

MOLECULAR INVESTIGATION OF VALONEA TANNIN

by

H. OZGUNAY¹, O. SARI¹ AND M. TOZAN²

¹Ege University Faculty of Engineering Department of Leather Engineering
35100 BORNOVA-IZMIR - TURKEY

²TFL Deri Teknolojisi San. ve Tic. A. S.
34957 TUZLA-ISTANBUL - TURKEY

ABSTRACT

Valonea is one of many well-known vegetable tannins used in tanning and retanning processes for leather making. Although it is used extensively in the leather industry, its main components and chemical structure are only gradually being identified. In order to investigate the chemical structure of valonea with novel techniques, MALDI-TOF (Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight) and FTIR (Fourier Transform Infrared) Spectroscopy were used. The MALDI-TOF spectrum showed the presence of low molecular weight fraction of hydrolysable tannins such as nonahydroxytriphenoic, flavogallonic acid, ellagic and gallic acid, pentagalloylglucose and all sorts of degradation and oxidation products which are thought to be major components of valonea tannin. Moreover, castalagin/vescalagin and those added by a few residual structures/atoms, thus creating larger molecule and greater mass than castalagin/vescalagin, derived by internal rearrangements of a larger molecule constitute major components of valonea.

ABSTRACTO

Valonea es uno de los taninos vegetales más conocidos, utilizado en curtido y reurtido en la fabricación del cuero. Aunque es extensamente utilizado en la industria del cuero, sus componentes principales y las estructuras químicas apenas están gradualmente siendo identificados. Para poder investigar la estructura química de la Valonea por medio de técnicas nuevas, MALDI-TOF (Desabsorción/Ionización de la Matriz Tiempo-en-Ruta Asistida por Láser) y Espectroscopia FTIR (Transformación de Furrier en Infrarrojo) fueron utilizadas. El espectro MALDI-TOF demostró la presencia de fracciones de bajo peso molecular de taninos hidrolizables como nonohidroxitriprnóico, ácido flavogalónico, ácidos elágico y gálico, pentagaloglucosa y todo tipo de

productos de la degradación y oxidación tal y cual son considerados como componentes principales del tanino de la Valonea. Más aun, castalegina/vescalegina, y aquellos residuos añadidos por unas cuantas estructuras/átomos, creando una molécula más grande y con más peso que las de castalegina/vescalegina, y derivada de reestructuraciones internas en moléculas más grandes, constituyen los principales componentes de la valonea.

INTRODUCTION

The leather industry is looking at options for metal-free tanning systems and especially tanning materials based on natural products such as vegetable tannins, which have gained in importance. It is known that plants synthesize different polyphenolic substances, some of which may contribute to the formation of tannins. Tannins are one of the many types of secondary compounds found in plants and widely distributed throughout the plant kingdom¹.

Vegetable tannins are one of the oldest materials used for tanning hides and skins. White² defined the term tannin as the substance which converts the putrefiable hide or skin into imputrifiable leather. Probably the most acceptable and simple definition for tannins is that of Bate-Smith and Swain: "water soluble phenolic compounds having molecular weights between 500-3000 and, besides giving the usual phenolic reactions, processing special properties such as the ability to precipitate alkaloids, gelatin and other proteins"³.

According to their chemical nature and structural characteristics vegetable tannins are subdivided into two groups⁴:

- Condensed tannins
- Hydrolysable tannins

Chestnut, tara, valonea, sumac, divi diva (*sic*), algarobilla and myrabolans are the most commonly used hydrolysable tannins in the leather industry.

Valonea tannin, obtained from tannin rich fruits of the acorn cups of the *Quercus* species, has been used extensively to tan

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* Corresponding Author - E-mail: hasan.ozgunay@ege.edu.tr

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hide and skin either alone or accompanied by other tanning agents in the Turkish leather industry. Besides giving excellent firmness and fullness and better light fastness and lower water absorption properties to leather than many other vegetable tannins, the use of valonea in the tanning process presents some problems such as sludge formation in the process pits, high astringency of tanning, and unfavorable color of leather⁵.

The TEMA Foundation (a non-profit organization dedicating itself to the reforestation and protection of natural habitats in Turkey) has started a campaign to plant planting 10 billion oak trees including the tannin rich *Quercus* species all over Turkey. When the project is completed, huge amounts of raw materials will be available for valonea extraction. For this reason, a research project has been started to improve the properties of valonea in order to extend its use to the worldwide tanning industry.

The first part of this project aims to investigate valonea tannin at the molecular level by using the rather novel technique called matrix-assisted laser desorption/ionisation-time-of-flight mass spectrometry (MALDI-TOF-MS) and FTIR spectroscopy.

EXPERIMENTAL

Material

Acorn cups and beards used for extraction were obtained from the fruits of oak trees growing around Salihli-Manisa, Turkey.

Method

Extraction

A dried crude sample (100 g cups and beards) was cut into small pieces. Then the crude sample was placed in a Koch extractor and extracted with deionized water at 70°C in 4 hours. The extract was concentrated under pressure at 50°C. The concentrated extract was dried by using a LAB-PLANT SD-04 spray drier.

MALDI-TOF-MS

The sample was dissolved in acetone (4 mg/ml). Then the sample solution was mixed with an acetone solution (10 mg/ml) of the

matrix, for which 2,5 dihydroxy benzoic acid was used. The solutions of the sample and the matrix were mixed in equal amounts and 0.5 to 1 µl of the resulting solution was placed on the MALDI target.

After evaporation of the solvent, the MALDI target was introduced into the spectrometer. The spectra were recorded on a Kratos Kompact MALDI 4 instrument (Kratos Analytical Instruments, Ramsey, NJ). The irradiation source was a pulsed nitrogen laser with a wavelength of 337nm. The length of one laser pulse was 3 ns. The measurements were carried out using the following conditions: positive polarity; linear flight path; high mass (20 kV acceleration voltage); 100-150 pulses per spectrum. The delayed extraction technique was used, applying delay times of 200-800 ns⁶.

FTIR spectrophotometer

FTIR spectra were recorded on a Perkin Elmer-Spectrum Bx FT-IR System spectrophotometer, in a matrix of KBr (0.5-1 mg tannin sample in 100-200 mg dried KBr).

RESULTS AND DISCUSSION

From the spectrum obtained for the valonea extract, mass peaks for ellagic acid at 326-Da, flavogallonic acid at 492-Da and nonahydroxytriphenoic acid at 523-Da were found in the spectra. These chemical species are characteristic of the low molecular weight fraction of hydrolysable tannins as cited before by Tang et al⁷. In addition, mass increments of roughly 170-Da, 302-Da and 471-Da were found in the spectra. (see Fig.1-4).

Evaluation of some of the mass peaks obtained from the valonea MALDI spectrum showed us that 655, 673 and 769 peaks are respectively obtained from the 959-Da, 976-Da and 1071-Da peaks by loss of an ellagic acid structure. The peaks at 959 and 976 are, respectively, the 935-Da castalagin or pentagalloylglucose to which has been added a C-O-C grouping from a split off (976) (Figure 5) and a 935-Da to which, again, a C-O-C remains attached and an -OH has been subtracted (959).

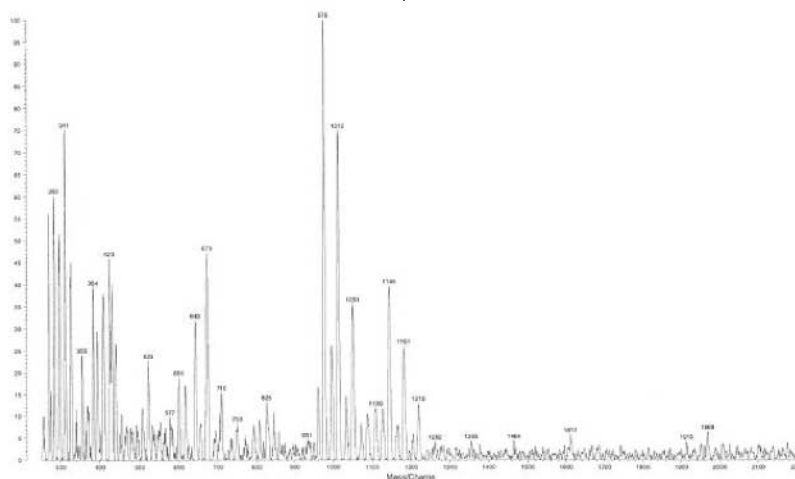


Figure 1. MALDI mass spectrum of valonea

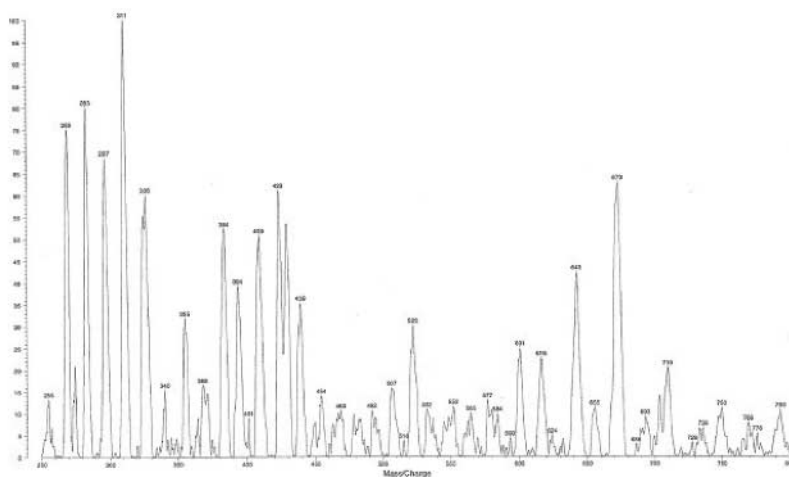


Figure 2. Details of the 250-800 Da range of valonea mass spectrum

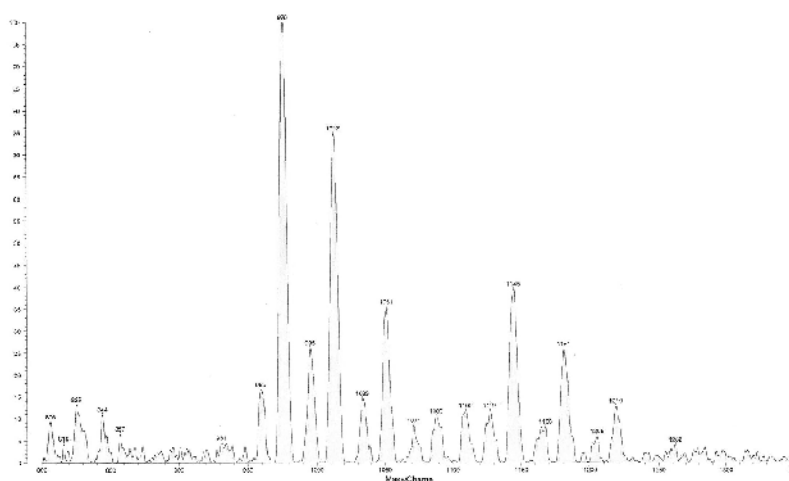


Figure 3. Details of the 800-1350 Da range of valonea mass spectrum

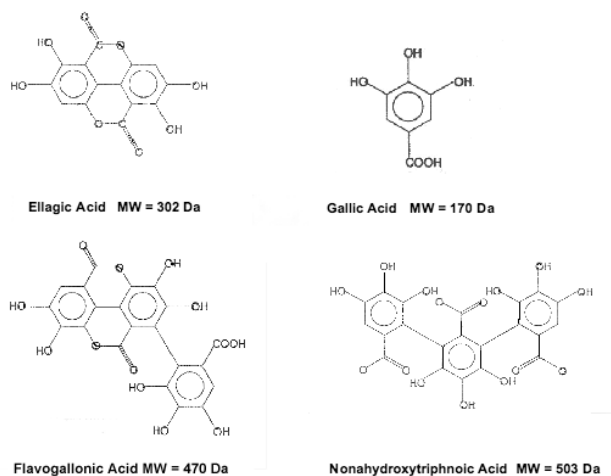


Figure 4. Low molecular weight fragments of valonea tannin extract

Another major peak at 1012 is thought to correspond to the structure of a glucose core holding a favogallonic acid (470-Da), an ellagic acid (302-Da) and a gallic acid. The 1088 peak corresponds to either a castalagin (935-Da) structure added by a gallic acid residue that esterifies the only free alcoholic -OH group of the structure.

The 1071 peak is the same as the 1088 peak by the loss of an -OH group. The 1109-Da peak is the same as the 1088 peak but more likely presents an -OH group. The 1127 peak would be vescavaloneicacid/ castavaloneicacid⁸ (Figure 6). The peak 1219 corresponds to either a castalagin or a vescalagin (935-Da) structure having been added to by an ellagic acid (302-Da) with loss of an -OH group from the structure.

Evaluation of the findings showed us that valonea has a similar structural configuration with chestnut tannin, considering Pash and Pizzi's study results, except that chestnut has tridimensional macromolecular chains⁶. This means that pentagalloylglucose, castalagin/vescalagin and their rearranged derivatives are held to be one of the main constituents of valonea extract.

FTIR spectroscopy

As is known, FTIR is used to determine characteristics of a compound which depend on its functional groups appearing in the FTIR fingerprint region. As pointed out by Nakagawa and Sugita it is believed that characterization of vegetable tannins by spectroscopy stands a good chance of success and in

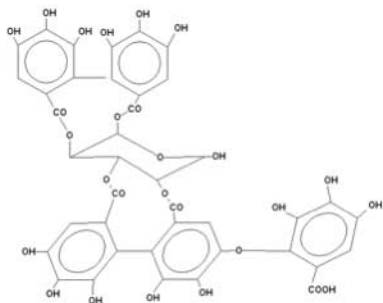


Figure 5. One of the main constituent structures of valonea (959 and 976-Da)

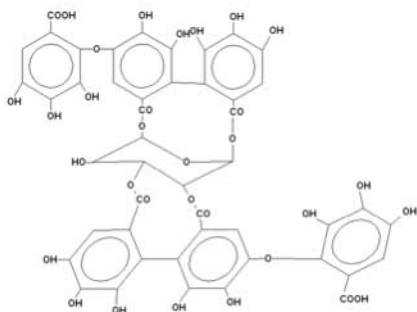


Figure 6. Vescavaloneicacid/ Castavaloneicacid8 (1127-Da)

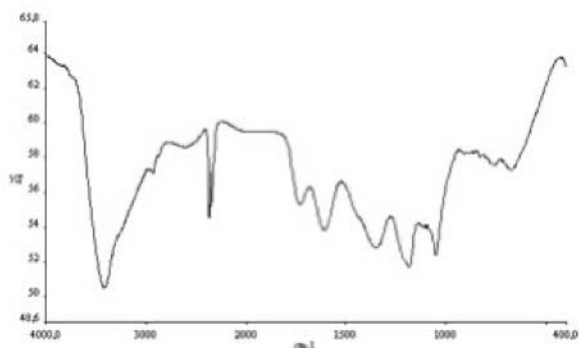


Figure 7. FTIR spectrum of valonea tannin extract

the study results they carried out, they cited that FTIR spectra of each tannin showed characteristic absorption patterns, which makes it possible for us to characterize each tannin⁹.

From evaluation of FTIR spectra of valonea presence of -OH at 3420 cm⁻¹, C=C at 1610 cm⁻¹, C=O at 1734 cm⁻¹, CH₂ at 2939 cm⁻¹, C-OH at 1340 cm⁻¹, C-O-C at 1185 cm⁻¹ and ester bonds at 1045 cm⁻¹ are detected (Fig.4). From the results it is clearly seen that valonea tannin has a very complex structure.

CONCLUSION

Upon evaluation of the results obtained from MALDI spectra of valonea, it is clearly seen that valonea has the same chemical species as is characteristic of the low molecular weight fraction of other hydrolysable tannins as cited before by Tang et al. Most of these low molecular weighted structures are thought to be extraction induced degradation products of valonea tannin. Higher molecular weight structures detected in valonea especially pentagalloylglucose and all sorts of degradation and oxidation products of it are thought to be major components of valonea tannin. Some of the other molecular structures determined in tannin are thought to be related with internal rearrangements of degradation products with higher molecular structures. But most of these structures are indeed castalagin/vescalagin ones added to by a few residual structures/atoms, thus creating larger molecules and greater mass than castalagin/vescalagin, derived by internal rearrangements of a larger molecule. Additionally, by the FTIR spectrum of the valonea it is detected that valonea has a complex structure having -OH at 3420 cm⁻¹, C=C at 1610 cm⁻¹, C=O at 1734 cm⁻¹, CH₂ at 2939 cm⁻¹, C-OH at 1340 cm⁻¹, C-O-C at 1185 cm⁻¹ and ester bonds at 1045 cm⁻¹ holding the structure.

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