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Ultraviolet spectroscopy and chemometrics for the identification of vegetable tannins

Fábio dos Santos Grasel[a,b,*], Marco Flôres Ferrão[c], Carlos Rodolfo Wolf[a]

[a] Tanac S/A, Montenegro, RS, Brazil
[b] Programa de Pós-graduação em Engenharia e Tecnologia de Materiais, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil
[c] Instituto de Química, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

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ABSTRACT

In this study six different commercial vegetable tannins were analysed by ultraviolet spectroscopy and multivariate analysis. In addition, also it was determinated a specific absorptivity and quantified the total polyphenols and tannin content of each extract by filter and Folin Ciocalteu methods, respectively. The lowest values of specific absorptivity were for extracts of condensed tannins and the highest for the hydrolysable tannins. At analysis of total polyphenols, the condensed tannins showed the highest percentage, 90% and 80% for the quebracho and mimosa, respectively. Hydrolysable tannins were in the range 60–67%. In the evaluation of tanning percentage, the quebracho, chestnut and mimosa presented a percentage around 80%, followed by valonea, tara and myrobalan, 74, 56 and 41%, respectively. At multivariate analysis, a well-defined separation can be seen through both principal component analysis (PCA) and hierarchical cluster analysis (HCA), between condensed (quebracho and mimosa) and hydrolysable (valonea, chestnut, myrobalan, and tara) tannins. In hydrolysable tannins, it was also possible to observe the formation of two different subgroups between samples of chestnut and valonea and between samples of tara and myrobalan. Of all samples analysed, the chestnut and valonea showed the greatest similarity, indicating that these extracts contain equivalent chemical compositions and structure and, therefore, have similar properties.

1. Introduction

Vegetable tannins are polyphenolic substances that can be easily extracted with water from almost all plants (Tondi and Petutschnigg, 2015) and have traditionally been used to tan leather (Falcão and Araújo, 2013; Venter et al., 2012). Tannins are a heterogeneous group of polyphenols widely present in the plant kingdom as secondary metabolites for protective purposes, with molecular weight between 500 and 30,000 Da (Falcão and Araújo, 2014). They occur in bark, wood, fruits, fruit pods, leaves, roots, and plant galls (Mané et al., 2007; Ricci et al., 2015).

Tannins are classified into hydrolysable and condensed tannins (Radebe et al., 2013). The hydrolysable tannins are composed of a polyol central core acylated by a variable number of gallic or ellagic acid units and derivatives (Falcão and Araújo, 2013, 2014; Mané et al., 2007; Radebe et al., 2013). The condensed tannins, also called proanthocyanidins are oligomers and polymers formed in the flavan-3-ol basic structure (Falcão and Araújo, 2013, 2014; Mané et al., 2007; Radebe et al., 2013; Ricci et al., 2015; Venter et al., 2012).

These compounds are used for many different applications, such as flocculants (Beltrán-Heredia et al., 2011, 2012), anti-corrosion (Peres et al., 2012), tanning (Falcão and Araújo, 2013, 2014), adhesives (Spina et al., 2013a,b), pharmaceutical agents (Frazier et al., 2010; Quideau et al., 2011), and foams (Basso et al., 2011, 2013; Jana et al., 2014; Yuso et al., 2014).

Due to the complexity of the composition of vegetable tannins, the identification of the nature of these extracts has been very difficult; requiring the use of advanced techniques with mass analysis, MALDI-TOF is one of the most common (Falcão and Araújo, 2013; Mané et al., 2007; Radebe et al., 2013; Shen et al., 2010; Vázquez et al., 2013; Zhang and Lin, 2008).

Among the simplest techniques for identifying the nature of the tannin extracts is the spot test. Falcão and Araújo (2011) developed a methodology to identify the nature of extracts in historic vegetable leathers. Chemical spot tests allow the characterisation of

* Corresponding author at: Tanac S/A, Montenegro, RS, Brazil.
E-mail addresses: fsgrasel@gmail.com, fsgrasel@tanac.com.br (F.d.S. Grasel).

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tannins in leather fibres and, indirectly, the approximate vegetable sources used both in the past and nowadays to produce leather. This often necessarily involves a rapid assessment of the quality and origin of the hides, and spot tests are a rapid, inexpensive and easy to use tool (Falcão and Araújo, 2011).

In more recent work, the ATR-FTIR technique has also been used successfully for characterisation of tannins (Falcão and Araújo, 2014; Grasel et al., 2016; Ricci et al., 2015Tondi and Petutschnigg, 2015). As the functional groups in the tannins are the same in general, a careful evaluation of the fingerprint area and comparison with the known standards of each group is required. It is possible to come to erroneous conclusions because some extracts have very similar chemical composition, such as valonea and chestnut (Grasel et al., 2016; Ozgunay et al., 2007; Hassanat and Benchaar, 2013; Wischer et al., 2013; Wischer et al., 2014).

Recently an FTIR technique associated with multivariate analysis for identification of the nature of polyphenolic extracts was published (Grasel et al., 2016). Chemometric tools used for pattern recognition were principal component analysis (PCA) and hierarchical cluster analysis (HCA) (Brereton, 2003; Bro and Bro, 2014; Véras et al., 2012; Wold et al., 1987). These unsupervised techniques are intended to verify the natural formation of a similar set of samples or groups. The HCA technique is based on the similarity and dissimilarity between samples, by calculating the distance between them. The PCA technique is based on the mathematical manipulation of raw data in order to obtain new variables that are linear combinations of the original variables and which have orthogonality to each other, called principal components (PCs) (Bro and Bro, 2014; Véras et al., 2012; Wold et al., 1987). The results showed two well-defined groups of condensed and hydrolysable tannins. In the group of hydrolysable tannins, the results for valonea and chestnut are very similar, and in accordance with those reported in the literature by MALDI-TOF (Ozgunay et al., 2007; Pasch and Pizzi, 2002).

Among the most popular techniques used for the characterisation of tannins in analytical chemistry, it can be mentioned the ultraviolet and visible spectrophotometer (UV–vis) (Rice et al., 2012). This is used for a simple and robust technique for analytical determinations in several areas. This can be used in organic chemistry in the quantification of polyphenolic compounds in natural extracts (Dall'Acqua et al., 2012; de Oliveira et al., 2012; Cádiz-Gurrea et al., 2014; Blainski et al., 2013), such as for the qualitative analysis of these compounds in leathers (Falcão and Araújo, 2013; Giurginca et al., 2007; Plavan et al., 2010). Falcão and Araujo (2013) used the UV–vis technique in association with ATR-FTIR techniques and chemical tests to identify the nature of the vegetable tannins in leathers. The authors concluded that the tannins are transparent in the visible region. These extracts absorb in the ultraviolet region (204–284 nm). The tannins absorb in the aromatic characteristic region, being the aromatic rings the main chromophore groups of these extracts (Pavia et al., 2010; Solomons and Fryhle, 2015). The conjugated π electrons in an aromatic ring provide characteristic absorptions of moderate intensity at around 205 nm and a less intense band in the range of 250–275 nm.

The UV–vis technique associated with multivariate analysis can be a very interesting tool for vegetable tannin identification. A methodology combining UV–vis techniques and an associated multivariate analysis to determine the nature of the vegetable tannins is presented in this work. Among the instrumental techniques, analysis by UV–vis is one of the fastest and most popular, requiring no reagents or pre-treatment of samples, in order to provide information on the main absorption bands. The HCA and PCA were used for multivariate analysis of the ultraviolet spectra. In addition, also it was determinated a specific absorptivity and quantified the total polyphenols and tannin content of each extract by filter and Folin Ciocalteu methods, respectively.

2. Experimental section

2.1. Standards and chemicals

All chemicals were analytical-reagent grade and the water was distilled. The chemicals included *2 N* Folin-Ciocalteu reagent (Dinâmica®, Diadema, Brazil), sodium carbonate-tartrate, formaldehyde, hydrochloric acid (Synth®, Diadema, Brazil), and gallic acid (Sigma-Aldrich®, St. Louis, MO, USA).

2.2. UV-vis

Ultraviolet spectra were measured on a double beam spectrophotometer with ultraviolet-visible spectroscopy, PG Instruments, T80+ model in the spectral range of 190–380 nm, with 1 cm quartz cells. The extracts with a concentration of 0.01 g L^{-1} were prepared in 100 mL volumetric flasks. The analyses were performed in triplicate using a water solvent and the average spectra.

2.3. Determination of specific absorptivity

The specific absorptivity of an extract is the relationship between maximal absorbance by the concentration of the solution. Natural extracts are complex mixtures, with molar concentration not defined wherein the concentrations of the solutions are usually expressed in g L^{-1}. The calculation is performed over the absorptivity observed at the wavelength of maximum absorption by the sample concentration and optical path.

2.4. Determination of total polyphenols

The total polyphenols were determinated by the Folin-Ciocalteu colorimetric method according previously described in the literature (Blainski et al., 2013; DiCiaula et al., 2014). The Folin-Ciocalteu reagent consists of a mixture of phosphotungstic and phosphomolybdic acids, which in basic medium are reduced to oxidize the polyphenol, yielding blue oxides, which are quantified by its absorbance. Color development consists in addition of 1 mL of Folin-Ciocalteu reagent and 10 mL sodium carbonate-tartrate reagent in 50 mL of sample, after 30 min in repose was measured the absorbance. A calibration curve was done with seven points from 0.02 to 10 ppm ($R^2 = 0.9981$ and $y = 0{,}043x + 0.0095$) using a standard gallic acid. Absorbance measures were performed at wavelength of 725 nm.

2.5. Reference methods for tannin determination

Analysis of the parameters of insoluble matter and tannin content were carried out as per the procedure given in ISO/IUL International Standard (2011). This procedure was performed in duplicate and the result matched the arithmetic average on a dry basis.

2.6. Samples

Six of the most commonly used industrial tannin extracts were investigated. For condensed tannins, ten samples of mimosa (*Acacia mearnsii*) and eight of quebracho (*Schinopsis lorentzii*) were analysed. For hydrolysable tannins, eight samples of tara (*Caesalpinia spinosa*), nine of chestnut (*Castanea sativa*), seven of myrobalan (*Terminalia chebula*), and five of valonea (*Quercus aegilops*) were analysed. The extracts were provided by Tanac (Brazil), Silvateam

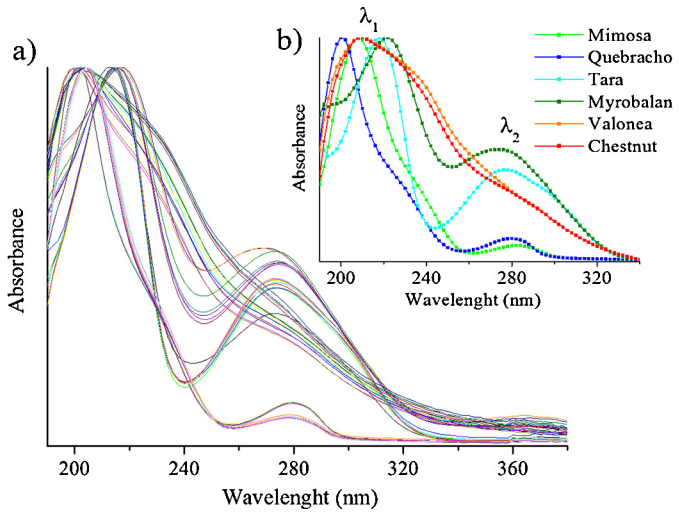

Fig. 1. Ultraviolet absorption spectra of vegetable tannin extracts. a) All ultraviolet spectra analysed. b) Average spectrum analysed.

(Argentina), Exandal (Peru), Tannin Sevnica (Slovenia), Samrat Chemical (India), and Air-tu Kymia (Turkey).

2.7. Multivariate analysis

The software Matlab 7.11 (MathWorks Inc., USA) and PLS-TOOLBOX 6.2.1 (Eigenvector Research Inc., USA) were used (Wise et al., 2006). Before multivariate analysis, the ultraviolet spectra were smoothed (1nd polynomial and 5 points per window) and normalised (inf-Norm, maximum = 1). Hierarchical cluster analysis (HCA) (Brereton, 2003) and principal component analysis (PCA) (Wold et al., 1987), were used for multivariate analysis of the ultraviolet spectra. For PCA in addition to the previous data, it was also mean-centred.

3. Results and discussion

3.1. Ultraviolet spectrometry

Fig. 1 shows the ultraviolet absorption spectra relating to polyphenolic extracts.

The vegetable tannin extracts, even with structures with molecular weight up to 3000 Da, absorb at lower wavelengths compared with other smaller molecules. This is due to the fact that the main chromophore groups of these extracts are aromatic rings, which are subject to change in the absorption spectrum according to the auxochrome groups present. Aromatic rings do not have large electronic displacement, resulting in a shifted absorption for the ultraviolet region (Fig. 1). Analysing the absorption spectra of the extracts, tare, myrobalan, quebracho and mimosa resulted in two absorption bands, while valonea and chestnut presented only one band broader. The first group presented an absorption maximum in the region of 274–282 nm and a more intense absorption between 200–216 nm. The valonea and chestnut only have an absorption maximum at 210 nm which extends to 340 nm, possibly due to a signal overlap of the different structures present in these extracts. The maximum absorption results for each vegetable extract are reported in Table 1.

The quebracho and mimosa, both condensed tannins, have very similar profiles of absorption spectra. These two extracts feature the most intense absorption band (λ_1), shifted to shorter wavelengths than the remaining extracts, 200 nm for quebracho and, 208 nm to mimosa. For absorption bands at longer wavelengths (λ_2), the quebracho absorbs at 280 nm, and the mimosa absorbs at 282 nm, with the lowest intensities of all the extracts examined. The results of absorption at most wavelengths are similar to those reported in the literature for quebracho and mimosa, 279 and 281 nm, respectively. In the analysis of hydrolysable tannins, tare and the myrobalan also are very similar in the absorption spectrum (Fig. 1). The band of highest intensity absorption (λ_1) is shifted to longer wavelengths than the remaining extracts, 214 and 216 nm for the tare and myrobalan, respectively. The absorption band at highest wavelengths (λ_2) for both extracts is located at 274 nm, which was more intense than in the other extracts in this region. The literature presents for the tare, λ1 of 213–215 nm and λ2 of 273–274 nm, and results found are in accordance with these (Falcão and Araújo, 2013). The extracts with greater similarity on the absorption spectrum were the chestnut and valonea, where the absorption spectra were practically overlapping (Fig. 1). Studies carried out by MALDI-TOF-MS (Ozgunay et al., 2007; Véras et al., 2012) and FTIR (Grasel et al., 2016) demonstrated that chestnut and valonea have similar structural conformation, except for the arrangement of three-dimensional polymer structures. These two extracts absorb at 210 nm with an extension up to 340 nm, probably due to structural diversity. The results for the valonea were in accordance with those cited in the literature (212 nm) (Falcão and Araújo, 2013).

Table 1
Spectroscopic data for the absorption maxima obtained by ultraviolet spectroscopy.

Polyphenolic	λ_1	λ_2
Extract	nm	nm
Quebracho	200	280
Mimosa	208	282
Tara	214	274
Myrobalan	216	274
Chestnut	210	–
Valonea	210	–

3.2. Specific absorptivity and active material determination of tannin extracts

The specific absorptivity and active material determination of tannin extracts are shown in Table 2. Specific absorptivity is an intrinsic property of the substance which is measured by its ability to absorb light at a given wavelength. For pure substances this property is called the molar absorptivity, but for complex substances are called the specific absorptivity, where the concentration is determined in $g\,L^{-1}$ instead of $mol\,L^{-1}$. The lowest values of specific absorptivity were for extracts of condensed tannins being $8.6\,L\,g^{-1}\,cm^{-1}$ for mimosa and $12.8\,L\,g^{-1}\,cm^{-1}$ to Quebracho. For hydrolysable tannins, the lowest values were for the myrobalan ($16.3\,L\,g^{-1}\,cm^{-1}$) and tara ($24.9\,L\,g^{-1}\,cm^{-1}$) and the highest values for the valonea ($50.0\,L\,g^{-1}\,cm^{-1}$) and chestnut ($63.1\,L\,g^{-1}\,cm^{-1}$). At analysis of total polyphenols, the condensed tannins showed the highest percentage, 90% and 80% for the quebracho and mimosa, respectively. Hydrolysable tannins were in the range 60–67%, with the lowest results for the myrobalan. The determination of tannin content by filter analysis consists in determining the content of active material capable of reacting with skin collagen. The insoluble matter is the content of the extract which lacks the ability to penetrate the skin during the tanning process. This analysis is widely used for tanning industry to determine the percentage of active material in tanning extracts for the transformation of hides into leather. All insoluble contents were below 2.35% except tara and myrobalan, who had percentages above 30%. This is due the fact that the fruits of the tara and myrobalan are marketed in micronized form and not extracted in aqueous solution as the others, thus resulting in a higher percentage of insoluble.

Table 2
The specific absorptivity and active material determination of tannin extracts.

Extracts	Tannin content (%)	Insolubles (%)	Total polyphenols (%)	Specific absorptivity ($L g^{-1} cm^{-1}$)
Chestnut	78.47 ± 1.04	2.35 ± 0.61	66.97 ± 0.95	63.13 ± 0.62
Mimosa	77.00 ± 0.71	1.85 ± 0.10	80.04 ± 2.48	8.17 ± 0.30
Myrobalan	41.22 ± 1.16	47.17 ± 1.57	59.80 ± 1.83	16.34 ± 0.15
Quebracho	81.72 ± 1.24	2.08 ± 0.41	89.89 ± 1.70	12.79 ± 0.11
Tara	55.75 ± 0.67	30.02 ± 0.68	64.15 ± 2.07	24.92 ± 0.34
Valonea	74.08 ± 0.84	1.53 ± 0.34	69.21 ± 2.65	49.97 ± 0.21

Table 3
Variance explained by the principal components (PCs) obtained by decomposition of data using principal component analysis (PCA).

PC number	Variance (%)	Cumulate Variance (%)
1	76.87	76.87
2	21.58	98.45
3	1.02	99.47
4	0.32	99.79
5	0.12	99.91
6	0.04	99.96
7	0.03	99.98
8	0.01	99.99

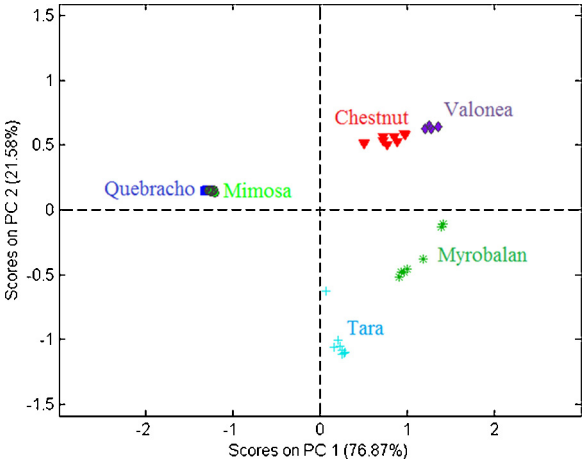

Fig. 2. Score plots of principal component 1 versus principal component 2.

In the evaluation of tanning percentage, the quebracho, chestnut and mimosa presented a percentage around 80%, followed by valonea, tara and myrobalan, 74, 56 and 41%, respectively. The tara and myrobalan have the lowest percentages due to contain higher fraction of insoluble.

3.3. Multivariate analysis

3.3.1. Principal component analysis

Table 3 presents the results of the decomposition of the spectral data through PCA. The first column indicates the principal component (PC) number, the second column indicates the percentage of variance explained by that PC, and the third indicates the accumulated variance (the sum of the percentage of variance explained by that PC and the preceding one). It can be seen that with three PCs, 99.47% of the total variance of the data was accumulated.

Fig. 2 shows the graph of the PC1 scores (76.87%) versus those of PC2 (21.58%).

Fig. 2 shows that PC1 separates the mimosa and quebracho (negative scores) from the tara, myrobalan, chestnut, and valonea (positive scores), the former being condensed tannins and the latter hydrolysable tannins. Principal Component 2 separates the tara and myrobalan (negative scores) from the quebracho, chestnut, mimosa, and valonea (positive scores).

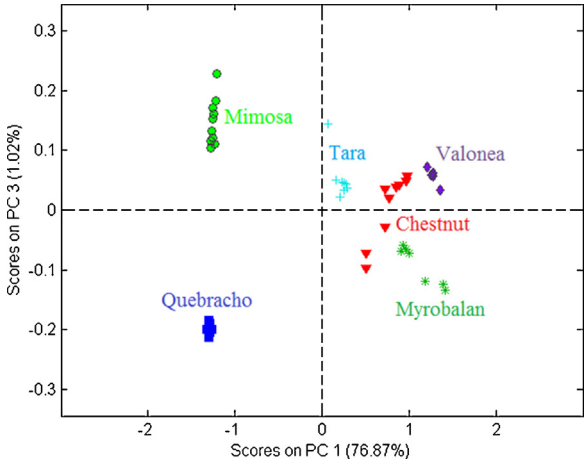

Fig. 3. Score plots of principal component 1 versus principal component 3.

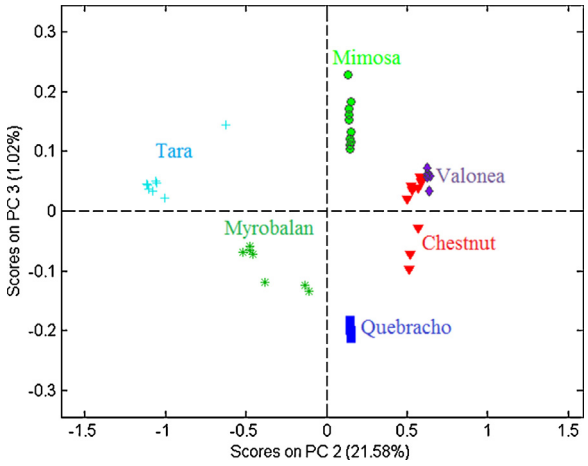

Fig. 4. Score plots of principal component 2 versus principal component 3.

Once the values of scores for mimosa and quebracho are very close to zero at PC2, the main contribution of this PC is the necessary information to characterise differences between the group formed by chestnut and valonea and group formed by tare and myrobalan samples.

Fig. 3 shows the graph of the PC1 scores (76.87%) versus those of PC3 (1.02%).

Fig. 3 shows that PC3 separates the myrobalan, and quebracho (negative scores) from the mimosa, tara, and valonea (positive scores). In PC3, the chestnut was split between positive and negative scores. It is noteworthy that the PC3 has information representing the separation of mimosa and quebracho. Fig. 4 is a graph of the PC2 scores (21.58%) versus those of PC3 (1.02%).

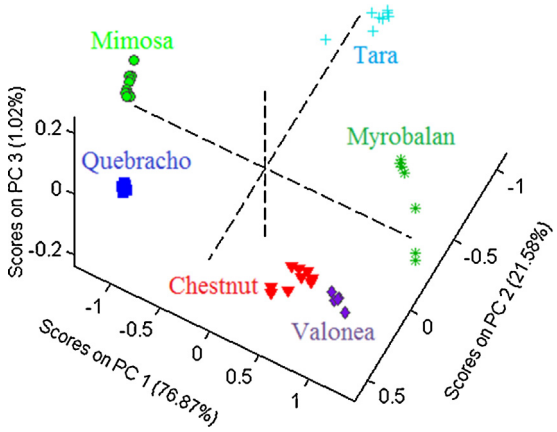

Fig. 5. Three-dimensional score plots of principal component 1 × principal component 2 × principal component 3.

Fig. 4 confirms what is seen in Figs. 2 and 3; however, there is evidence that both PC2 as PC3 are important components for the differentiation between different tannins whether these are hydrolysable or condensed. Fig. 5 shows the graph of the PC1 × PC2 × PC3 in 3D. It can be seen that the chestnut and the valonea extracts exhibited greater difficulty of separation with the first three PCs. According to the UV–vis analysis, these two extracts showed greater structural similarity, which agreed with reports in the literature. Ozgunay et al. (2007) investigated the chemical structure of valonea with MALDI-TOF and FTIR spectroscopy. The authors concluded that valonea has a similar structural configuration as chestnut tannins; however, in Pash and Pizzi's study results, an exception is that the chestnut tannin has tri-dimensional macromolecular chains (Pasch and Pizzi, 2002).

Fig. 6 shows the loadings of the PC1, PC2, and PC3 graphics. It is important to emphasise that the formation of groups, as well as their separation as seen in Fig. 5, are directly related to the signals observed in their loadings. Although very similar the spectra of Fig. 1, the loadings of the PCA allow identification of the small displacements of these signs between the extracts, due to the small structural differences they present.

At analysis of PC1 with negative values of scores are the condensed tannins (quebracho and mimosa) and positive values, the hydrolysable tannins (tare, myrobalan, valonea and chestnut). Based on the region of highest intensity in absorbency of the extracts (λ_1) in Fig. 6a, the mimosa and the quebracho absorb at wavelengths shorter than the others, with the unique signal at 200 nm (showing negative loading) that distinguishes condensed and hydrolysable tannins. At PC2, the positive scores are chestnut and valonea and negative scores tare and the myrobalan, which are the hydrolysable tannins (Fig. 2). The region of more intense absorptivity of the extracts (λ_1) is from 190 to 220 nm. Around 200 nm (Fig. 6b), signals with positive loadings refer to extracts that absorb at lower wavelengths (chestnut and valonea), and at around 215 nm (with negative loadings) are the characteristic signals of extracts that absorb larger ones (tare and the mirabolano). At 235 nm (with positive loadings), signs of absorption shoulders can be seen on the mimosa, quebracho, chestnut and valonea. In this region at higher wavelengths, the signal of tare and mirabolano is at 280 nm (negative loading), being extracts that absorb more strongly in this region. As already discussed, the main information provided by the PC3 is the separation between samples of mimosa and quebracho. In the quebracho samples, the λ_1 is below 200 nm with negative loadings (Fig. 6c), and in the samples of mimosa, the λ_1 is above 200 nm with positive loadings. With negative loadings

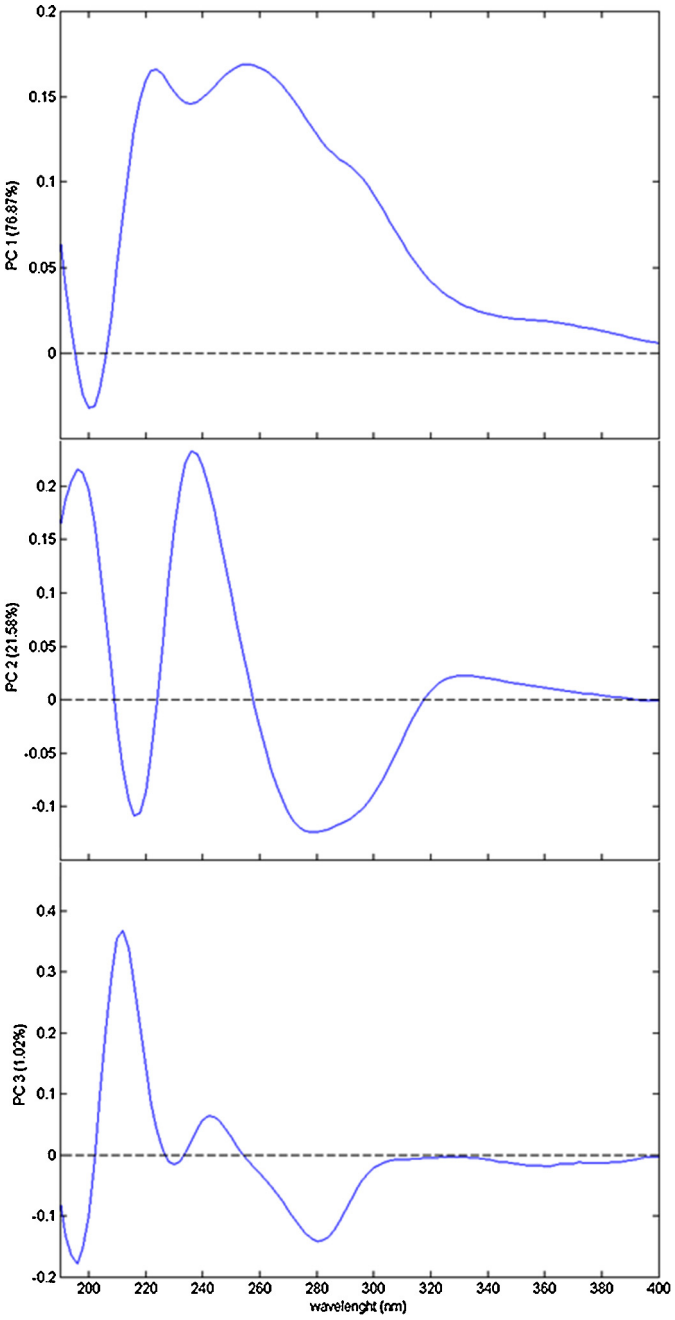

Fig. 6. Loading plot of principal component 1 (a), principal component 2 (b) and principal component 3 (c).

at 280 nm, the λ2 signal for quebracho is more intense than the mimosa samples, as shown in Fig. 3.

3.3.2. Hierarchical cluster analysis

In order to confirm the results obtained by PCA, HCA was performed to provide a clearer view of the similarities and differences in the extracts studied. The dendrogram obtained is shown in Fig. 7.

Analysis of the top-down dendrogram shows the group of condensed tannins in light green followed by blue. The condensed tannins are mainly composed of flavonoids, which are represented by the mimosa extract (light green) and the quebracho extract (blue). Continuation of the analysis shows another large group that is divided into two. Within the first subgroup, the tara (cyan) and myrobalan (dark green) can be seen. In the second subgroup, in orange and red, the upper part is valonea and the bottom is chest-

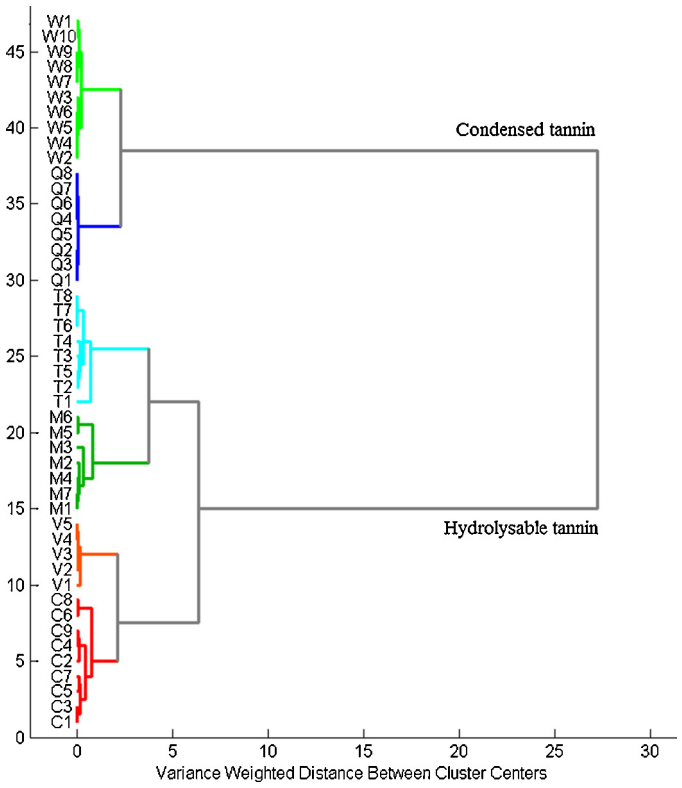

Fig. 7. Dendrogram of ultraviolet spectra of tanning extracts. Mimosa (W). Quebracho (Q). Tara (T). Myrobalan (M). Valonea (V). Chestnut (C).

nut. Although the ultraviolet spectroscopy presents information as more simplified when compared with other molecular techniques such as FTIR and Raman, the HCA clearly separated the valonea and chestnut samples.

Falcão and Araújo (2013) developed a methodology using two molecular spectroscopic techniques, ATR-FTIR and UV–vis, and three specific chemical tests to identify different tannins. The technique developed by authors depends largely on the sensitivity of the analyst in interpreting the ATR-FTIR and UV–vis spectra, without mentioning that chemical tests. In our methodology we use only molecular spectroscopic techniques UV–vis associated with multivariate analysis. The methodology proposed is much more accurate, without requiring ATR-FTIR and specific chemical tests analysis to identify different tannins. Our methodology depends only of the UV–vis analysis and statistical treatments carried out correctly. Regardless of the analyst who performs the analysis, considering that the software and the equipment of UV–vis are working correctly, the results will be always equals.

4. Conclusions

In this study, 47 samples of vegetable tannin extract, representing six commercially available types, were analysed by ultraviolet and multivariate analyses. The ultraviolet technique is one of the fastest and cleanest techniques. It requires no reagents or pretreatment of the sample, and it proved useful in the differentiation of the extracts, as it provided information on the main absorption bands, which are related to chemical composition and structure. In addition, also it was determinated a specific absorptivity and quantified the total polyphenols and tannin content of each extract by filter and Folin Ciocalteu methods, respectively. The lowest values of specific absorptivity were for extracts of condensed tannins being 8.6 $L\,g^{-1}\,cm^{-1}$ for mimosa and 12.8 $L\,g^{-1}\,cm^{-1}$ to Quebracho. For hydrolysable tannins, the lowest values were for the myrobalan (16.3 $L\,g^{-1}\,cm^{-1}$) and tara (24.9 $L\,g^{-1}\,cm^{-1}$) and the highest values for the valonea (50.0 $L\,g^{-1}\,cm^{-1}$) and chestnut (63.1 $L\,g^{-1}\,cm^{-1}$). At analysis of total polyphenols, the condensed tannins showed the highest percentage, 90% and 80% for the quebracho and mimosa, respectively. Hydrolysable tannins were in the range 60–67%, with the lowest results for the myrobalan. At filter analysis, all extracts showed insoluble contents below 2.35% except tara and myrobalan, who had percentages above 30%. In the evaluation of tanning percentage, the quebracho, chestnut and mimosa presented a percentage around 80%, followed by valonea, tara and myrobalan, 74, 56 and 41%, respectively. At multivariate analysis, a well-defined separation between condensed and hydrolysable tannins can be seen through both PCA and HCA. Among the hydrolysable tannins, the chestnut and valonea samples showed the greatest similarity, indicating that these extracts have equivalent chemical compositions and, therefore, similar properties. The ultraviolet technique and associated multivariate analysis were effective in identifying the nature of the polyphenolic extracts of chestnut, myrobalan, quebracho, tara, valonea, and black wattle used in this study.

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