Industrial Crops and Products 91 (2016) 279–285

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

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

ARTICLE INFO

Article history:
Received 11 March 2016
Accepted 19 July 2016
Available online 26 July 2016

Keywords:
Ultraviolet
Vegetable tannins
Multivariate analysis
Principal component analysis
Hierarchical cluster analysis

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).

http://dx.doi.org/10.1016/j.indcrop.2016.07.022
0926-6690/

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