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USE OF VISIBLE AND NEAR-INFRARED SPECTROSCOPY FOR DISCRIMINATION OF EUCALYPT SPECIES BY EXAMINATION OF SOLID SAMPLES

S Nisgoski[1], *, GIB De Muñiz[1], TAP Gonçalves[2] & AW Ballarin[3]

[1]*Department of Forest Engineering and Technology, Federal University of Paraná, Av Pref Lothário Meissner, 900, Jardim Botânico, 80.210-170–Curitiba–PR, Brazil*
[2]*Museu Paraense Emílio Goeldi, Botanic Coordination, Institutional Capacity Program, Av Perimetral 1901, Terra Firme, 66077-830, Belém, PA, Brazil*
[3]*Department of Rural Engineering, Universidade Estadual Paulista/UNESP. Fazenda Lageado, Rua José Barbosa de Barros, 1780, Caixa Postal 237, 18603-970 Botucatu, SP, Brazil*

**silvana.ufpr@gmail.com*

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Eucalypt species have high importance in the forestry sector in Brazil. The wood has wide range of uses, including pulp, charcoal, plywood panels and treated boards. However, eucalypt species are hard to identify in lumber, which can cause problems in industrial use. So, the aim of this study was to combine visible and near-infrared spectroscopy to discriminate wood samples of two *Corymbia* spp. and five *Eucalyptus* spp. Visible spectroscopy was performed according to the CIELab standard. The data were analysed using descriptive statistics and regression analysis. Near-infrared analyses were based on ASTM E1655-05. The data were analysed with the Unscrambler X chemometric program, in the raw form and after pre-processing with second derivative of Savitzky-Golay. Visible and near-infrared spectroscopy can be successfully applied to separate *Eucalyptus* and *Corymbia* species. In the visible spectroscopic analyses, it was possible to separate the wood samples into two colour groups, i.e. red/rose and grey/yellow/brown. In the near-infrared spectra it was possible to discriminate samples, and three groups associated with density of the species were formed. Both techniques were appropriate to distinguish species and their industrial use might solve practical problems.

Keywords: *Eucalyptus, Corymbia*, colorimetry, NIR, identification

INTRODUCTION

More than 72% of plantations in Brazil are planted with *Eucalyptus* spp. and *Corymbia* spp. The average yield of eucalypts in Brazil is 36 m^3 ha^{-1} $year^{-1}$ while in Australia, it is 22 m^3 ha^{-1} $year^{-1}$ (IBÁ 2015, 2016). In general, most eucalypt wood is used for pulp, industrial firewood and charcoal, although its use for construction lumber, plywood panels and treated wood is also important (IBÁ 2016).

Colour is very important to determine the final use of eucalyptus wood, and to evaluate this characteristic, visible spectroscopy can be a rapid and non-destructive alternative to verify adequate application, for example, in construction or furniture. The industry typically considers wood colour and grain pattern to be important features for large-scale use of any species. These features can increase the commercial value of some species based on consumer preferences for colour patters of certain species such as mahogany, cherry or sucupira (Camargos & Gonçalez 2001). Consumers have mistaken *E. grandis* as mahogany from its rose–red colour (Gonçalez et al. 2006). Colour classification of species and natural variability of the tree are important for manufacturers and consumers of parquet floors and furniture because they influence the aesthetic characteristics and final value of the product (Defoirdt et al. 2012, Csordós et al. 2014).

Near-infrared (NIR) spectroscopy is a non-destructive technique that can be applied on an industrial scale to monitor wood processing and also for classification of some characteristics of raw material (Tsuchikawa & Schwanninger 2013). This technique has been effectively used to discriminate species using wood and leaf samples (Pastore et al. 2011, Espinoza et al. 2012, Nisgoski et al. 2015a), and data for classification can be

analysed after different pre-treatment methods (Tominaga 1999, Tsuchikawa et al. 2003, Oliveira et al. 2015). For *Eucalyptus*, Castillo et al. (2008) used NIR spectroscopy for fast discrimination of *Eucalyptus globulus* and *E. nitens*.

The technique can also be applied to distinguish different geographical origins (Sandak et al. 2011, Nisgoski et al. 2016), although some responses are influenced by hybridisation (Meder et al. 2014) and genetic factors (Hein & Chaix 2014). When wood material is analysed, face, shape and particle size influence the spectra, and measurements taken at different points of a sample can produce variations (Brunner et al. 1996, Braga et al. 2011, Nisgoski et al. 2015b) but still allow distinction from other species.

Considering the commercial importance of eucalyptus in Brazil, the objective of the present study was to combine visible and NIR spectroscopy to discriminate species based on solid samples.

MATERIALS AND METHODS

Wood samples of *Corymbia* spp. and *Eucalyptus* spp. lumber were obtained from Prema, a forestry company in the city of Rio Claro, São Paulo. Table 1 shows the data of the species studied and Figure 1 illustrates them. For each species, four samples were analysed and 10 spectra of each were obtained by visible and NIR spectroscopy from the longitudinal surface, for a total of 40 spectra per species.

Colorimetric evaluation was performed using spectrophotometer with a spectral range from 400–750 nm, D65 light source and 10° observation angle (CIELab standard). Forty measurements of each species were taken, from which lightness (L*), green–red chromatic

Table 1 Geographic location and density of species studied

Species	Origin (municipality, state)	Planted	Average wood density* (kg m^{-3})
Corymbia citriodora	Floresta Estadual de Pederneiras, São Paulo (22°22' S, 40°44' W)	1966	980
Corymbia maculata	Floresta Estadual de Rio Claro, São Paulo (22° 25' S, 47° 33' W)	1975	810
Eucalyptus dunnii	Reflorestamento Klabin, Telêmaco Borba, Paraná (24° 16' S, 50° 31' W)	1987–1990	750
Eucalyptus microcorys	Floresta Estadual de Rio Claro, São Paulo (22° 25' S, 47° 33' W)	1975	770
Eucalyptus saligna	Fazenda Mariana, Araras, São Paulo (22° 17' S, 47° 15' W)	1960	690
Eucalyptus tereticornis	Fazenda Santa Elisa, Campanha, Minas Gerais (22° 25' S, 47° 33' W)	1970	950
Eucalyptus viminalis	Fazenda Santa Maria, Guarapuava, Paraná (25° 7' S, 51° 30W)	1990	720

*Density is based on mass and volume at 12% moisture content (Ballarin et al. 2015)

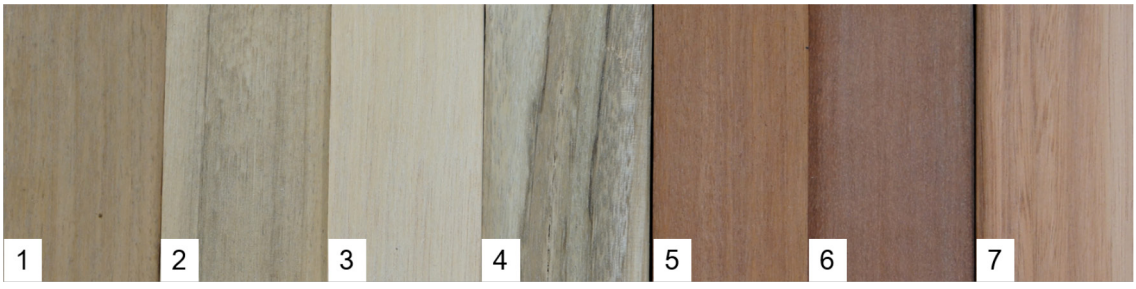

Figure 1 Wood species used in discrimination study: *Corymbia citriodora* (1), *C. maculata* (2), *Eucalyptus dunnii* (3), *E. microcorys* (4), *E. saligna* (5), *E. tereticornis* (6) and *E. viminalis* (7)

coordinate (a*) and blue–yellow chromatic coordinate (b*) were obtained. The data were analysed using descriptive statistics and regression analysis. In addition, the Tukey test was performed to verify the possible grouping of species in each parameter. Analysis was performed with 95% probability.

NIR analyses were performed in a Bruker Tensor 37 spectrometer equipped with integrating sphere and operating in reflectance mode. There were 64 scans with resolution of 1 nm and a spectral range of 1000–2500 nm. Spectral analysis was based on ASTM E1655-05 (ASTM 2000).

The Unscrambler X chemometric program (CAMO Software AS, version 10.1) was used to analyse the data. Exploratory modelling was done by analysing the score and factor loading graphs obtained by principal component analysis for 280 spectra, 40 per species in NIR and colour analysis to verify possible differences between *Eucalyptus* and *Corymbia* solid samples. Data were analysed in raw form and after preprocessing by the second derivative of Savitzky-Golay (polynomial order = 2, smoothing point = 5).

RESULTS AND DISCUSSION

Colorimetry

The colorimetric parameters of *Eucalyptus* and *Corymbia* species (Table 2) showed differences in lightness between most species except *C. citriodora*, *E. viminalis* and *C. maculata* which had the same tint for white and black colours. For the intensity of green–red chromatic coordinate there were no significant differences between *C. citriodora*, *C. maculata* and *E. dunnii*. Intensity of the blue–yellow chromatic coordinate separated the species into two distinct groups, i.e. one comprising *E. dunnii* and *E. viminalis* and other, *E. microcorys* and *E. saligna*. For the rest of the species, *C. citriodora* was similar to *E. dunnii* and *E. viminalis* while *C. maculata* and *E. tereticornis* are both unique, not similar to the rest of the species.

Based on the timber colour chart (Camargos & Gonçalez 2001), the species were classified as (1) olive yellow—*C. citriodora* and *C. maculata*, (2) grey–red—*E. dunnii*, (3) grey rose to olive yellow—*E. microcorys*, (4) brown–red to red—*E. saligna*, (5) dark brown—*E. tereticornis* and (6) rose—*E. viminalis*. For *E. grandis*, Gonçalez et al. (2006) observed rose–red color and grey olive for *E. cloeziana* and reported differences in colour parameters between radial/tangential surfaces. *Eucalyptus grandis* has been reported to have lightness = 46.77 and coordinates green–red = 10.57 and blue–yellow = 14.08 (Amorim et al. 2013). They elaborated that the green–red chromatic coordinate is mainly responsible for red colour and yellow pigmentation.

Eucalyptus dunnii was classified in this study as grey–red, while Vanclay et al. (2008) classified it as pale yellowish and uniformity in colour within trees and a small distinction between families related to yellowness (b*) of the wood was reported. *Corymbia citriodora* showed an olive yellow colour, contrary to Garcia et al. (2014) who observed that parameters related to grey–red colour and its green–red chromatic coordinate had more influence on colour variation between species and also in variation of lightness of radial/tangential surface. Colorimetry has potential to be applied as an auxiliary tool for species identification but it must be done

Table 2 Mean (standard deviation) values of CIELab parameters of species

Species	L*	a*	b*
Corymbia citriodora	63.16 (6.29) b	7.26 (2.25) c	21.05 (2.02) b
Corymbia maculata	61.78 (4.43) bc	8.03 (1.22) c	22.91 (3.15) a
Eucalyptus dunnii	74.02 (2.01) a	7.21 (0.44) cd	20.28 (0.88) bc
Eucalyptus microcorys	59.73 (5.35) c	6.38 (0.97) d	18.79 (0.80) c
Eucalyptus saligna	51.25 (2.15) d	16.52 (1.62) a	19.50 (1.40) c
Eucalyptus tereticornis	45.91 (4.46) e	15.25 (1.11) b	16.08 (2.07) d
Eucalyptus viminalis	63.91 (2.53) b	15.83 (0.85) ab	20.60 (1.03) bc

Mean values followed by the same letter in the same column do not differ statistically by the Tukey's test at 95% probability; L* = lightness, a* = green–red chromatic coordinate and b* = blue–yellow chromatic coordinate

carefully and more studies should be carried out (Garcia et al. 2014). Adequate cutting angle and the observation of the differences in longitudinal and radial position of eucalypt clones are recommended for more homogeneity based on the final colour desired (Mori et al. 2005).

Colour variation within a species is frequent and can be related to genetic and environmental characteristics (Bradbury et al. 2011). Significant difference in the L*a*b* values between species, provenances and sites as well as between young trees from plantations and old trees from natural stands was described by Gierlinger et al. (2004). The authors explained that differences in origins and sites are associated with phenolic content. The reflectance curves of visible spectra showed the same behaviour between species (Figure 2). *Eucalyptus viminalis, E. saligna* and *E. tereticornis* are species classified visually as reddish and showed a distinct line compared with the rest of the species, with less reflectance in the 480–580 nm region.

Principal component analysis was able to distinguish all species (Figure 3). A single principal component explained 99% of the variation between analysed materials based on data without mathematical treatment, enabling species discrimination. *Corymbia citriodora* and *E. microcorys* presented a tendency to form two groups because of differences in samples. From visual evaluation, it was clear that first group had contrast between sapwood and heartwood while the second presented natural variety of colour bars. *Corymbia maculata* and *E. dunnii* also exhibited separation between samples. This might be the result of irregular surface or differences between radial and tangential section in light reflection. However, discrimination of the studied species can be done based on reflectance curves of visible light.

It is possible to distinguish the distribution of *Eucalyptus* and *Corymbia* species into two groups based on colour (Figure 3), namely, red/rose and grey/yellow/brown, and also verify the influence of surface orientation when samples of one species are more separate in function of data position, i.e. radial or tangential section. Wavelengths, which represent rose and red colour (640–790 nm) and chromatic coordinate a*, presented influence on PC2 (loading graphs not shown) and are responsible for the division in two groups based on colour, even representing only 1%.

Colour differences between radial and tangential sections can be ascribed to anatomical characteristics such as arrangement of cells, large rays and spiral grain (Nishino et al. 2000). The texture of a wood surface has important influence on its colour, and a difference with magnitude of 1 to 2 in colour parameters is common and accepted (Buchelt & Wagenführ 2012). Drying, thermal modification and

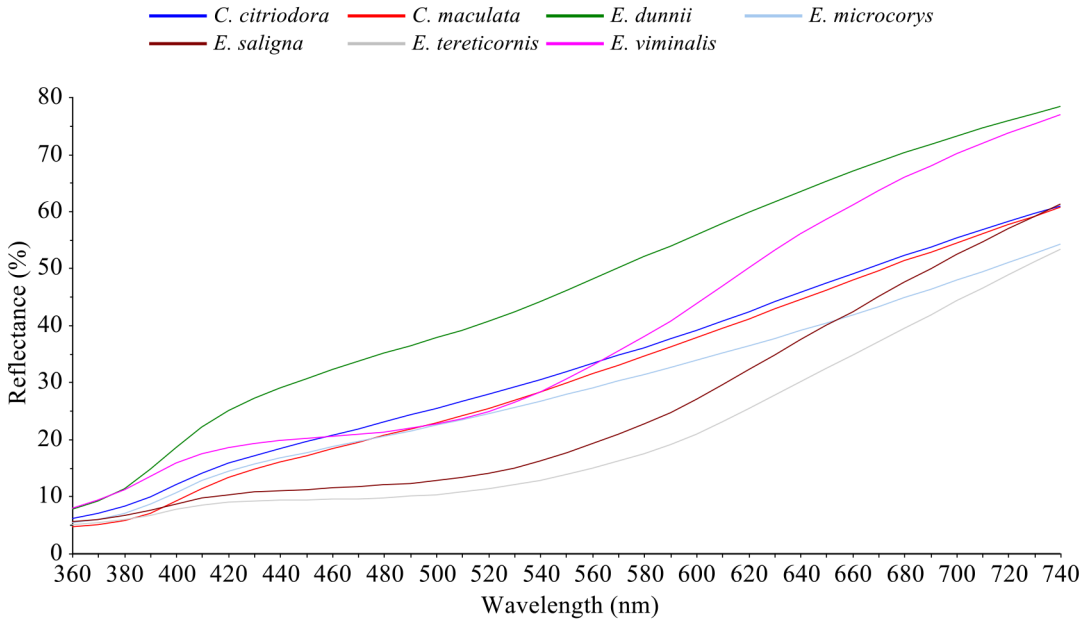

Figure 2 Reflectance curves in the visible range for five *Eucalyptus* and two *Corymbia* species

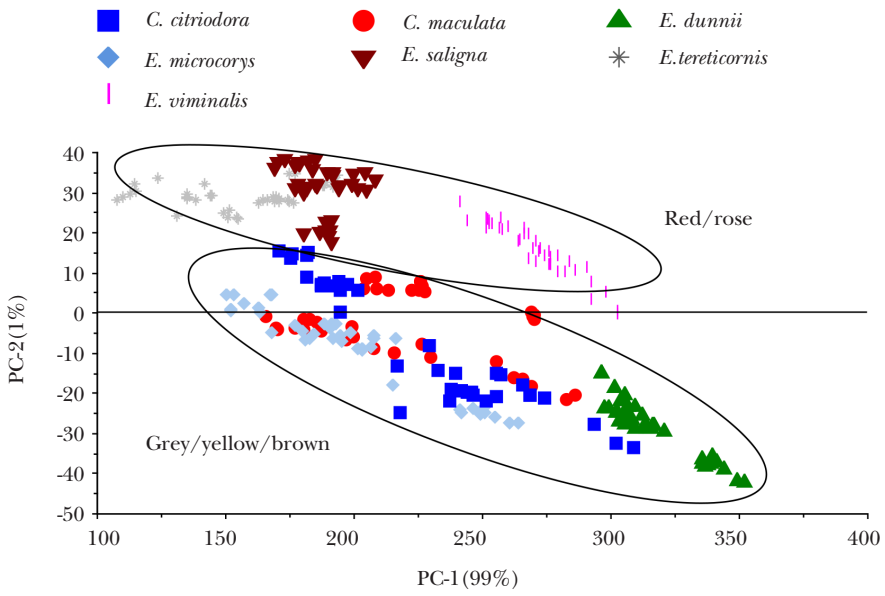

Figure 3 Score plot from principal component analysis with visible light data of five *Eucalyptus* and two *Corymbia* species

natural or biological degradation can cause changes in wood colour as a result of chemical modification and are examples of processes that can be monitored by colorimetry (Torres et al. 2012, Cademartori et al. 2013, Stangerlin et al. 2013). The use of colour in distinguishing wood species is presented in studies generally based on images and with classification methods such as artificial neural networks (Bombardier & Schmitt 2010, Peng 2013). The use of CIELab values for distinction of tree species based on leaves were reported by Richardson et al. (2003) and Nisgoski et al. (2015a) and with wood samples of *Eucalyptus* spp. by Garcia et al. (2014), which verified the potential of this technique.

Near-Infrared spectroscopy

The NIR spectra of the species were similar (Figure 4). As found for chemical and anatomical characteristics of species, NIR absorbance values can show variation and for discrimination, some spectral regions can have more influence (Pastore et al. 2011, Durgante et al. 2013, Nisgoski et al. 2016) than the rest. To eliminate noise and remove additive and multiplicative effects in the spectra and also improve analysis, the second derivative was applied (Figure 5). Second derivative preprocessing has already been applied in other studies of foliage and wood species discrimination (Sandak et al. 2011, Meder et al. 2014, Zhang et al. 2014). The informative wavelength was related to peaks in the spectra based on interaction of the infrared radiation with the cell compounds (Figure 4). *Corymbia citriodora* had more intense peaks in all spectra and *E. dunnii* and *E. saligna* presented less variation in NIR absorbance. Some differences between species were observed in regions related to cellulose, hemicelluloses and lignin which depended on cell wall composition and extractives, and also can influence species discrimination.

The spectral region between 1445 and 1450 nm and near 2134 nm presented bands related to lignin and extractives content. The region from 1470 to 1490 nm was principally assigned to cellulose and hemicelluloses. Bands at 2267 nm are related to lignin while at 2270 nm, the bands can be assigned to cellulose components. Between 1800 and 1900 nm, it was possible to verify greater distinction of absorbance intensity of peaks between species. This region is related to all cell wall components, while bands between 1916 and 1942 nm are associated with OH from water (Schwanninger et al. 2011).

Principal component analysis was carried out to verify the distribution of wood samples with the second derivative of Savitzy-Golay (Figure 6). The first principal component explained 87% of variation between the analysed materials,

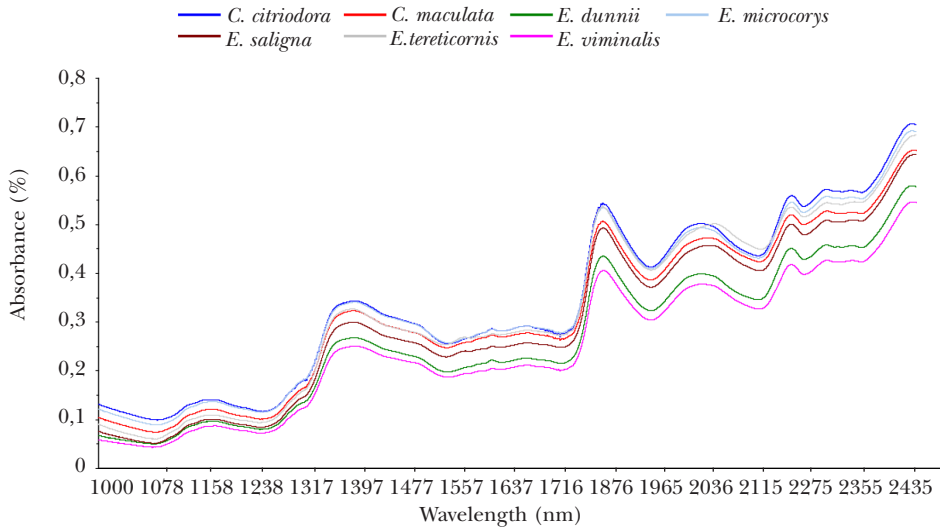

Figure 4 Mean near-infrared spectra from five *Eucalyptus* and two *Corymbia* species

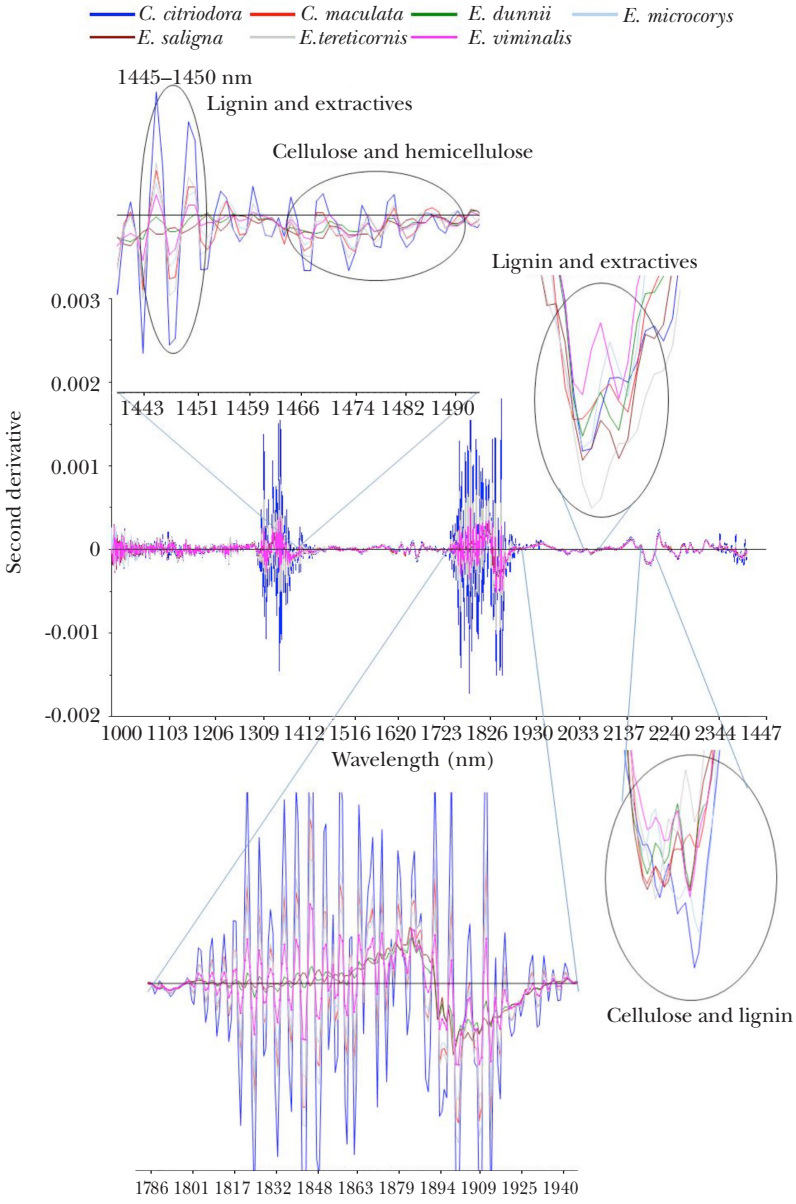

Figure 5 Mean second derivative near-infrared spectra from five *Eucalyptus* and two *Corymbia* species

enabling species discrimination. We expected some groups related to colour to be formed but this was not observed. One group was formed comprising *C. maculata* and *E. microcorys,* another *E. dunnii*, *E. saligna* and *E. viminalis*, and a third group was formed of *C. citriodora* and *E. tereticornis*.

Groups based on NIR spectra can be the result of chemical composition and also be influenced by genetics, age, forest conditions, adaptations and natural hybridisation. Some characteristics from phylogeny can explain why *E. dunnii* and *E. viminalis* presented similarity in data position in PCA graphic. Both are from subgenus *Symphyomyrtus*, section Maidenaria. In contrast, *E. saligna* is from the same subgenus but from section Latoangulatae and *E. tereticornis* belongs to Exsertia. On the other hand, results from NIR analysis that group *C. maculata* and *E. microcorys* are not influenced by these characteristics because the latter is phylogenetically classified in subgenus *Blakella* and section Maculatae, while *E. microcorys* is from subgenus *Alveolata* (Steane et al. 2011, Bayly et al. 2013). Wood density of the species also had an influence on spectra. The groups observed in Figure 6 are: (1) 690–750 kg m^{-3} for *E. dunii*, *E. saligna* and *E. viminalis*, (2) 770–810 kg m^{-3} for *C. maculata* and *E. microcorys* and (3) 950–980 kg m^{-3} for *C. citriodora* and *E. tereticornis* (Ballarin et al. 2015).

CONCLUSIONS

Visible and NIR spectroscopy can be successfully applied to separate eucalypt species. In the visible spectroscopic analyses, lightness and intensity of green–red chromatic coordinate presented differences between most of the species, while intensity of the blue–yellow chromatic coordinate was similar for *E. dunnii* and *E. viminalis,* and for *E. microcorys* and *E. saligna*. Principal component analysis was able to distinguish all species. Just one principal component explained 99% of the variation of the analysed materials. It was possible to separate the wood samples into two colour groups, i.e. red/rose and grey/yellow/brown.

The NIR spectra were similar for the species. In the principal component analysis, the first component explained 87% of the variation between the species and it was possible to discriminate them. Three groups associated with the density of the species were formed, i.e. (1) *Corymbia maculata* and *Eucalyptus microcorys,* (2) *E. dunnii*, *E. saligna* and *E. viminalis* and (3) *C. citriodora* and *E. tereticornis*.

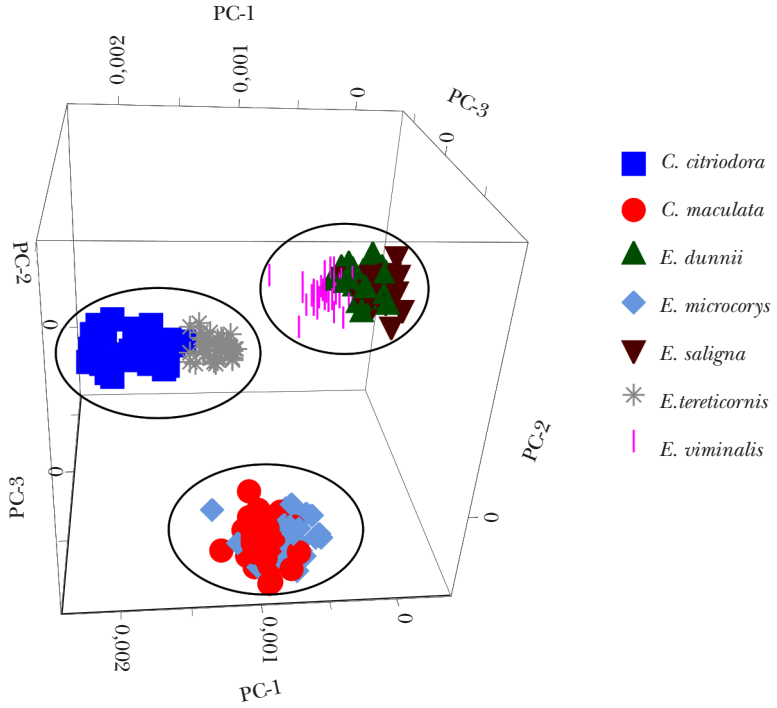

Figure 6 Score plot from principal component analysis with second derivative of near-infrared data of five *Eucalyptus* and two *Corymbia* species

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