids may also absorb. Indeed, it is well known that carbohydrate levels are higher in non-defective beans. It was also reported in the literature that lipid content is significantly higher in non-defective beans than defective ones (OLIVEIRA et al., 2006, VASCONCELOS et al., 2007, RAMALAKSHMI et al., 2007, FRANCA et al., 2005b, MAZZAFERA, 1999). In the study by RIBEIRO et al. (2011), coffee samples with different cup qualities were analyzed by NIR. Based on an ordered predictor selection algorithm, some regions were selected as relevant contributors for the overall quality. Among them, the lipid regions 1412-1444 nm, 1704-1720 nm and 2126-2132 nm were selected as significant. The same regions were associated in the present work with non-defective coffee. Negative coefficients were found at regions 2, 6, 11, 22 and 26. Region 2 is possibly related to CH vibration, but, from our knowledge, no chemical assignment of a possible coffee compound in this region has been documented in the literature so far. Regions 6, 11, 22 and 26 could be related to the absorption of caffeine, chlorogenic acids and proteins. Previous studies found slightly lower levels of caffeine in non-defective coffees, and in comparison to immature coffee, non-defective beans present lower levels of chlorogenic acids (MAZZAFERA, 1999, FRANCA et al., 2005b). VASCONCELOS et al. (2007) also found lower level of protein in non-defective coffee than defective ones, agreeing with the results obtained in this study.

Correct classification of light sour coffee was associated with 12 regions. The positive regions 3, 5, 15, 19, 25 and 28 could be associated with high contents of caffeine, chlorogenic acids, lipids and/or proteins in light sour coffee. The regions with negative coefficients, 4 and 10, corresponded to regions where lipids and proteins absorb, respectively. Region 21 and 22 could be related to lower content of moisture or chlorogenic acids, and protein and/or nitrogenous compounds respectively in light sour coffee. In the case of dark sour, correct classification was associated with seven regions of the spectrum. The positive region 12 was previously related in the literature to proteins, although ESTEBAN-DIEZ et al. (2004b) attributed a peak at 1584 nm to carbohydrates. The selected regions with negative coefficients were the regions 1, 5, 7,

24 and 27. The lower absorption in the first two regions and at the regions 24 and 27 may occur due to a lower content of compounds such as quinic acid, carbohydrates, amino acids, caffeine, lipids and/or chlorogenic acids in dark sour coffees. Both dark and light sour coffee exhibited positive coefficients at region 19, while non-defective coffee exhibited a negative coefficient. According to WORKMAN and WEYER (2007) the C=O overtone of carboxylic acids occur at 1900 nm. Indeed, studies form the literature suggest that sour beans contain higher level of acids than other coffee classes, which is in accordance with the literature (FRANCA et al., 2005a, RAMALAKSHMI et al., 2007, VASCONCELOS et al., 2007).

With regard to black coffee, the correct classification was related to five positive peaks including regions 1, 7, 8, 9 and 23. The first one occurs due to the 2nd overtone of CH in CH_2 and CH groups that has been related in the literature to many coffee compounds such as quinic acids, carbohydrates, amino acids and caffeine. Nevertheless, amino acids might have a higher contribution to this region considering that black coffee was previously characterized by its higher content of proteins in comparison to other coffee classes (OLIVEIRA et al., 2006, MAZZAFERA, 1999, VASCONCELOS et al., 2007). The positive regions 8 and 9 might be associated with the presence of chlorogenic acids or phenols, which is in agreement with the study by FRANCA et al. (2005b), where roasted black coffee exhibited a significantly higher content of chlorogenic acids than other coffees (FRANCA et al., 2005b). The negative peak at region 13 was assigned to caffeine; however the opposite was expected because roasted black coffee was previously associated with higher levels of caffeine by FRANCA et al. (2005b). Regarding region 15, assigned to lipids, according to the literature, black coffee contains lower level of lipids than non-defective coffee (OLIVEIRA et al., 2006) and possibly light sour coffees, but a slightly higher lipid content than other defective beans (OLIVEIRA et al., 2006, MAZZAFERA, 1999).

Nine regions of the spectrum were associated with the correct classification of immature coffee, including the three positive regions 13, 20 and 27, assigned in the literature to caffeine and chlorogenic acids. RIBEIRO et al. (2011) correlated beverage acidity and chlorogenic acids with the region of 2246-2270 nm because of the O-H combination bands. This region overlaps exactly the region 27 selected by Elastic net. However, a negative peak at the region 8, where chlorogenic acids or phenols may absorb, was also observed, disagreeing with the previous assignment. An evaluation of the published works on chlorogenic acids in relation to coffee quality indicates that high

levels seem to be associated with the presence of immature beans (MAZZAFERA, 1999, FRANCA et al., 2005a, FARAH et al., 2005). After roasting, FRANCA et al. (2005b) found a lower level of chlorogenic acids in immature coffee than in non-defective, black and sour. No significant difference in the level of caffeine among defects was found by MAZZAFERA (1999) and FRANCA et al. (2005b), but the ATR-FTIR results obtained in this study are in agreement with the selected positive coefficients related to caffeine, reinforcing the fact that immature coffee may contain a higher content of caffeine than non-defective and sour beans. Negative peaks at the regions 14, 16, 17 and 18 suggest that immature coffee contains a lower amount of lipids and carbohydrates than other coffee classes, which is in accordance with the studies by MAZZAFERA (1999) and OLIVEIRA et al. (2006).

A comparison between the results obtained for ATR-FTIR (see section 4.2.3) and NIR indicated that ATR-FTIR can provide more information and selectivity on the group frequencies present in the samples. It is well known that precise band assignments are difficult in the near-infrared region because of the fact that a single band may be attributable to several possible combinations of fundamental and overtone vibrations overlapped (WORKMAN and WEYER, 2007). The use of Elastic net, a statistical technique that provides the selection of specific and sparse variables, provided insights to the interpretation of NIR spectra.

Although some differences in the chemical compounds assigned in the ATR-FTIR and NIR variable selection results were noticed, most of compounds assigned for the NIR spectra were in agreement with those assigned for ATR-FTIR spectra. A compilation of the major chemical compounds assigned and in conformity with both techniques is shown in Table 16. A valuable note from this Table is that lipids were the major compounds strictly related to coffee quality, with high levels observed for non-defective and low levels for dark sour, black and immature.

The discussion presented in sections 4.2.3 and 4.3.3 also evidenced that, under the same roasting conditions, the higher extent of roasting attained by non-defective and light sour and the lesser attained by dark sour, black and immature beans was a key factor for the discrimination of these beans. The high content of free sugars available for reactions in healthy beans resulted in an efficient roasting, with extensive degradation of compounds such as amino acids and, consequently, large production of aroma compounds, including ketones and aldehydes. The opposite was observed for defective beans. In particular, the low extent of roasting attained by black beans

resulted in a reduced degradation of chlorogenic acids and trigonelline. However, the establishment of the roasting degrees was based only in the color measurements, and since the black beans exhibit naturally low luminosity before roasting, this results could be influenced by an incomplete roasting. It is also important to mention that the roasting processes were conducted in laboratory scale using a convective oven with fixed temperature and no motion of the beans. In this system, the heat transfer is not as efficient and homogeneous (BONNLANDER et al., 2005). Thus, it is reasonable to consider that the roasting results obtained in this study could be changed if an industrial coffee roaster was used.

Table 16. Chemical compounds assigned in ATR-FTIR and NIR variable selection

Compounds	**ND**	**LS**	**DS**	**BL**	**IM**
Carbohydrates	+	+			
Proteins and/or amino acids	-			+	+
Lipids	+		-	-	-
Caffeine	-	+			+
Chlorogenic acids				+	
Aliphatic acids			+		

LS = light sour, DS = dark sour, BL = black, ND = non-defective, IM = immature

A positive peak indicates higher level than other classes; a negative peak indicates lower level

Table 17. Tentative chemical assignment of significant NIR bands selected by Elastic net (α = 0.75) for the classification of defective and non-defective coffees

Region selected	Gereral region ranges	ND	LS	DS	BL	IM	Vibration modes	Compounds	References
1	1215-1224			-	+		2nd overtone CH in CH_2 and CH groups	Quinic acid, carbohydrates, amino acids, caffeine	1, 2, 3
2	1264-1276	-					CH vibration		
3	1398		+				2x CH str + 2x CH def (=CH)	Caffeine	3
4	1411	+	-				1st overtone OH	ROH, oil	4
5	1420-1425		+	-			1st overtone OH in aromatic, 1st overtone OH NH	CGA	2
6	1427-1445	-					1st overtone OH NH	CGA	2
7	1451-1461	+		-	+	-	1st overtone OH str	Water	
8	1462-1473				+	-	1st overtone OH	CGA and phenols	2
9	1473-1491				+		1st overtone OH	CGA and phenols	2
10	1494-1500		-				1st overtone NH str in aromatic amine	Aromatic amine	5
11	1504-1527	-					1st overtone NH str in proteins	Proteins	1, 5
12	1556-1601			+			1st overtone NH str 1st overtone of OH str	CONH Carbohydrates	1, 4
13	1672-1711				-	+	1st overtone CH (=CH)	Caffeine	2, 3
14	1716-1733	+				-	1st overtone CH_2 (double band)	Lipids	1, 6
15	1738-1755	+	+		-		1st overtone CH_2 (double band)	Lipids	1, 6
16	1760-1791	+				-	1st overtone CH str (CH_2 sym)	Carbohydrates, amino acids	1, 3
17	1795-1830					-	1st overtone CH str (CH_2 sym)	Carbohydrates, amino acids	1, 3
18	1830-1871	+				-	OH str + 2x CO str	Carbohydrates (fibre content)	1, 4
19	1892-1899	-	+	+			1st overtone C=O str (.C=OOH)	Carboxylic acids	5
20	1902-1913					+	2nd overtone C=O str (CO_2H)	CGA	1, 2
21	1937-1959		-				2nd overtone C=O in CO_2R OH str + OH def	CGA Water	1
22	1970-1993	-	-				NH asym str + amide II C=O str second overtone	Protein and nitrogenous comp Caffeine	1, 3
23	2085-2114				+		1st overtone C=O and OH comb bands	Caffeine, CGA	2

24	2132-2166	+		-			comb =CH str + C=C str (HC=CH)	Lipids, caffeine	1, 7, 8
25	2170-2230		+				2x amide I + amide III NH bend 2nd overtone, CH str/C=O str comb, C=O str/amide III comb 1st overtone of OH in RNH_2 CC CHO	Protein and/or CGA	1, 2, 4
26	2238-2241	-					NH and OH comb in RNH2 CHO CH_3 and CH_3	Caffeine, CGA	2
27	2242-2266			-		+	NH and OH comb in RNH_2 CHO CH_3 and CH_3 comb OH str OH def	Caffeine, CGA	1, 2, 8
28	2283-2293		+				CH + CH comb (CH_2 CH_3)	Caffeine or CGA	2, 6

LS = light sour, DS = dark sour, BL = black, ND = non-defective, IM = immature.

comb = combination, def = deformation, str = stretching

CGA = chlorogenic acids

List of references:

1. ESTEBAN-DIEZ et al. (2004b)
2. RIBEIRO et al. (2011)
3. PIZARRO et al. (2007b)
4. JOHN et al. (2007)
5. WORKMAN and WEYER (2007)
6. HUCK et al. (2005)
7. LAASONEN et al. (2003)
8. DOWNEY and BOUSSION (1996)

4.5. Quantitative analysis of defective and non-defective coffees: A comparative evaluation between ATR-FTIR and NIR

In the previous sections, it was demonstrated that defective and non-defective coffees can be discriminated according to chemical differences between the samples. These differences were evidenced taking into account the selected discriminating variables (wavelengths or wavenumbers) and how these variables contributed to the discrimination of defective and non-defective coffees. The assignment of these variables to chemical compounds provided a powerful tool for the characterization of each of the sample classes. Nonetheless, it may be taken into account that, in practice, a 100% non-defective coffee, as well as a 100% defective coffee, would not be found in the market for two reasons. First, the electronic sorting machine employed in cooperatives of producers and industries to separate defective beans is not completely efficient in the separation of beans that have similar color to non-defective ones, such as immature and sour beans (FRANCA et al., 2005b, FARAH et al., 2006, VASCONCELOS et al., 2007). Also, since defective beans represent an investment in growing, harvesting and handling in the coffee production chain, coffee producers have adopted the practice of dumping the separated beans in blends with non-defective ones, giving rise to a low-grade roasted and ground coffee (OLIVEIRA et al., 2006). In view of the aforementioned, in this section a comparative evaluation of the performances of ATR-FTIR and NIR techniques for the development of quantitative models to predict the percentage of defective coffee in admixture with non-defective coffee is presented.

The results from PCA discussed in sections 4.3.2 and 4.4.2 suggested that the variability among spectra of defective and non-defective coffees was higher than the variability due to roasting condition (roasting degree and temperature), i.e. samples were essentially discriminated by sample class. Therefore, the quantitative analysis was conducted using only one sample from each sample class to produce the coffee blends. The samples used corresponded to those roasted to a medium degree and at a medium roasting temperature (235 °C). As described in section 3.1, the following blends were produced: (a) defects (25% of each defect) in admixture with non-defective coffee, (b) light sour in admixture with non-defective coffee, (c) dark sour in admixture with non-

defective coffee, (d) black in admixture with non-defective coffee and (e) immature in admixture with non-defective coffee.

To find the best correlation between spectra and percentage of defective coffee, besides the original spectra, two preprocessings were applied to ATR-FTIR spectra (baseline correction and area normalization, and 1st derivative) and to NIR spectra (baseline correction and MSC). The quantitative datasets were split into calibration (43 spectra) and validation (15 spectra) sets. PLSR was the regression technique of choice in this study. Leave-one-out cross-validation was used to automatically select the optimum number of latent variables, based on the minimum value of RMSECV.

Samples from the calibration set with *Q* or *T2* statistics values over the threshold of 99% were classified as outliers and removed from the data set. The procedure for outlier removal was performed and repeated until no outliers were identified. Table 18 shows the number of outliers removed from each of the PLSR models developed, where it is evidenced that more outliers were identified in the ATR-FTIR than in the NIR models. In this scenario, the presence of outliers can be related to operation errors, instrumental noise or abnormal observations originated from errors or differences during the sample weighing and production of the mixtures.

Table 18. Outliers detected and removed from the PLSR models developed from ATR-FTIR and NIR spectra

ATR-FTIR	**Defects**	**LS**	**DS**	**BL**	**IM**
Original	0	4	3	0	0
Baseline correction + area normalization	0	0	3	2	0
1st derivative	1	2	1	1	0
NIR	**Defects**	**LS**	**DS**	**BL**	**IM**
Original	0	0	0	1	0
Baseline correction	0	0	0	1	0
MSC	0	0	0	1	1

The scatter plots of actual and calculated values for percentage of defects in admixtures with non-defective coffee for both ATR-FTIR and NIR models are shown in Figures 22 and 23. The scatter plots of individual defects (black, light sour, dark sour and immature) in admixture with non-defective coffee are displayed in Annexes A and

B. A visual inspection of the scatter plots suggests that higher predictability was achieved with the NIR models. Also, from a visual inspection it is tough to determine whether the application of preprocessings improved the results.

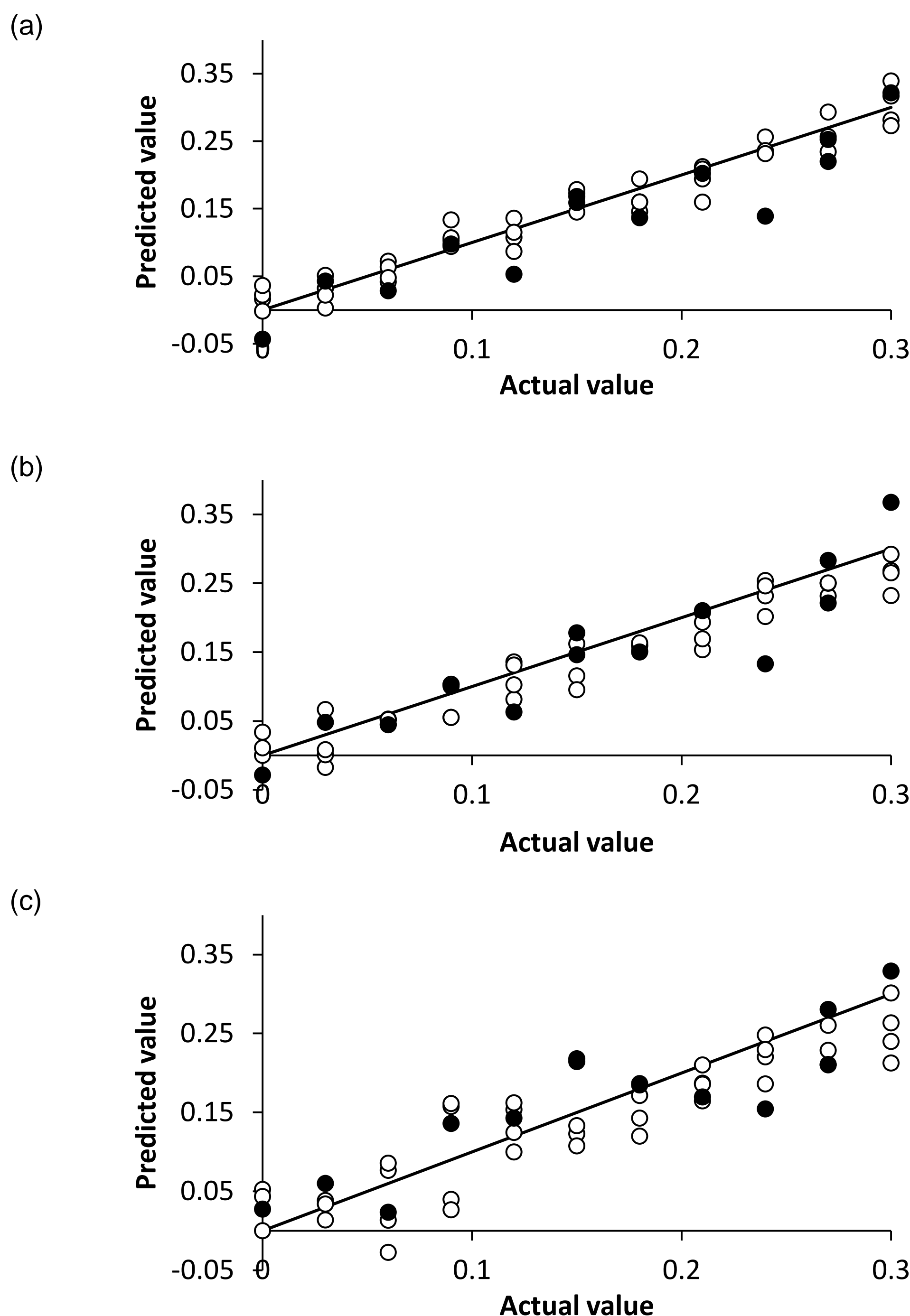

Figure 22. Actual x predicted percentage of defective coffee in admixture with non-defective coffee from PLSR models developed with ATR-FTIR spectral data. (a) original, (b) baseline correction and area normalization, (c) 1st derivative. ○ calibration set, ● validation set.

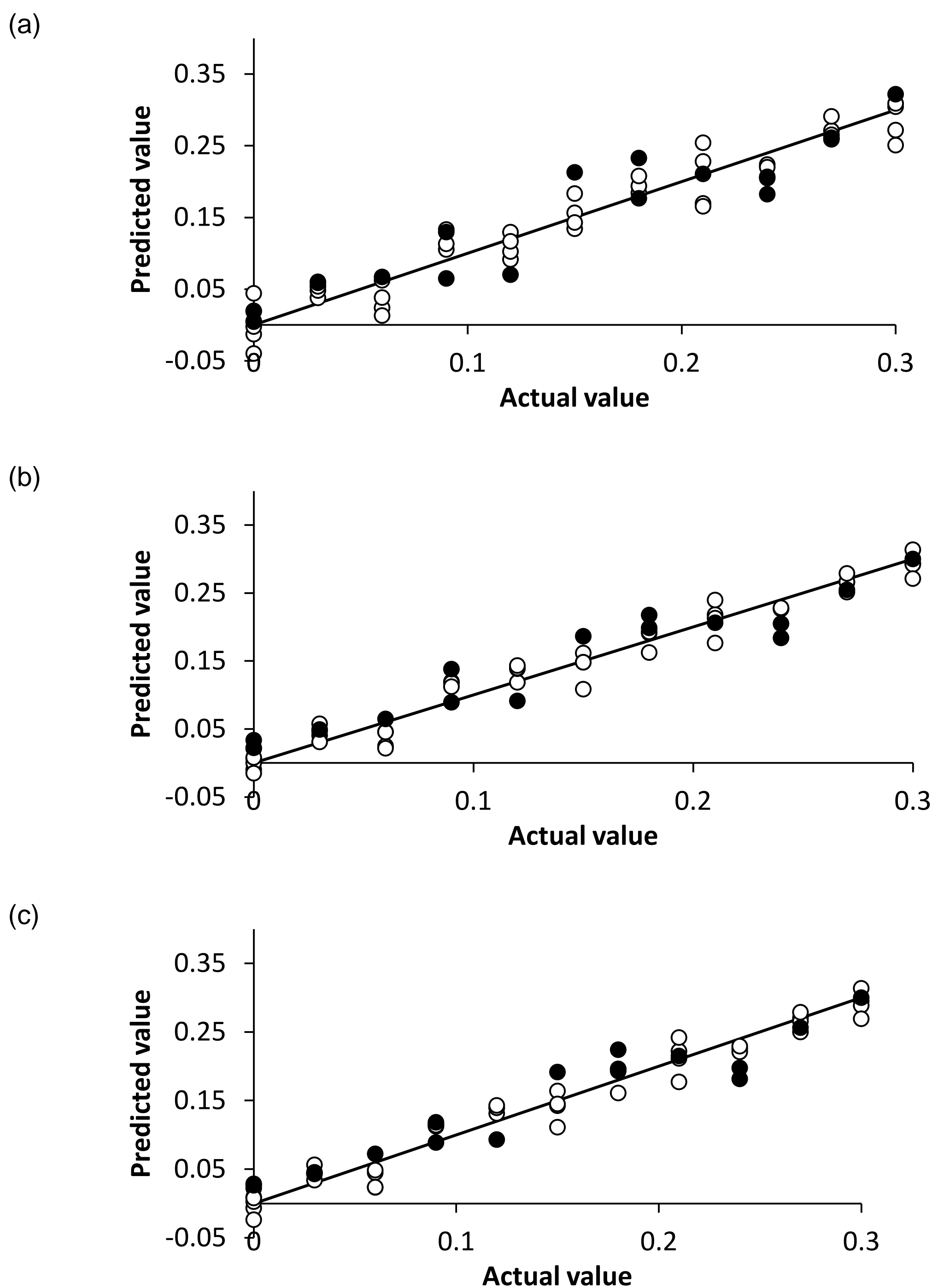

Figure 23. Actual x predicted percentage of defective coffee in admixture with non-defective coffee from PLSR models developed with NIR spectral data. (a) original, (b) baseline correction, (c) MSC. ○ calibration set, ● validation set.

Tables 19-23 summarize the prediction results obtained by PLSR for both ATR-FTIR and NIR spectra. A comparison between the ATR-FTIR models constructed with

original (only mean centered) spectra and spectra submitted to baseline correction and area normalization and 1st derivative shows that the application of preprocessings provided potential benefits to the regression models. Apart from black coffee, where the opposite was observed, the application of mathematical preprocessings, increased the robustness of the models, reducing the number of latent variables included. The same observation was made for all NIR models: although similar values of RMSEP and R^2 were obtained at original and pretreated spectra models, the use of preprocessings reduced the number of latent variables included in the models. In Table 16, particularly, the application of MSC improved the predictability of the results, even using a lower number of latent variables.

Taking into account the results from the predictive parameters RMSEC, RMSEP and R^2 shown in Tables 19-23, it can be concluded that NIR models provided more accurate and robust models than ATR-FTIR models. Beyond that, the number of latent variables was a key feature. To achieve satisfactory predictive results, a considerably higher number of latent variables were used in the development of the ATR-FTIR models. In reference to ASTM (2012), the determination of the number of latent variables to be used in a model is a critical step in the model development. In general, if too few variables are used, a less accurate model will result. If too many variables are used, the estimates from the model will be unstable, which means that small changes in the spectrum, on the order of the spectral noise, may produce statistically significant changes in the estimates. Thus, models with fewer factors are less likely to exhibit over fitting and tend to have better generalization ability.

Overall, NIR spectroscopy was superior to ATR-FTIR in the quantitative analysis of defective and non-defective coffees. All defect classes could be accurately quantified in admixtures with non-defective coffee, except for light sour coffee. Both ATR-FTIR and NIR models for light sour coffee quantification exhibited moderate results even employing a higher number of PLSR latent variables. Nevertheless this achievement was expected considering that previous studies employing ATR-FTIR suggested that light sour coffee is chemically similar to non-defective coffee and these classes are usually clustered together (CRAIG et al., 2012b, CRAIG et al., 2012a).

Table 19. Results from the PLSR models developed from ATR-FTIR and NIR spectra for predicting the percentage of defects in admixture with non-defective coffee

	PLS-LVs	Calibration			Validation	
		RMSEC (%)	R^2	RMSECV (%)	RMSEP (%)	R^2
ATR-FTIR						
Original	8	0.021	0.949	0.039	0.042	0.863
Baseline correction + area normalization	7	0.021	0.932	0.036	0.044	0.832
1st derivative	5	0.026	0.926	0.037	**0.032**	**0.891**
NIR						
Original	5	0.024	0.94	0.032	0.034	0.871
Baseline correction	5	0.018	0.964	0.030	**0.029**	**0.913**
MSC	4	0.019	0.963	0.029	0.030	0.906

Table 20. Results from the PLSR models developed from ATR-FTIR and NIR spectra for predicting the percentage of light sour coffee in admixture with non-defective coffee

	PLS-LVs	Calibration			Validation	
		RMSEC (%)	R^2	RMSECV (%)	RMSEP (%)	R^2
ATR-FTIR						
Original	10	0.014	0.977	0.036	**0.043**	**0.786**
Baseline correction + area normalization	8	0.014	0.977	0.036	0.045	0.784
1st derivative	9	0.048	0.978	0.045	0.048	0.747
NIR						
Original	7	0.015	0.977	0.056	**0.038**	**0.837**
Baseline correction	6	0.018	0.968	0.055	0.041	0.818
MSC	6	0.017	0.97	0.054	0.043	0.799

Table 21. Results from the PLSR models developed from ATR-FTIR and NIR spectra for predicting the percentage of dark sour coffee in admixture with non-defective coffee

	PLS-LVs	**Calibration**			**Validation**	
		RMSEC (%)	R^2	RMSECV (%)	RMSEP (%)	R^2
ATR-FTIR						
Original	7	0.021	0.949	0.033	**0.033**	**0.881**
Baseline correction + area normalization	5	0.023	0.943	0.036	0.034	0.857
1st derivative	5	0.018	0.965	0.028	0.039	0.847
NIR						
Original	6	0.016	0.975	0.029	**0.026**	**0.953**
Baseline correction	5	0.017	0.97	0.029	0.027	0.943
MSC	4	0.019	0.963	0.029	0.029	0.941

Table 22. Results from the PLSR models developed from ATR-FTIR and NIR spectra for predicting the percentage of black coffee in admixture with non-defective coffee

	PLS-LVs	**Calibration**			**Validation**	
		RMSEC (%)	R^2	RMSECV (%)	RMSEP (%)	R^2
ATR-FTIR						
Original	3	0.028	0.91	0.032	0.042	0.817
Baseline correction + area normalization	5	0.025	0.93	0.040	0.042	0.839
1st derivative	10	0.005	0.997	0.030	**0.039**	**0.847**
NIR						
Original	6	0.019	0.964	0.037	**0.028**	**0.918**
Baseline correction	5	0.021	0.958	0.038	0.029	0.915
MSC	4	0.022	0.979	0.037	0.030	0.905

Table 23. Results from the PLSR models developed from ATR-FTIR and NIR spectra for predicting the percentage of immature coffee in admixture with non-defective coffee

	PLS-LVs	**Calibration**			**Validation**	
		RMSEC (%)	R^2	RMSECV (%)	RMSEP (%)	R^2
ATR-FTIR						
Original	7	0.035	0.889	0.065	0.039	0.865
Baseline correction + area normalization	6	0.034	0.884	0.060	0.043	0.871
1st derivative	6	0.029	0.903	0.056	**0.034**	**0.9**
NIR						
Original	5	0.019	0.962	0.028	0.055	0.723
Baseline correction	5	0.012	0.986	0.030	**0.029**	**0.903**
MSC	5	0.007	0.995	0.028	0.031	0.895

5. CONCLUSION

The main objective of this study was to evaluate the potential of FTIR and NIR spectroscopy for the classification and quantification of defective (black, immature, light and dark sour) and non-defective roasted coffees. Considering the results obtained and presented, it can be concluded that the methodologies employed were adequate for the proposed objective.

Results from the exploratory analysis by PCA indicated that, based on the spectra obtained by DRIFTS, it was possible to discriminate the samples into four major groups: (a) non-defective, (b) black, (c) dark sour and (d) light sour, with immature beans scattered among sour samples. Classification models were developed by LDA and provided high levels of correct classification in both calibration and validation sets. In spite of the interesting results achieved at a classification level, DRIFTS possess some drawbacks such as the time demanded to prepare the samples, which limits its applicability for routine analyses, and the considerably small amount of sample analyzed, that limits its applicability for quantitative analyses. Thus, in this study a quantitative assay was not attempted by DRIFTS.

With regard to ATR-FTIR and NIR results, an exploratory analysis of the ATR-FTIR spectra indicated that this technique provided, although not clearly, the discrimination of the coffee samples into two groups: (a) non-defective and light sour and (b) black, dark sour and immature. Results from NIR spectroscopy, on the other hand, demonstrated that the coffee samples could be discriminated into three major groups: (a) non-defective, light sour and immature, (b) dark sour, and (c) black. In both cases the variance among the samples led to the discrimination of the coffees primarily by their classes, regardless of roasting degree.

The classification models for ATR-FTIR and NIR spectra were developed based on Elastic net and exhibited excellent classification results. The best results, which achieved up to 100% of correct classification, were obtained at α levels ranging from 0.25 to 1, indicating that accurate classification can be obtained from relatively small regions of the spectrum, by means of imposing penalties on the models to reduce the number of explicit variables. The discriminating variables that contributed to the correct classification of defective and non-defective coffees were extracted and provided an interesting interpretation of the models. As expected, ATR-FTIR provided more

information and selectivity on the group frequencies present in the coffee samples. The use of Elastic net to select specific and sparse variables provided insights in the interpretation of the complex NIR spectra dominated by broad combination and overlapping bands.

A summarized compilation of the major chemical compounds assigned and in agreement in both ATR-FTIR and NIR variable selection results suggested that correct classification of non-defective coffee was directly related to high levels of carbohydrates and lipids and lower levels of proteins and/or amino acids and caffeine. Correct classification of light sour was related to high levels of carbohydrates and caffeine. Dark sour coffee was directly associated with high levels of aliphatic acids and low levels of lipids, and black coffee with high levels of proteins and/or amino acids, and low levels of lipids. Finally, correct classification of immature coffee was related to high levels of proteins and/or amino acids and caffeine and low levels of lipids. A valuable note from these results were the fact that lipids were the major compound strictly related to coffee quality, with high levels observed for non-defective and low levels for dark sour, black and immature.

A comparative evaluation between ATR-FTIR and NIR for the quantification of defective and non-defective coffees based on PLSR indicated that NIR was superior to ATR-FTIR providing more robust and accurate models. Indeed, PLSR models constructed with NIR spectra provided excellent predictive results.

6. FUTURE WORK

The positive results obtained for the classification and quantification of defective and non-defective coffees presented in this study indicated that the employed techniques are suitable for the assessment of the coffee quality in terms of the presence of defective beans, regardless of roasting degree. Thus, one can expect that this research should be expanded to the analysis of roasted and ground coffees with different cup qualities and further validation. Methodologies based on infrared spectroscopy, especially NIR, could be potential alternatives to the current cup test employed in regulation organizations and industry. For this purpose it would be primarily required to evaluate the effect of including more variants in the model, i.e. to include coffees from different lots, crops and geographical origin in the study. In addition, coffees used in the present study were roasted in a convection oven that, although simulates coffee roasting process on a laboratory scale, does not correspond to the process employed in the industry. Then, in a future work, defective and non-defective coffees, coffees with different cup quality, from different lots, crops or geographical origin would need to be processed in a coffee roaster instead.

Elastic net, the technique applied to the classification of defective and non-defective coffees and variable selection could also be used for the quantitative analysis of blends in an effort to provide better predictive results. For this purpose, two strategies could be tested. The first is simply applying Elastic net to fit a linear regression model, instead of fitting a multinomial model for classification. In this case the variable response would be quantitative instead of categorical. A second option would be applying Elastic net to select the variables that best represent each sample class of mixture and use these variables as inputs to develop conventional regression models such as PLSR, or ideally MLR.

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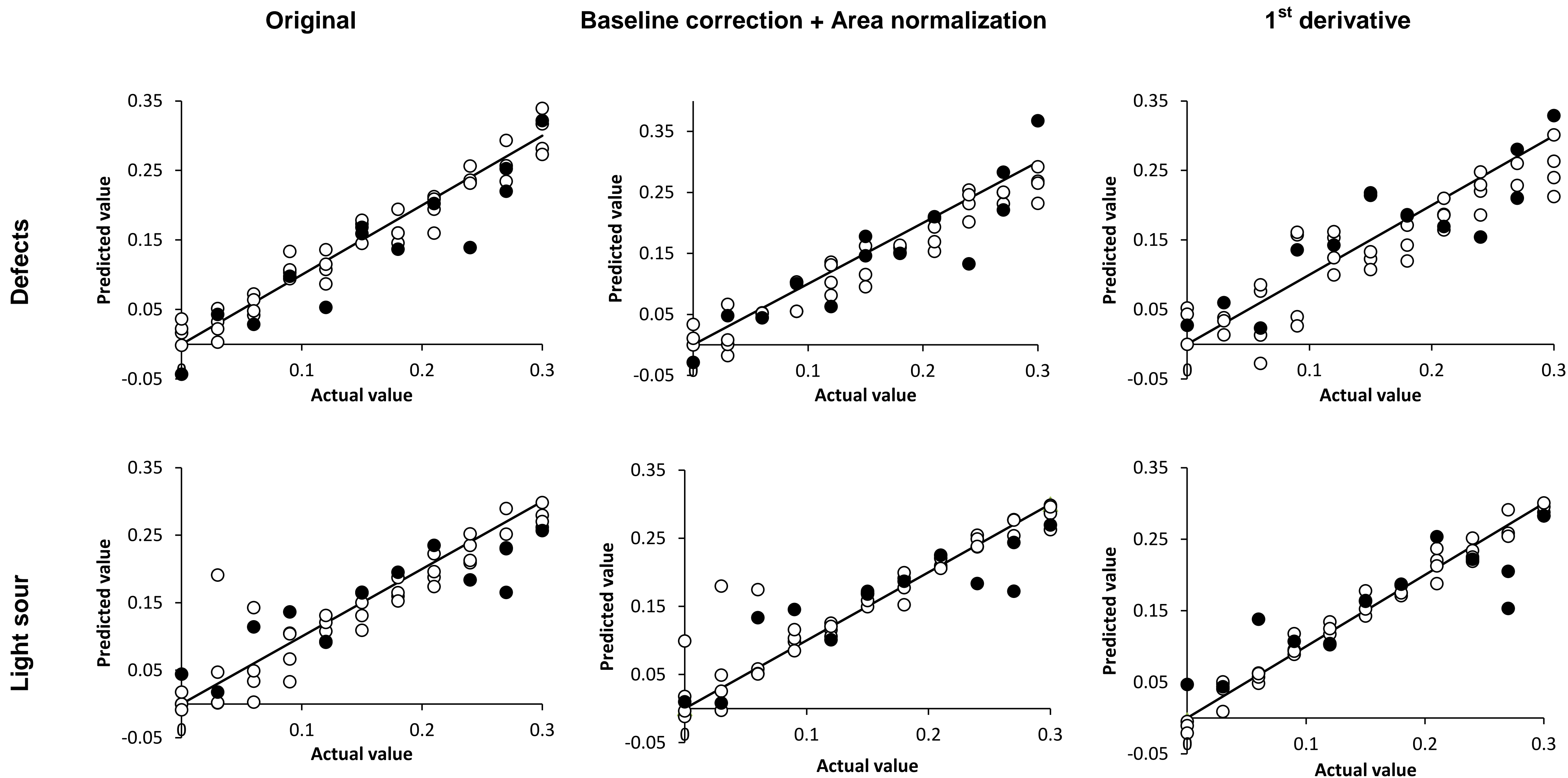
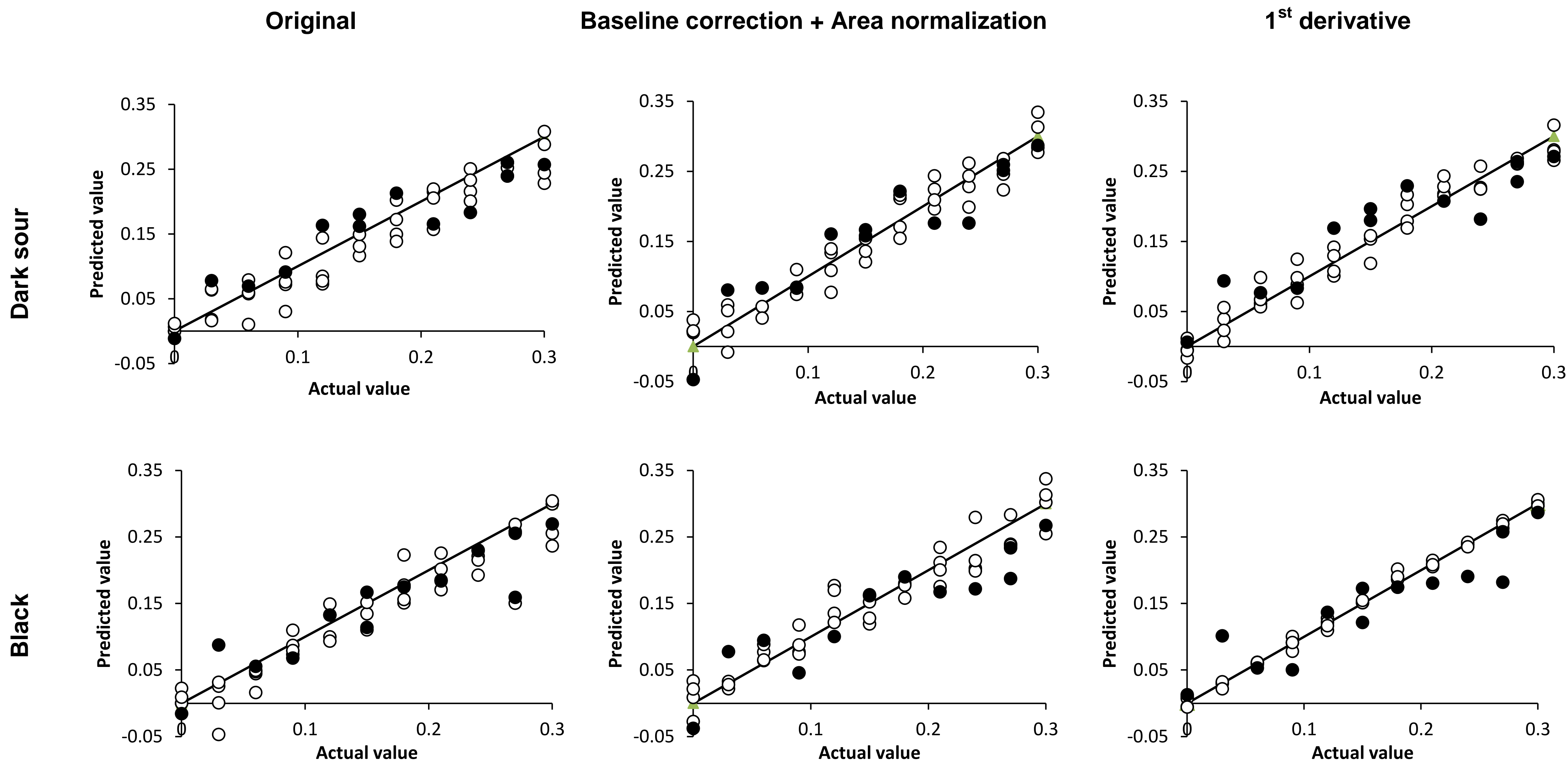
ANNEX

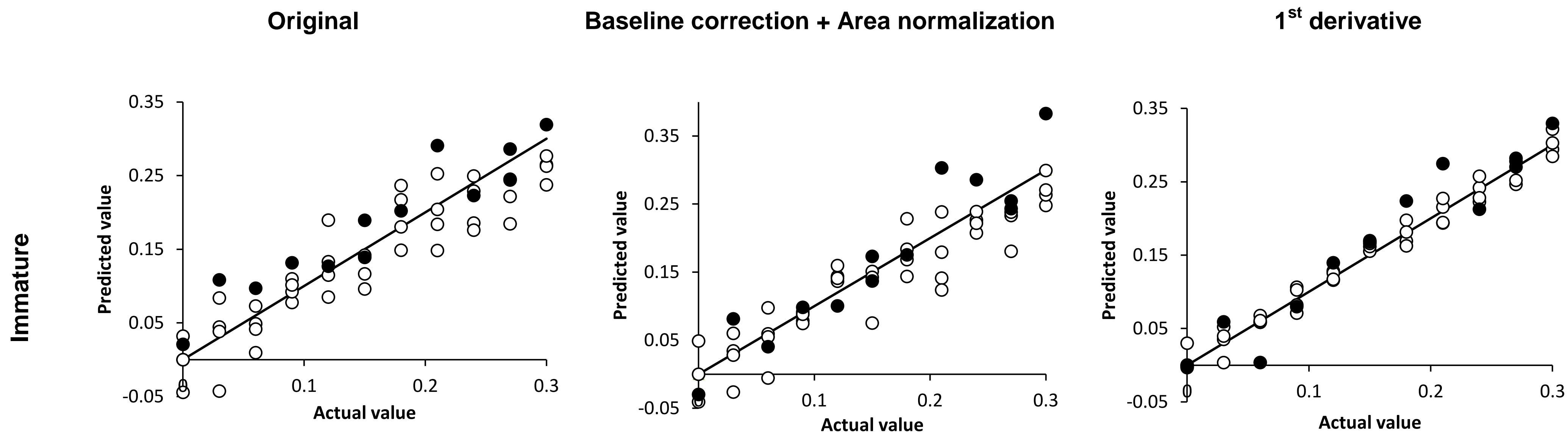
ANNEX A. Actual x predicted percentage of defective coffee in mixture with non-defective coffee from PLSR models developed with FTIR spectral data. (a) original, (b) baseline correction and area normalization, (c) 1st derivative. ○ calibration set, ● validation set.



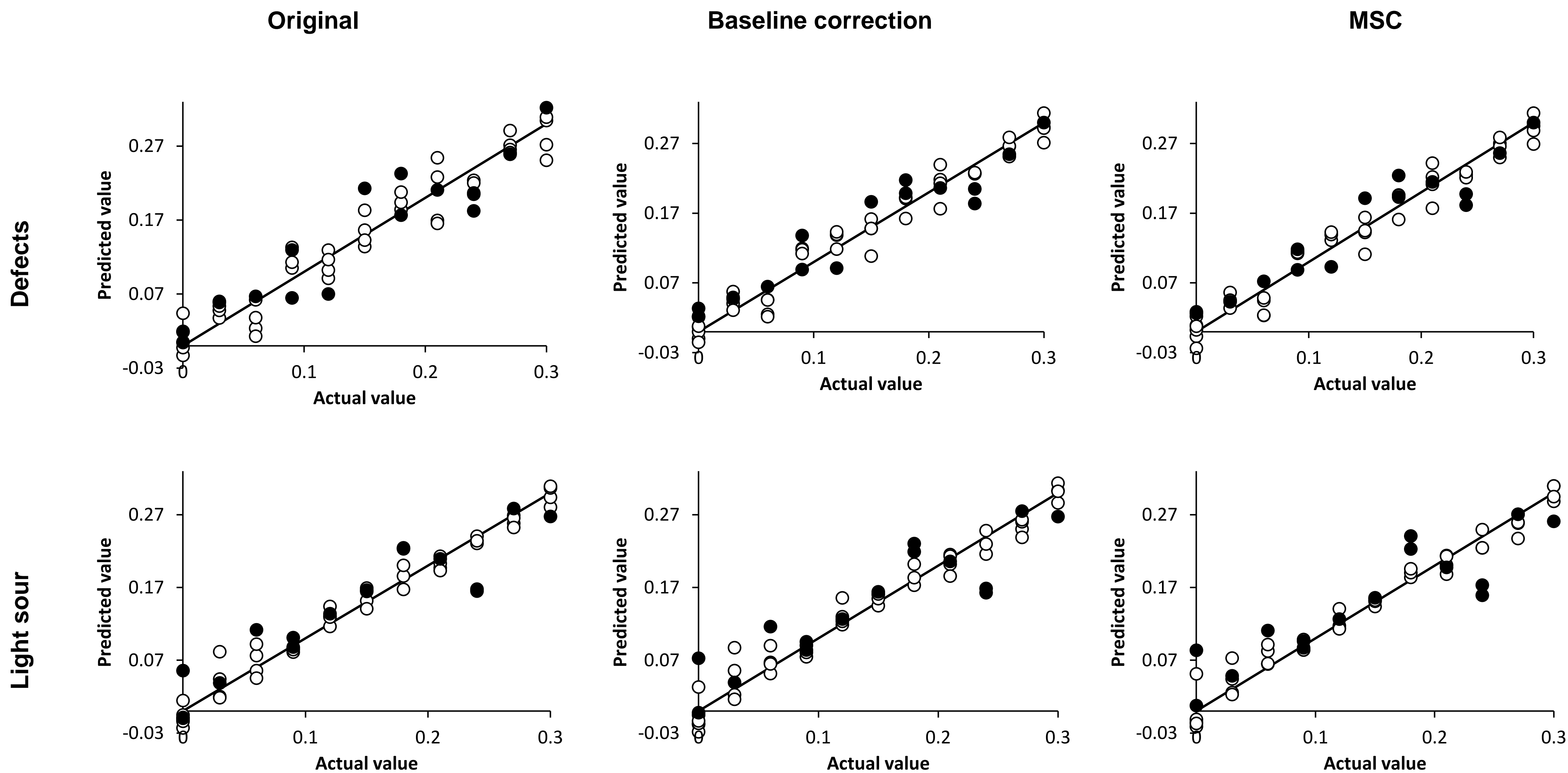
ANNEX A. Actual x predicted percentage of defective coffee in mixture with non-defective coffee from PLSR models developed with FTIR spectral data. (a) original, (b) baseline correction and area normalization, (c) 1st derivative. ○ calibration set, ● validation set. (continued)



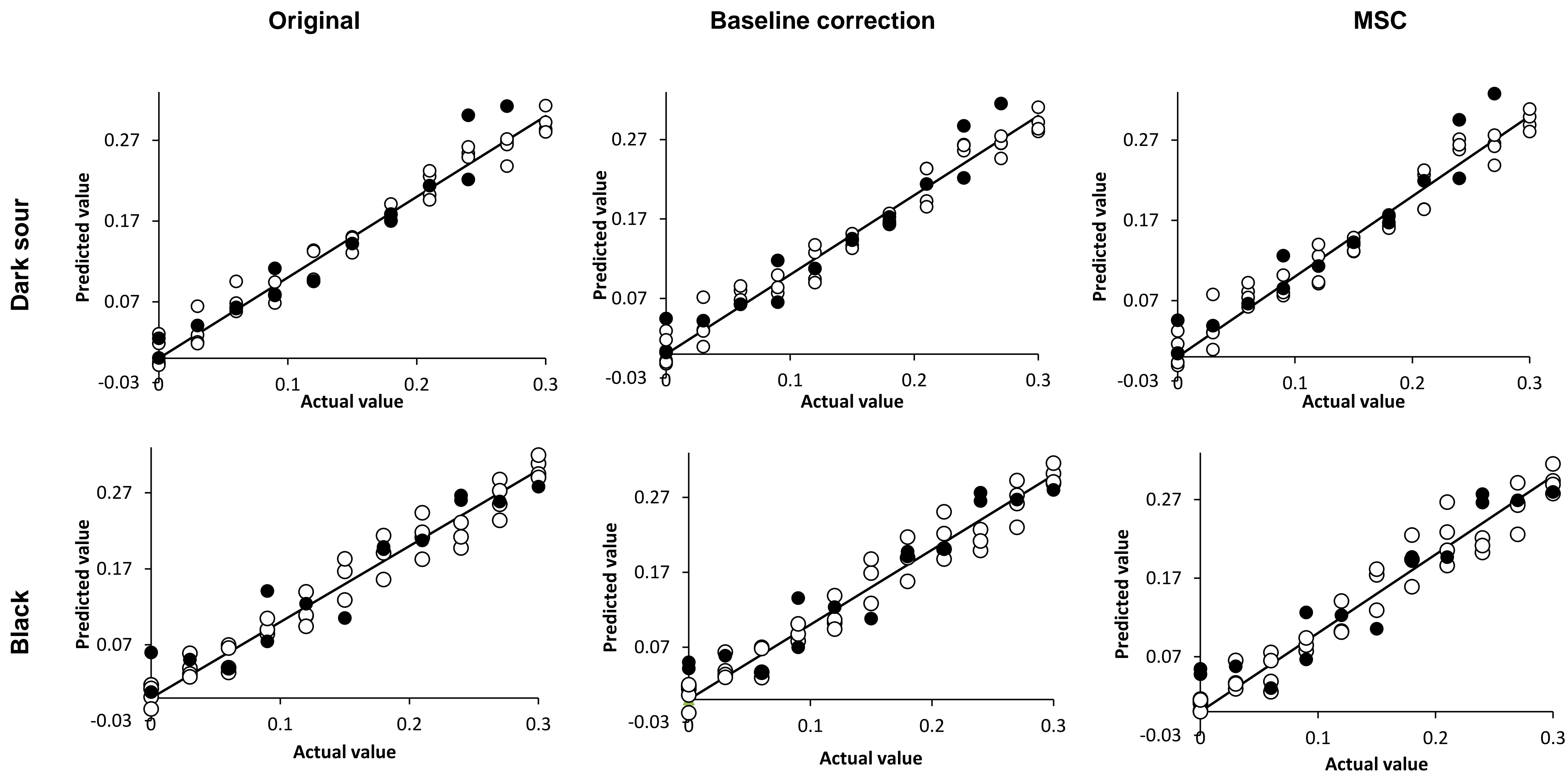
ANNEX A. Actual x predicted percentage of defective coffee in mixture with non-defective coffee from PLSR models developed with FTIR spectral data. (a) original, (b) baseline correction and area normalization, (c) 1st derivative. ○ calibration set, ● validation set. (continued)



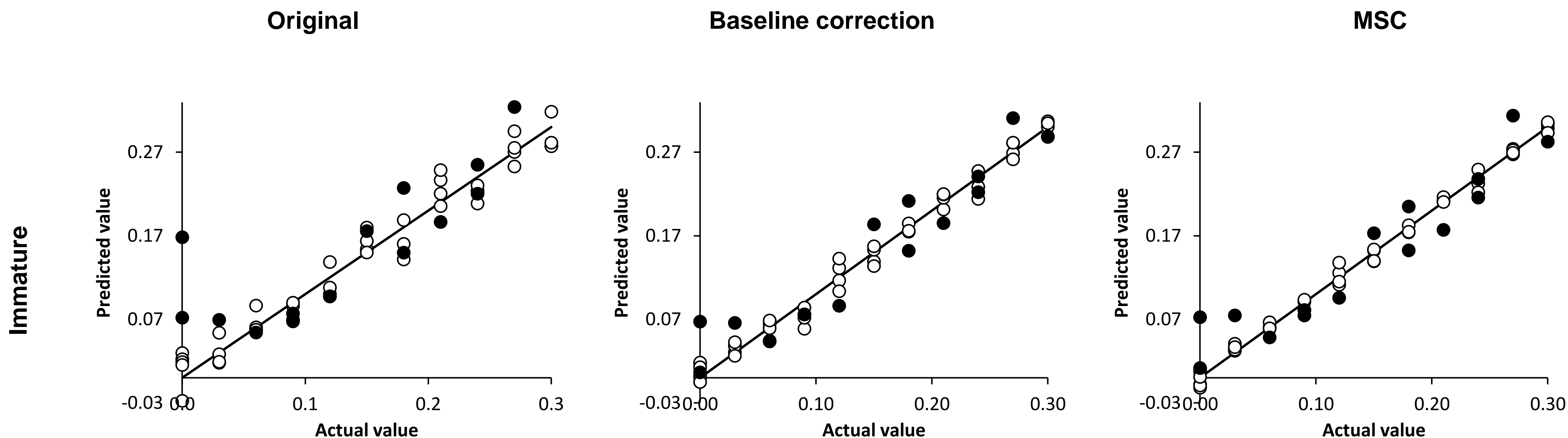
ANNEX B. Actual x predicted percentage of defective coffee in mixture with non-defective coffee from PLSR models developed with NIR spectral data. (a) original, (b) baseline correction, (c) MSC. ○ calibration set, ● validation set.



ANNEX B. Actual x predicted percentage of defective coffee in mixture with non-defective coffee from PLSR models developed with NIR spectral data. (a) original, (b) baseline correction, (c) MSC. ○ calibration set, ● validation set. (continued)



ANNEX B. Actual x predicted percentage of defective coffee in mixture with non-defective coffee from PLSR models developed with NIR spectral data. (a) original, (b) baseline correction, (c) MSC. ○ calibration set, ● validation set. (continued)



ANNEX C. Discrimination between defective and non-defective roasted coffees by DRIFTS.

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Discrimination between defective and non-defective roasted coffees by diffuse reflectance infrared Fourier transform spectroscopy

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ABSTRACT

The objective of this work was to evaluate the feasibility of employing Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) for discrimination between defective and non-defective coffees after roasting and grinding. Defective (black, immature and sour) and non-defective Arabica coffee beans were submitted to light, medium and dark roasts at 220, 235 and 250 °C. Principal Components Analysis of the DRIFTS spectra (normalized or not) and of the first derivatives of the spectra provided separation of the samples into four groups: non-defective, black, dark sour and light sour, with immature beans scattered among the sour samples. Classification models were developed based on Linear Discriminant Analysis and recognition and prediction abilities of these models ranged from 95 to 100%. Such results indicate that DRIFTS presents potential for the development of a fast and reliable analytical methodology for discrimination between defective and non-defective coffee after roasting and grinding.

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1. Introduction

The presence of defective coffee beans depreciates the quality of the coffee beverage consumed worldwide. These beans represent about 20% of the total coffee produced in Brazil and similar amounts can be expected in other producing areas around the world (Mendonça, Franca, Oliveira, & Nunes, 2008; Ramalakshmi, Kubra, & Rao, 2007). Although separated from the non-defective beans prior to commercialization in external markets, the majority of the defective beans are dumped in the Brazilian internal market and, overall, a low-grade roasted coffee is consumed in the country (Craig, Franca, & Oliveira, 2011). The negative effect that such beans have on coffee quality can be associated to 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 et al., 2008). The chemical changes due to the extraneous factors acting upon the beans (e.g., microbial fermentation) and due to the maturity stage of the beans (e.g., immature vs. mature) exert a perceptive effect in the sensory quality of the coffee beverage when determined by a trained sensory panel, but can be subtle enough not to be detected by analytical instruments depending on the technique being employed for that purpose. Considering that the defective coffee is separated from the non-defective prior to commercialization, and is also cheaper than non-defective coffee, the amount of defective beans to be used for roasting is dependent exclusively on the types of blends defined by the roasters themselves. Thus, the ultimate quality of a brand of coffee will be dictated by the amount of defective beans used for roasting, with higher qualities being expected for blends with small amounts of these beans and lower qualities for blends with greater amounts. The presence of black beans in a roasted batch usually imparts a heavy flavor to the beverage; sour beans contribute to sour and oniony tastes, while immature beans impart astringency (Clarke & Macrae, 1987).

Research interest on defective and low quality coffee beans has intensified over the past years, given the increasing awareness regarding the negative aspects they impart to the quality of the roasted and ground coffee used for beverage preparation and consumption (Craig et al., 2011; Craig, Franca, & Oliveira, 2012;

Abbreviations: ATR-FTIR, Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy; DR, diffuse reflectance; DRIFTS, Diffuse Reflectance Infrared Fourier Transform Spectroscopy; DLATGS, Deuterated Triglycine Sulfate Doped with L-Alanine; ESI-MS, electrospray-ionization mass spectrometry; LDA, linear discriminant analysis; FTIR, Fourier Transform Infrared Spectroscopy; PCA, principal components analysis; PR, pattern recognition.

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Farah, Monteiro, Calado, Franca, & Trugo, 2006; Franca, Mendonça, & Oliveira, 2005; Franca, Oliveira, Mendonça, & Silva, 2005; Mancha Agresti, Franca, Oliveira, & Augusti, 2008; Mendonça et al., 2008; Mendonça, Franca, & Oliveira, 2009; Mendonça, Franca, Oliveira, & Afonso, 2009; Oliveira, Franca, Mendonça, & Barros-Junior, 2006; Ramalakshmi et al., 2007; Vasconcelos, Franca, Glória, & Mendonça, 2007). Such studies have shown that there are physical and chemical differences between defective and non-defective coffee beans prior to roasting, but only a few have attained some success regarding discrimination of defective and non-defective coffees after roasting. Mancha Agresti et al. (2008) showed that roasted defective and non-defective coffees could be separated into two distinct groups based on their volatile profiles: immature/black beans and non-defective/sour coffees. Mendonça, Franca, and Oliveira (2009) showed that, for Arabica coffees, defective and non-defective roasted coffees could be separated by sieving. However, the majority of the commercially available roasted coffee is ground. Mendonça et al. (2008) and Mendonça, Franca, Oliveira et al. (2009) attempted to employ electrospray-ionization mass spectrometry (ESI-MS) for discrimination of defective and non-defective coffees before and after roasting. ESI-MS profiles in the positive mode (ESI(+)-MS) provided separation between defective and non-defective green coffees prior to roasting, but could not provide separation of roasted coffees.

Recent studies have shown that methods based on Fourier Transform Infrared spectroscopy (FTIR) in combination with chemometric techniques have been successfully applied for food quality evaluation (Rodriguez-Saona & Allendorf, 2011). FTIR-based methods are fast, reliable and simple to perform. They can be based on transmittance or reflectance readings, and although both techniques are appropriate for analyzing either solid or liquid samples, reflectance-based methods require none or very little sample pretreatment, being thus more commonly employed as routine methodologies for food analysis (Bauer et al., 2008; Rodriguez-Saona & Allendorf, 2011). Reflectance methods that are appropriate for non specular solid samples are divided into Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) and Diffuse Reflectance Fourier Transform Infrared Spectroscopy (DRIFTS). While ATR collects information mainly from the solid surface, DRIFTS provides information from the entire solid matrix, given that it is a combination of internal and external reflection. Both techniques have been employed for coffee quality analysis, with most of the ATR-based studies focusing on analysis of liquid samples, i.e., the coffee beverage (Briandet, Kemsley, & Wilson, 1996; Lyman, Benck, Dell, Merle, & Murray-Wijelath, 2003; Wang, Jun, Bittenbender, Gautz, & Li, 2009). DRIFTS has been also successfully applied for analysis of coffee, specifically targeting discrimination between Arabica and Robusta varieties (Kemsley, Ruault, & Wilson, 1995), detection of glucose, starch or chicory as adulterants of freeze-dried instant coffees (Briandet et al., 1996) and separation between decaffeinated and regular roasted coffees (Ribeiro, Salva, & Ferreira, 2010). We have shown, in recent studies, that DRIFTS provides satisfactory discrimination of non-defective/defective and immature/mature coffees prior to roasting (Craig et al., 2011, 2012). In view of the aforementioned, the objective of this work was to evaluate the potential of this technique in the discrimination of defective and non-defective coffee beans after roasting and grinding.

2. Materials and methods

Arabica green coffee samples were acquired from a Coffee Roasting Company located in Minas Gerais (MG) State, Brazil (Café Fino Grão, Contagem, MG). The samples consisted of three 60 kg bags of coffee beans (harvested by the strip-picking method) that were rejected by color sorting machines. Four samples of 2 kg of whole beans were randomly taken from each bag, mixed and their beans were manually sorted (by a professional trained and certified for green coffee classification) into five lots: non-defective, immature, black and sour (separated into light and dark colored). Coffee samples (25 g) were taken from each lot and submitted to roasting in a convection oven (Model 4201D Nova Ética, São Paulo, Brazil), at 220, 235 and 250 °C. After roasting, the samples were ground ($D < 0.5$ mm) and submitted to color evaluation. Color 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°. Measurements were based on the CIE $L^*a^*b^*$ three dimensional cartesian (xyz) color space represented by: Luminosity (L^*), ranging from 0 (black) to 100 (white) – z axis; parameter a^*, representing the green–red color component – x axis; and parameter b^*, representing the blue–yellow component-y axis. Roasting conditions were established for each specific lot, given that defective coffee beans have been reported to roast to a lesser degree than non-defective coffee beans when submitted to the same processing conditions (Mancha Agresti et al., 2008). Roasting degrees were then defined according to luminosity (L^*) measurements similar to commercially available coffee samples ($19.0 < L^* < 25.0$), corresponding to light ($23.5 < L^* < 25.0$), medium ($21.0 < L^* < 23.5$) and dark ($19.0 < L^* < 21.0$) roasts. The corresponding roasting times ranged from 7 to 10 min (250 °C), 9–16 min (235 °C) and 12–33 min (220 °C), with the smaller and larger times for a given temperature corresponding to the light and dark roasts, respectively.

A Shimadzu IRAffinity-1 FTIR Spectrophotometer (Shimadzu, Japan) with a DLATGS (Deuterated Triglycine Sulfate Doped with L-Alanine) detector was used in the measurements that were all performed in a dry controlled atmosphere at room temperature (20 ± 0.5 °C). Diffuse reflectance (DR) measurements were performed in diffuse reflection mode with a Shimadzu sampling accessory (DRS8000A). The ground coffee sample 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). All spectra were recorded within a range of 4000–400 cm^{-1} with a 4 cm^{-1} resolution and 20 scans, and submitted to background subtraction. The spectra were also truncated to 2500 data points in the range of 3100–600 cm^{-1}, in order to eliminate noise readings present in the upper and lower ends of the spectra. Preliminary tests were performed in order to evaluate the effect of particle size (0.39 mm $< D <$ 0.5 mm; 0.25 mm $< D <$ 0.39 mm; 0.15 mm $< D <$ 0.25 mm; and $D <$ 0.15 mm) and coffee/KBr mass ratio (2, 5, 10, 20, 30, 40 and 50%) on the quality of the obtained spectra. The conditions that provided the best quality spectra (higher intensity and lower noise interference) were $D <$ 0.15 mm and 10% coffee/KBr mass ratio. In order to improve performance of prediction models, the following data pretreatment techniques were evaluated: (0) no additional processing (raw data), (1) mean centering, (2) normalization, (3) baseline correction, (4) first derivatives and (5) second derivatives. Mathematical treatments such as mean centering and normalization are commonly applied to data in order to remove redundant information and enhance sample-to-sample differences (Wang et al., 2009). Mean centering corresponds to subtraction of the average absorbance value of a given spectrum from each data point. Normalization is calculated by dividing the difference between the response at each data point and the minimum absorbance value by the difference between the maximum and minimum absorbance values. Baseline correction and derivative transformations are usually performed in order to compensate for baseline offset between samples and also to reduce instrument variations (Esteban-Díez, González-Sáiz, Sáenz-González, & Pizarro, 2007).

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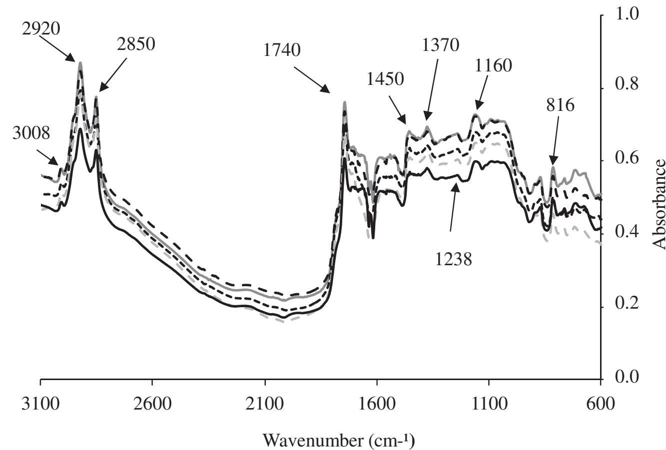

Fig. 1. Average DR spectra obtained for defective and non-defective roasted coffee beans (—— non-defective; - - - - immature; — — — sour (light); — — — sour (dark); —— black; each spectrum represents an average of 25 samples).

The statistical software XLSTAT Sensory 2010 (Addinsoft, New York) was employed for all the chemometric calculations.

3. Results and discussion

Average spectra obtained for defective and non-defective roasted coffee samples are shown in Fig. 1. A comparative evaluation of these spectra indicates that they are quite similar, although variations in band intensity are perceived, with absorbance values being higher for non-defective and light sour beans and lower for black beans. The two sharp bands at 2920 and 2850 cm^{-1} have been previously identified in Arabica and Robusta roasted coffee samples (Kemsley et al., 1995) and also on Arabica green coffee samples (Craig et al., 2011, 2012), in association to asymmetric and symmetric stretching of C–H bonds. Studies of FTIR analysis of caffeine on soft drinks have reported two sharp bands at 2882 and 2829 cm^{-1}, with the latter being due to the asymmetric stretching of C–H bonds of methyl ($-CH_3$) group in the caffeine molecule (Paradkar & Irudayaraj, 2002). Other FTIR studies on corn and corn flour have also reported two bands at 2927–2925 and 2855 cm^{-1}, being respectively attributed to asymmetric and symmetric C–H stretching in lipids (Cremer & Kaletunç, 2003; Gordon, Schudy, Wheeler, Wicklow, & Greene, 1997). Thus, the sharp bands at 2920 and 2850 cm^{-1} observed in the spectra presented for coffee in Fig. 1 can be attributed to combination bands to which both caffeine and lipids contribute. The sharp band at 1740 cm^{-1} was also reported on previous FTIR studies on roasted coffee, in association to

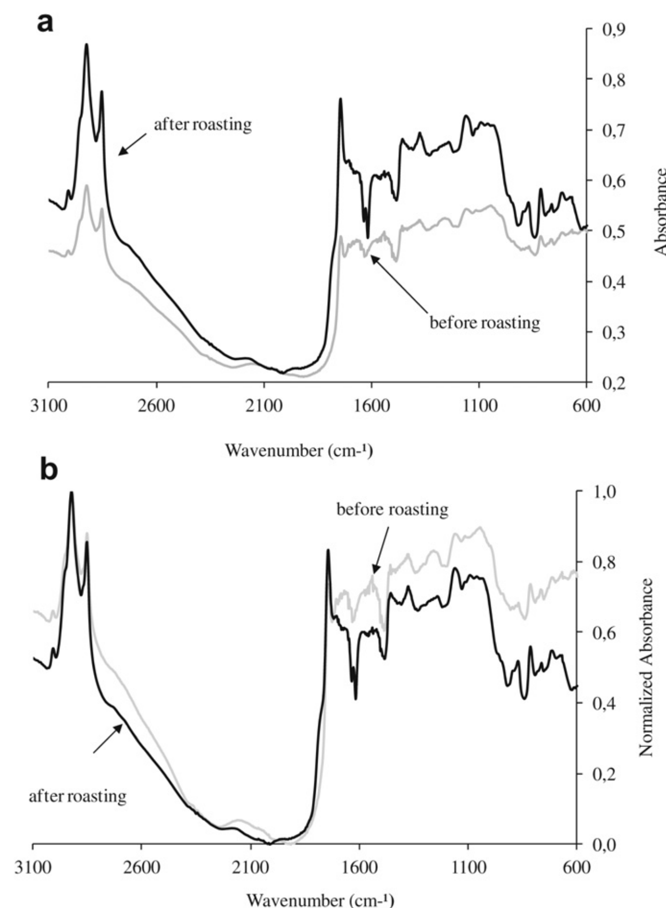

Fig. 2. Comparison of (a) raw and (b) normalized spectra obtained for coffee beans before (gray curves) and after roasting (black curves).

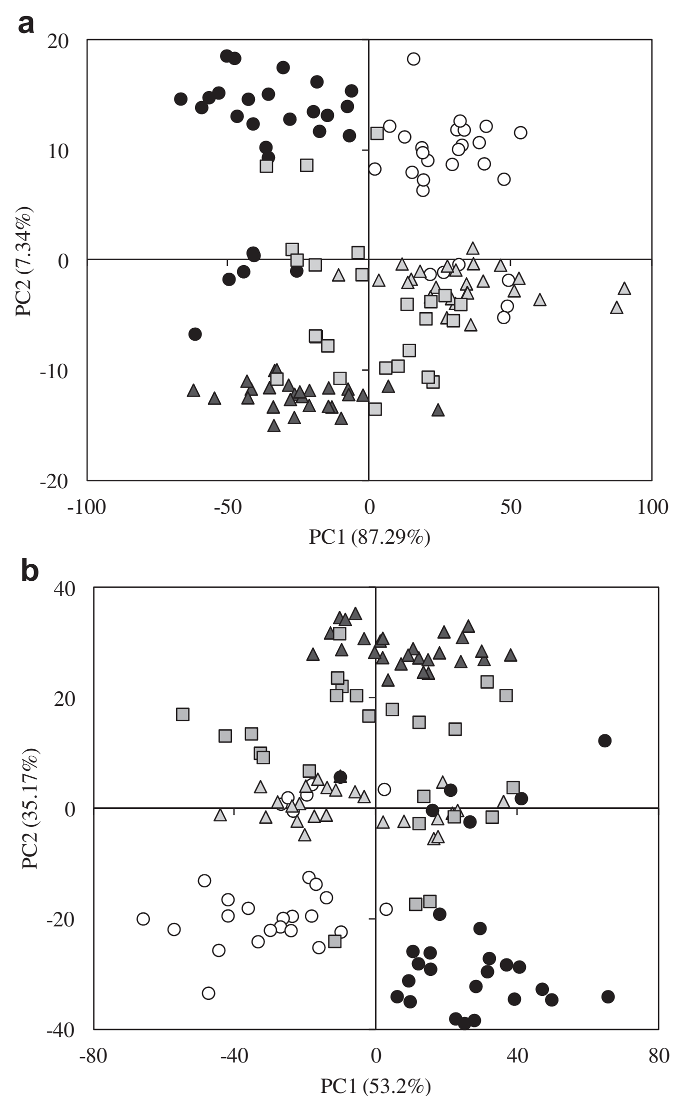

Fig. 3. PCA scores scatter plot (PC1 vs. PC2) of diffuse reflectance spectra (3100–600 cm^{-1}) (a) raw spectra (b) after mean centering; (c) after normalization; and (d) after first derivatives. ○ non-defective; □ immature; △ sour (light); ▲ sour (dark); ● black.

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carbonyl (C═O) vibration of the ester group in triglycerides (Kemsley et al., 1995) or to aliphatic esters (Lyman et al., 2003), indicating that this band could be associated to lipids. The combination of absorptions at 1740 cm^{-1} (C═O stretch) and at 2830-2695 cm^{-1} (H–C═O stretch) with a weak shoulder-type peak at 2725–2740 cm^{-1} could be interpreted as a presence of aldehydes (Miller, Mayo, & Hannah, 2003), which are volatile compounds found aplenty in roasted coffee, as a result of the thermal degradation of unsaturated fatty acids, such as linoleic acid, which is quite abundant in the coffee lipid fraction (Oliveira et al., 2006). The wavenumber 1659 cm^{-1} has been identified by Garrigues, Bouhsain, Garrigues, and De La Guardia (2000) as due to the presence of carbonyl groups in caffeine in their FTIR analysis of trichloromethane extracts of roasted coffee, and was further used as the determinant band in their quantitative analytical procedure for caffeine in roasted coffee samples. However, in our study, this band appears rather modestly in the spectra for roasted and ground coffee. Thus, it can be assumed that several other compounds in roasted coffee also absorb in that range of wavenumbers and that, apparently, trichloromethane does not extract them, since in the work by Garrigues et al. (2000) the 1659 cm^{-1} was quite sharp in the trichloromethane extract.

A comparison of average DR spectra obtained for green and roasted coffees is shown in Fig. 2a. The spectra are qualitatively similar, even though roasted coffees presented higher absorbance in comparison to green coffees. It is interesting to observe that, once the spectra were normalized (see Fig. 2b), all the previously cited bands (2920, 2850 and 1740 cm^{-1}) presented similar levels of absorbance in green and roasted coffees. This could be associated to the fact that both caffeine and lipids levels are not expected to vary significantly during roasting (Franca, Mendonça et al., 2005; Franca, Oliveira et al., 2005; Vasconcelos et al., 2007). Evaluation of Fig. 2b

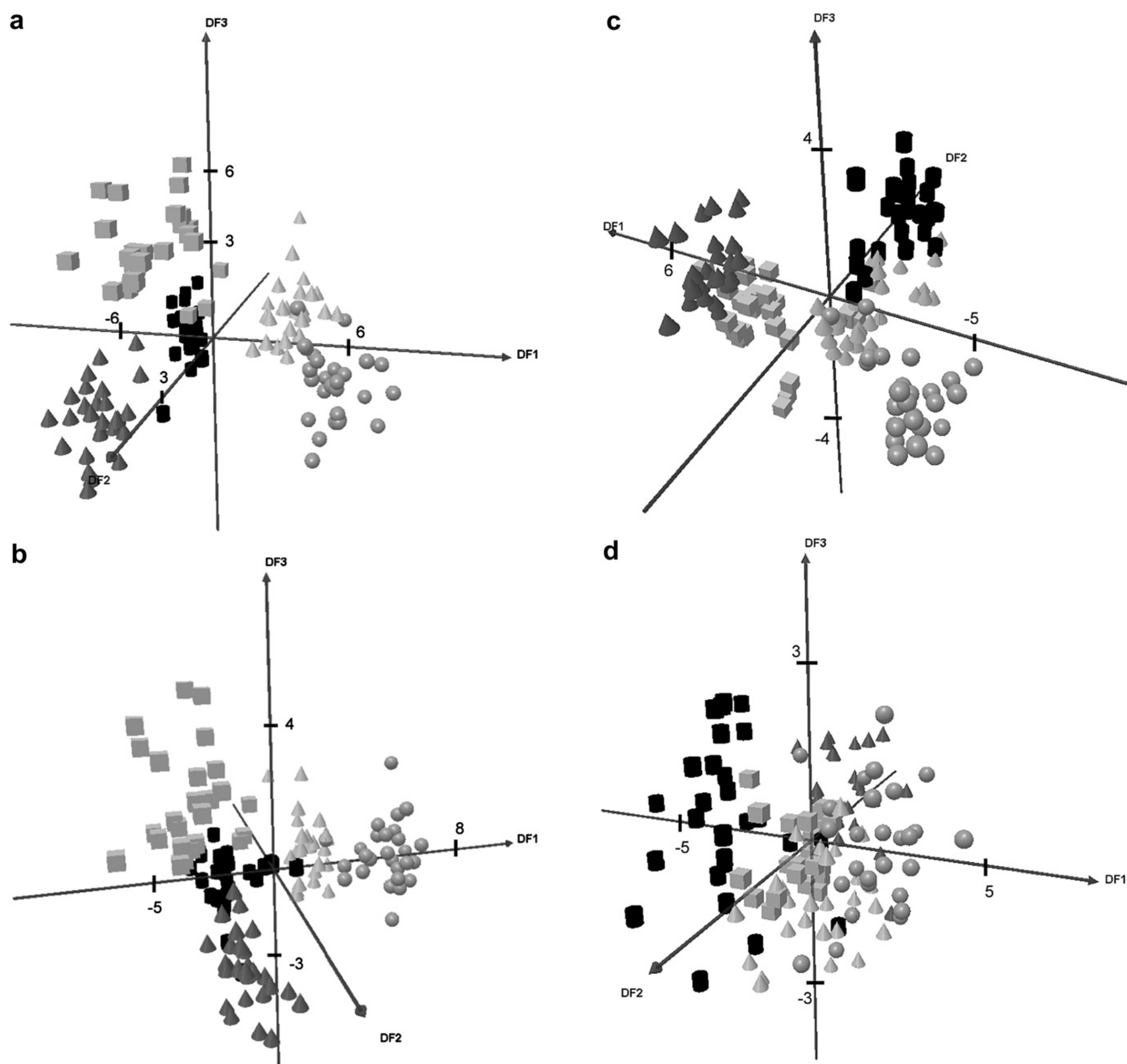

Fig. 4. Scores on the discriminant functions provided by the 8 variables LDA models of diffuse reflectance spectra (3100–600 cm^{-1}) (a) raw spectra (b) after mean centering; (c) after normalization; and (d) after first derivatives. ● non-defective; ■ immature; ▲ sour (light); ▲ sour (dark); ■ black.

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also shows no significant differences between green and roasted coffees regarding absorbance values of the small band at 3008 cm^{-1}. This band can be attributed to the symmetric stretching vibration of C–H cis-olefinic groups (=C–H in *cis* RHC = CHR) and can be also associated to the presence of lipids (Yang & Irudayaraj, 2001). The fact that it was not significantly changed from the spectrum for the green beans to that of roasted ones indicates that the double bonds of unsaturated fatty acids did not undergo isomerization from *cis* to *trans* during roasting.

Several bands can be viewed in the range of 1700–600 cm^{-1}. The wavenumber range of 1400–900 cm^{-1} is characterized 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). Other studies on FTIR analysis of roasted coffees (Briandet et al., 1996; Kemsley et al., 1995) have reported that carbohydrates exhibit several absorption bands in this region, so it is expected that this class of compounds will contribute to several of the observed bands. According to Kemsley et al. (1995), Briandet et al. (1996), and Lyman et al. (2003), chlorogenic acids also present absorption in the region of 1450–1000 cm^{-1}. Chlorogenic acids represent a 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}, and 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) and therefore the bands located in the range of 1450–1050 cm^{-1} could be partially due to chlorogenic acids. Hashimoto et al. (2009) studied the influences of coffee varieties, geographical origin and of roasting degree on the mid-infrared spectral characteristics of brewed coffee, and also developed a fast and reliable procedure to determine the caffeine and chlorogenic acid contents in brewed coffee using the ATR-FTIR method. In their method, developed based on the spiking of the coffee brew with different amounts of caffeine, they identified the band at 1242 cm^{-1} as the most relevant absorption band for characterization of the caffeine content in the brew. In the roasted and ground coffee IR spectra herein obtained for defective and non-defective coffee beans this peak appears shifted to a slightly lower band (1238 cm^{-1}), but it is present in all spectra. Another substance that can be associated to peaks in the 1600–1300 cm^{-1} range is trigonelline, a pyridine derivative that has been reported to present four bands in this range, due to axial deformation of C=C and C=N bonds (Silverstein et al., 2005). A comparison of the average spectra of green and roasted coffees presented in Fig. 2b shows a decrease in the relative absorbance of several bands in the 1700–600 cm^{-1} region after roasting. Several literature reports confirm that the levels of carbohydrates, trigonelline and chlorogenic acids diminish upon roasting (Farah et al., 2006; Franca, Oliveira et al., 2005), so such variations in chemical composition are expected to affect the spectra in the 1700–600 cm^{-1} range.

Using the DR spectra as chemical descriptors, pattern recognition (PR) methods (principal components analysis – PCA and linear discriminant analysis – LDA) were applied in order to establish whether or not specific types of beans could be discriminated within roasted coffee samples. Data matrices were constructed so that each row corresponded to a sample and each column represented the spectra datum at a given wavenumber, after processing as described in the previous section. The spectra pretreatment steps that provided a satisfactory level of discrimination between defective and non-defective coffees were the following: (0) no additional treatment of raw data, (1) mean centering, (2) normalization and (4) first derivatives. Pretreatments (3) and (5), baseline correction and second derivatives, did not provide satisfactory separation between defective and non-defective coffees. Furthermore, baseline correction (3) provided undesirable separation by roasting temperature.

Table 1
Calculated values of the first three discriminant functions at each sample group centroid.

Model	Non-defective	Immature	Dark sour	Light sour	Black
Raw spectra					
DF1	5.683	−2.422	−3.816	3.483	−3.013
DF2	0.432	0.314	2.544	−0.304	−3.122
DF3	−1.034	2.851	−1.479	0.996	−1.220
Mean-centered spectra					
DF1	4.695	−3.409	−1.918	1.614	−1.115
DF2	0.577	−0.040	2.880	−0.489	−2.975
DF2	0.454	2.121	−1.493	0.350	−1.437
Normalized spectra					
DF1	−3.621	3.274	2.847	−1.266	−0.945
DF2	−1.691	0.506	−1.588	0.828	2.513
DF2	−0.507	3.274	1.549	−0.531	1.283
First derivatives					
DF1	2.402	−0.711	0.094	0.376	−2.078
DF2	0.885	−0.696	−2.073	0.625	1.280
DF2	0.423	−0.410	0.388	−0.992	0.496

DF1, DF2 and DF3 represent the first, second and third discriminant functions, respectively.

The scatter plots obtained by PCA analysis are displayed in Fig. 3. A clear separation between categories can be observed, with four distinct major groups: non-defective (○), black (●), dark (▲) and light sour (△), with some outlier points. The few outlier samples from each group that were present in other classes (for example, a few non-defective and black beans in the light sour group) correspond to samples subjected to extreme roasting conditions (light roast/lower temperature and dark roast/higher temperature). Regardless of the employed spectra processing technique, immature beans (■) are somewhat scattered between light and dark sour defects. Clustering of immature and sour defects was also observed in the analysis of green coffees by ESI (+)-MS profiles (Mendonça et al., 2008) or DRIFTS (Craig et al., 2011), whereas Mancha Agresti et al. (2008) reported grouping of immature and black roasted coffee beans according to their volatile profiles.

A clear separation between non-defective and defective coffee beans can be observed in all the plots displayed in Fig. 3. Evaluation of the loadings plots obtained after PCA analysis of raw and processed spectra (not shown) indicated that the spectral ranges that presented the highest influence on PC1 and PC2 values in association with the non-defective coffees (PC1 and PC2 positive for spectra without further treatment, PC1 and PC2 negative for spectra submitted to mean centering, and PC1 negative and PC2 positive for normalized

Table 2
Correct classification rates (%) for the LDA models.

Model	Non-defective	Immature	Dark sour	Light sour	Black	Total
Raw spectra						
Recognition	87.0	90.9	100.0	100.0	100.0	95.5
Prediction	100.0	100.0	100.0	100.0	100.0	100.0
Mean-centered spectra						
Recognition	83.3	87.0	100.0	100.0	78.3	89.6
Prediction	100.0	100.0	100.0	100.0	100.0	100.0
Normalized spectra						
Recognition	84.0	90.0	100.0	100.0	72.7	89.3
Prediction	100.0	80.0	100.0	100.0	100.0	95.0
First derivatives						
Recognition	82.6	75.0	77.3	70.0	87.0	78.6
Prediction	75.0	100.0	100.0	66.7	75.0	80.0

Classification rates were evaluated as the percent ratio between the number of samples correctly classified in a specific group and the total number of samples of that group.

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Table 3
Model equations and correct classification rates (%) based on generic discrimination between defective and non-defective coffees.

Model			
Raw spectra: $DF = -5.0 + 93.7A_{2924} - 110.8A_{2852} + 53A_{1743} - 23.9A_{1541} - 86.4A_{1377} - 128.6A_{1076} + 25.4A_{910} + 9.4A_{816}$			
	Non-defective	Defective	Total
Recognition	84.0	100.0	96.4
Prediction	100.0	100.0	100.0
Mean-centered spectra: $DF = -6.7 + 63.3B_{2924} - 69.9B_{2852} + 66.9B_{1743} - 21.9B_{1541} - 60.7B_{1377} - 111.0B_{1076} + 49.3B_{910} + 19.7B_{816}$			
	Non-defective	Defective	Total
Recognition	87.5	100.0	97.3
Prediction	100.0	100.0	100.0
Normalized spectra: $DF = -251.2 + 175.4C_{2924} + 93.6C_{2852} - 36.0C_{1743} + 18.9C_{1541} - 58.8C_{1377} + 86.6C_{1076} - 29.4C_{910} + 3.0C_{816}$			
	Non-defective	Defective	Total
Recognition	84.0	100.0	96.4
Prediction	100.0	100.0	100.0
First derivatives: $DF = -6.2 - 109.0D_{2924} - 815.8D_{2852} - 433.5D_{1743} - 615.2D_{1541} - 715.4D_{1377} + 2560.3D_{1076} + 859.2D_{910} - 486.3D_{816}$			
	Non-defective	Defective	Total
Recognition	88.0	96.6	94.6
Prediction	94.0	100.0	95.0

DF represents the discriminant function. A_n corresponds to the absorbance value at wavenumber n; B_n corresponds to the absorbance value at wavenumber n, after mean centering; C_n corresponds to the absorbance value at wavenumber n, after normalization; and D_n corresponds to the absorbance first derivative at wavenumber n. Classification rates were evaluated as the percent ratio between the number of samples correctly classified in a specific group and the total number of samples of that group.

spectra) were the following: 1700–1500 and 970–600 cm^{-1}, in general representing the regions in which non-defective coffees presented higher absorbance intensity in comparison to all defective categories (see Fig. 1). Loadings obtained for first derivatives could not be associated to specific regions in the spectra.

Results from the principal components analysis indicate that the obtained spectra could provide enough information to develop classification models for non-defective and each specific class of defective roasted coffees. Thus, linear discriminant analysis (LDA) was performed with the purpose of obtaining classification models for assigning categories to samples. Model validation was performed using ~25% of the samples as the evaluation set. Recognition ability was calculated as the percentage of members of the calibration set that were correctly classified, and prediction ability was calculated as the percentage of members of the validation set that were correctly classified. LDA models were constructed employing different numbers of variables (wavenumbers), starting with the entire spectrum and decreasing the number of variables. It was observed that model recognition ability varied significantly with the number of variables, with the best correlations being provided by eight-variable models. In general the models were satisfactory (average recognition and prediction abilities above 75%) as long as the selected wavenumbers presented high loading values. Therefore, the following wavenumbers, that have been previously reported in other FTIR studies on coffee, were selected for the final models: 2924, 2852, 1743, 1541, 1377, 1076, 910 and 816 cm^{-1}, with possible association to caffeine, carboxylic acids, lipids, chlorogenic acids, trigonelline and carbohydrates. The score plots for the first three discriminant functions are shown in Fig. 4. The first three discriminant functions accounted for 96.2, 95.2, 95.3 and 97.6% of of the total sample variance, for the models based on raw spectra, media-centered spectra, normalized spectra and first derivatives, respectively. A clear separation of all groups (non-defective, black, immature, dark sour and light sour) can be observed for the models based on DR spectra (see Figs 4a–c), whereas some level of group overlapping was observed for the model based on spectra derivatives (Fig. 4d). The calculated values of each discriminant function at the group centroids are displayed in Table 1. It is interesting to point out that, for all the developed models, the first three discriminant functions are enough to provide sample classification. For example, considering the model based on the raw spectra, it can be observed that non-defective coffees present positive values for DF1 and DF2 and negative values for DF3, whereas black beans present negative values for DF1, DF2 and DF3. The corresponding values obtained for correct classification rates for each specific model and group are shown in Table 2. Recognition and prediction abilities were quite similar for all the developed models.

The data were further evaluated in order to develop a more generic classification model, i.e., only one discrimination function that would provide discrimination between non-defective and defective beans, without separating the defects into specific groups. The classification functions and respective correct classification rates are shown in Table 3. Respective average values of recognition and prediction abilities were 96.4 and 100%, for the model based on raw spectra, 97.3 and 100%, for the model based on media-corrected spectra, 96.4 and 100%, for the model based on normalized spectra, and 94.6 and 95%, for the model based on first derivatives. Such results confirm that DRIFTS provides satisfactory discrimination between defective and non-defective roasted coffees, demonstrating its potential for detection of defective beans in mixtures with non-defective ones after roasting. Regarding the application of such methodology for routine analyses of roasted coffee quality, further studies are still necessary, involving a trained panel of coffee tasters, to establish the minimum amount, if any, in which defective beans can be introduced to a non-defective coffee batch and changes in the beverage quality would still not be perceived in relation to one without defective beans. With the minimum amounts effectively established, mixtures of defective and non-defective roasted beans can be suitably prepared and duly tested for the discrimination capability of the developed models.

4. Conclusion

The feasibility of employing DRIFTS as a methodology for discrimination between defective and non-defective roasted coffees was evaluated. The obtained spectra were similar, with small differences in absorbance intensity between non-defective and defective coffees. PCA results based on DR spectra and first derivatives indicated separation of the samples into four major groups: non-defective, black, dark sour and light sour, with immature beans scattered among the sour samples. LDA classification models, based on absorbance readings and derivatives at eight wavenumbers (2924, 2852, 1743, 1541, 1377, 1076, 910 and 816 cm^{-1}), provided separation of the samples into five groups: non-defective, black, dark sour, light sour and immature beans. Average recognition and prediction abilities ranged from 79 to 96% and from 80 to 100%, respectively. Discrimination functions for

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generic classes of defective and non-defective coffee samples were also developed. For these generic models, recognition and prediction abilities ranged from 95 to 97% and from 95 to 100%, respectively. The results obtained in the present study confirm that DRIFTS provides satisfactory levels of discrimination between defective and non-defective coffee beans after roasting.

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