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IDENTIFICATION OF OENOLOGICAL TANNINS EXTRACTED FROM OAK WOOD

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ABSTRACT
Our study is dealing with the determination of the origin of oenological tannins using the modified OIV procedure. The method is based on HPLC analyses of proanthocyanidol content (catechin, epicatechin, epigallocatechin, epicatechin-3-O-gallate and epigallocatechin-3-O-gallate) after thioacidolysis - thiolytic cleavage of the flavonol intermonomer linkages in proanthocyanidols under heat in an acid medium. The purpose of the study was to differentiate oenological tannins gained from Quebracho cortex and tannins extracted from oak wood. As the results show, both groups of tannins do not produce any flavan-3-ols after thioacidolysis, but they show specific peaks, which enable determination of their origin.

Keywords: oenological tannin, thioacidolysis, oak wood, Quebracho, HPLC

INTRODUCTION
Oenological tannins are presented as a group of food additives that are extracted from different vegetable materials and are used in winemaking practises **(Vivas, 1997)**.
They are used to facilitate the clarification of wines and musts. The main use of oenological tannins is to eliminate unstable proteins and modify some organoleptic properties in wines (colour stabilisation in red wines, astringency and bitterness) **(Lurton et al., 2002)**. They are used to ensure wine palate balance and complexity. International organization of Vine and Wine (OIV) approved the use of enological tannins as fining agents for white wines. However, enological tannins also serve other applications **(Bautista-Ortín et al., 2007)**. They can be used to inhibit laccase in Botrytis-infected grapes **(Obreque-Sliér et al., 2009)**.

Tannins are primarily derived from the seeds and skin of the fruit during winemaking. As a result, wines made with little or no skin contact such as white and sparkling wines have low tannin levels, while red wines that are made with periods of skin contact ranging from a few days to several weeks can have quite variable tannin concentrations **(Harbertson et al., 2008)**.

In chemical terms, tannins are relatively bulky phenol molecules, produced by the polymerization of elementary molecules with phenolic functions **(Ribéreau-Gayon et al., 2006)**. The chemical composition of tannins changes notably with its botanical origin and the nature of the tissues **(Vivas et al., 2004)**. They consist of polyphenolic fractions belonging to different chemical classes of tannins, namely condensed tannins which are composed of flavan-3-ol monomer subunits, such as catechin, epicatechin and their gallates, prepared from grapes (seeds and skins) and quebracho wood; and hydrolysable tannins, such as gallotannins consisting of a central glucose molecule substituted with gallic acid fraction, from exotic wood and ellagic tannins, as gallic acid dimers, prepared from oak and chestnut materials **(Vivas, 1997; Haslam, 1998)**. Traditional source of hydrolysable tannins is the oak barrels where the wine is kept during the ageing process. Some tannin preparations are relatively pure extracts from single species, while others are mixtures from several species and may include both hydrolysable and condensed tannins **(Obreque-Slíer et. al., 2009)**.

Different chemical composition of tannins leads to differences in their chemical and biological activity, what requires the analytical characterisation of the enological tannins **(Laghi et al., 2010)**. A wide spectrum of oenological tannins is now available on the market, classified mainly according to the oenological properties. However, the tannins' chemical nature is not always clearly defined, and it is not always possible to know their botanical origin **(Obradovic et al., 2005)**. From an economical and technological point of view, it is important to know the differences among commercial tannins and to verify the information presented by suppliers **(Obreque-Sliér et al., 2009)**. In this study our point of view is identification and distinction between Quebracho and Oak wood tannins.

MATERIAL A METHODOLOGY
Tannin samples
Firstly, tannin extractions from different oak chips (Quercus robur, Quercus robur/petraea, Quercus alba) and Quebracho bark (Aspidosperma quebracho blanco) were prepared. 0,1 g of oak chips or quebracho was introduced into 10 ml methanol. After 24 hours, solutions were filtered and analysed using thioacidolysis and HPLC. Thereafter 21 preparations of oenological tannins from five suppliers available at Austrian market were analysed and their chromatograms were compared with chromatograms of oak wood- and quebracho extracts.
Chemicals
For our analyses we used: HPLC grade methanol (J.T.Baker, Deventer, Netherlands), distilled water, MilliQ water (device from TKA, Germany), formic acid (Merck, Darmstadt, Germany), toluene -α-thiol (CAS 100-53-8)

99 % (Sigma-Aldrich, St. Louis, USA), hydrochloric acid (12M) 37 % (Merck); standards: (+)-catechin, (-)-epicatechin (Sigma-Aldrich, USA), (-)-epigallocatechin, (-)-epicatechin gallate (Extrasynthese, Genay, France), epigallocatechin gallate (Roth, Karlsruhe, Germany), gallic acid and ellagic acid (Sigma-Aldrich, USA); tannin-methanol solutions with concentration 1 $g.l^{-1}$, 10mg tannin was introduces in 10 ml methanol.

Thioacidolysis

Thioacidolysis of tannin preparations was performed according to modified O.I.V. (2010) differentiations method for proanthocyanidin tannins by HPLC.

Thioacidolysis is a selective acidic depolymerisation method using a thiol as a nucleophilic agent for gaining monomeric composition and discrimination of polymeric proanthocyanidis. Condensed tannin is heated with toluene-α-thiol (benzyl mercaptan), which releases the terminal unit as a flavan-3-ol, while the extension units are released as toluene-α-thiol derivatives **(Rigaud et al., 1991; Matthews et al., 1997; Pash et al., 2001)**. 1 ml tannin-methanol solution and 1 ml thioacidolysis reagent (470 µl toluene-α-thiol introduced into hydrochloric acid solution - 140 µl 12M HCl in 10 ml methanol) were mixed together in a hydrolysis tube. The mixture was stirred and heated at 60 °C for 10 minutes. The tube was than cooled with air. After cooling 1 ml distilled water was added. Mixture was than analysed by HPLC.

HPLC analysis

The samples after thioacidolysis were analysed on HP system series II 1090 AminoQuant with DAD (Hewlett Packard, USA). Separation was performed by column LiChrospher 100, RP-18, 250 x 4 mm, 5 µm (Merck, Darmstadt, Germany), what was changed in comparison with original O.I.V. (2010) method. Mobile phase we used was also changed; instead of phosphoric acid we used 1% formic acid in MilliQ water as solution A and 1% formic acid in methanol as solution B. Separation was led by 40 °C in 55 minutes by following modified gradient: concentration of B solution started at 5 %, than it was lead from 5 % to 10 % in 14 minutes, from 10 % to 30 % in 20 minutes, from 30 % to 90 % in 6 minutes, than 10 minutes at 90 %, finally in 5 minutes returned back to 5 %, post run time was running 15 minutes. Flow rate was constant and the same as in original method: 1 ml/min. Samples were measured by wavelength 280 nm and injection volume 20 µl.

RESULTS AND DISCUSSION

Before analyses of commercial tannin preparations, standard solutions of catechin, epicatechin, epigallocatechin, epicatechin gallate and epigallo-catechin-3-gallate, gallic acid and ellagic acid were analysed. Except of standards, two more peaks (Figure 1) appeared on chromatogram as benzyl-thioether compounds and reagent residue, after thioacidolysis.

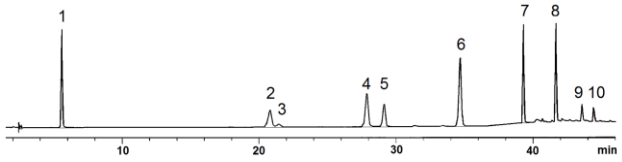

Fig. 1 Standard solution after thioacidolysis: 1-gallic acid, 2-catechin, 3-epigallocatechin, 4-epigallocatechin gallate, 5-epica-techin, 6-epicatechin gallate, 7-ellagic acid, 8 and 10 benzyl-thioether compounds, 9-reagent residue.

We could not identify each peak on samples' chromatograms, whereas we used HPLC system without Mass Spectrometry. In spite of this disadvantage we could recognize the origin of tannin preparations according to presence or absence of single peaks and overall features of chromatogram. Some peaks are namely specific only for definite botanical origin. Therefore the calibration of standards was not required.

According to **Vivas et al. (2004)**, tannins obtained from quebracho do not contain any flavan-3-ols as it is by grape tannins.

According to our results tannin samples acquired from quebracho bark offered typical chromatogram (Figure 2), without any proanthocyanidin or prodelphinidin compounds but we found out another specific peak at retention time between 38th and 39th minute.

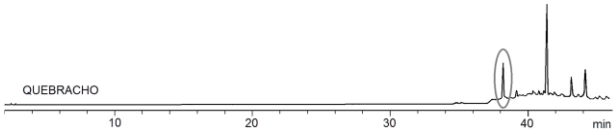

Fig. 2 Chromatogram of **que-bracho** tannin with specific peak.

Tannins extracted from oak wood show similar feature as quebracho tannins, they do not contain any proanthocyanidins as well. Difference was noticed in presence of gallic and ellagic acid. In addition, tannins obtained from toasted oak wood show specific double peak at retention time 44 minutes (Figure 3). For not toasted and medium toasted oak wood distinctive initial peak was typical.

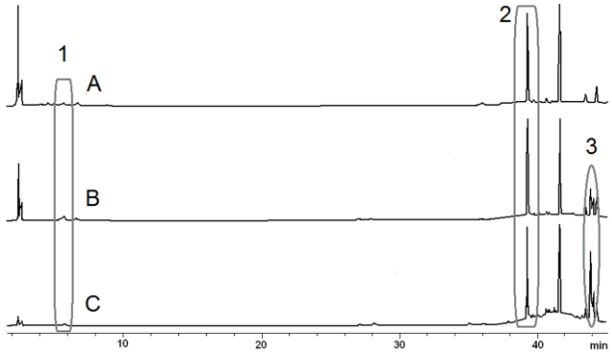

Fig. 3 Chromatograms of **oak wood** tannins, with gallic acid (1), ellagic acid (2) peaks and typical double peak (3) by toasted oak samples. A-not toasted oak wood, B-oak wood medium toasted, C-oak wood heavy toasted.

We analysed 20 samples of tannin preparations available at Austrians market (Table 1), which were marked by supplier as oak wood tannin, quebracho tannin, mixture of concrete tannins without marking of its origin and samples without any information about origin or chemical composition of tannin preparation.

According to the results - chromatograms with typical peaks in Figure 4, we noticed, that eleven samples (T1-T11) were obtained from not toasted oak wood,

Tab. 1 Samples of tannins with declaration of origin, marked by suppliers.

Sample	Origin (marked by supplier)	Sample	Origin (marked by supplier)
T 1	Oak wood	T 11	Oak wood
T 2	Oak wood	T 12	Oak wood medium toasted
T 3	Oak wood	T 13	Oak wood heavy toasted
T 4	-	T 14	Oak wood toasted
T 5	-	T 15	Ellagic tannins + Quebracho
T 6	French Oak wood	T 16	Quebracho
T 7	Oak wood	T 17	Ellagic, gallic, proanthoc. tannin
T 8	Limusin Oak wood	T 18	-
T 9	Oak wood air-dried	T 19	Catechinic tannin
T 10	Oak wood air-dried	T 20	Tropical tree + grape tannin

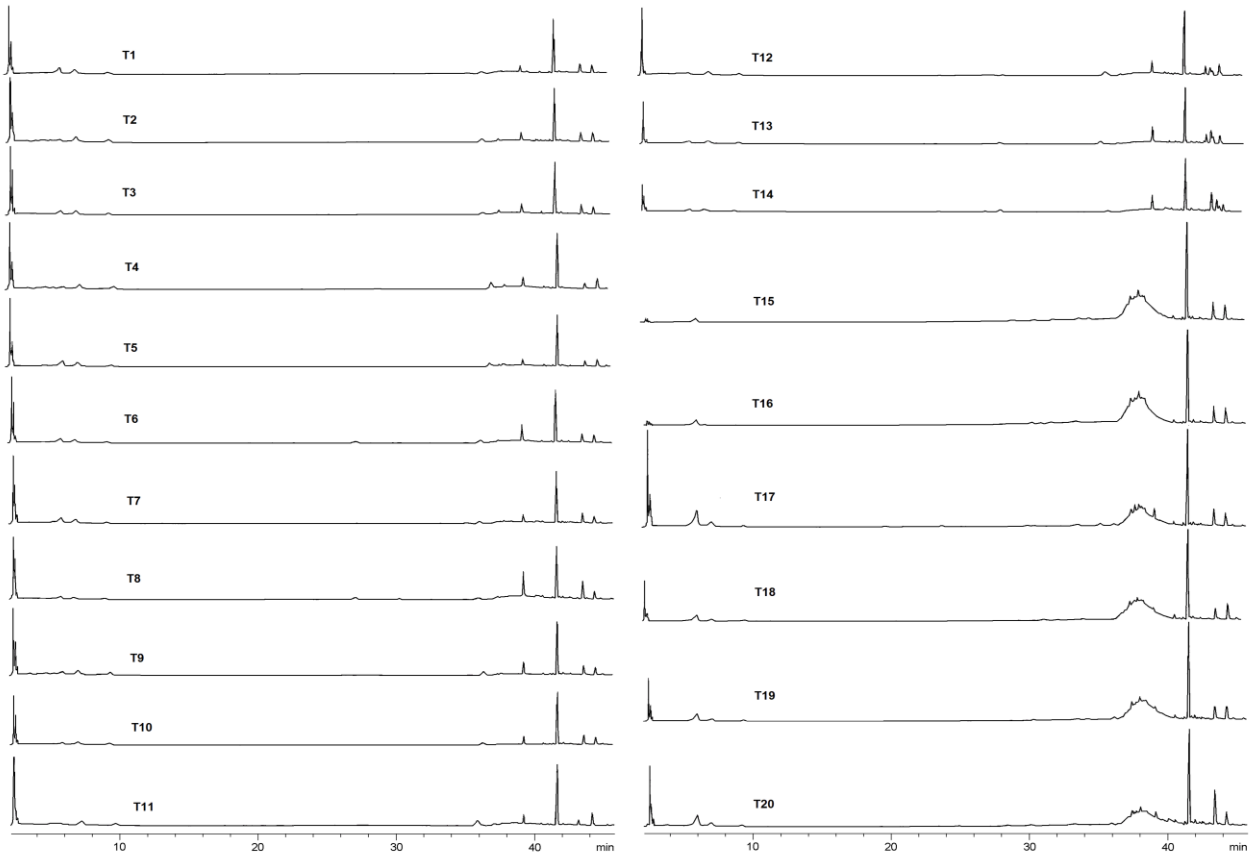

Fig. 4 Chromatograms of tannin samples. Not toasted oak tannins (T1-T11), toasted oak tannins (T12-T14), quebracho tannins T15, T16), tannin mixture (T17-T20).

samples T12-T14 from toasted oak wood and samples T15-T16 were extracted from quebracho wood. We assume, that samples T17-T20 are prepared as a mixture of quebracho and oak wood, because of presence of gallic and ellagic acid and distinctive initial peak specific for not-toasted oak wood. We do not agree with marking of sample T19 as catechinic tannin, as the chromatogram of the sample showed no peak of catechin and sample T17 and T20 as coming from grapes (proanthocianidic tannins), as we did not identify any flavan-3-ols.

CONCLUSION

Following our results, we can confirm, that the origin of most of tannin samples is labelled correctly. We also determined the origin in cases where it was unknown. Some oenological tannins are prepared from mixture of materials with different origin. In this case, the identification is more complicated, what requires further research in this area.

REFERENCES

BAUTISTA-ORTÍN A. B., FERNÁNDEZ-FERNÁNDEZ J. I., LÓPEZ-ROCA J. M., GÓMEZ-PLAZA, E. 2007. The effects of enological practices in anthocyanins, phenolic compounds and wine color and their dependence on grape characteristics. In *Journal of Food Composition and Analysis*, vol. 20, 2007, p. 546-552.

HARBERTSON, J. F., HODGINS, R. E., THURSTUN, L. N., SCHAFFER, L. J., REID, M. S., LANDON, J. L., ROSS, C. F., ADAMS, D. O. 2008. Variability of tannin concentration in red wines. In *American Journal of Enology and Viticulture*, vol. 59, 2008, no. 2, p. 210-214.

HASLAM, E. 1998. *Practical polyphenolics: From structure to molecular recognition and physiological function*. In Cambridge University Press. Cambridge, U.K.

LAGHI, L., PARPINELLO, G. P., DEL RIO, D., CALANI, L., MATTIOLI, A. U., VERSARI, A. 2010. Fingerprint of enological tannins by multiple techniques approach. In *Food Chemistry*, vol. 121, 2010, p. 783-788.

LURTON, L., LAURENT, M., GARNIER, C. 2002. Vinification en rouge: Des tanins de raisins pour stabiliser la couleur. In *La Revue des Oenologues*, vol. 104, 2002, p. 27-28.

MATTHEWS, S., MILA, I., SCALBERT, A., POLLET, B., LAPIERRE, C., HERVE DU PENHOAT, C. L. M., ROLANDO, C., DONELLY, D. M. X. 1997. Method for estimation of proanthocyanidins based on their acid depolymerization in the presence of nucleophiles. In *Journal of Agricultural and Food Chemistry*, vol. 45, 1997, p. 1195-1201.

OBRADOVIC, D., SCHULZ, M., OATEY, M. 2005. Addition of natural tannins to enhance the quality of red wine. In *Australian and New Zealand Grapegrower and Winemaker*, vol. 493, 2005, p. 52-54.

OBREQUE-SLIER, E., PEÑA-NEIRA, A., LOPEZ-SOLIS, R., RAMIREZ-ESCUDERO, C., ZAMORA-MARIN, F. 2009. Phenolic characterization of commercial enological tannins. In *European Food Research and Technology*, vol. 229, 2009, p. 859-866.

O.I.V. International Oenological Codex. Issue 2010. Paris. Capter: Oenological Tannins – COEI-1-TANINS: 2009. p. 5-13.

PASH, H., PIZZI, A., RODE, K. 2001. MALDI-TOF mass spectrometry of polyflavonoid tannins. In *Polymer*, vol. 42, 2001, no. 18, p. 7531-7539.

RIGAUD, J., PEREZ-ILZARBE, J., RICARDO DA SILVA, J. M., CHEYNIER, V. 1991. Micro method for identification of proanthocyanidin using thiolysis monitored by high-performance liquid chromatography. In *Journal of Chromatography*, vol. 540, 1991, p. 401-405.

RIBEREAU-GAYON, P., GLORIES, Y., MAUJEAN, A., DUBOURDIEU, D. 2006. *Handbook of Enology*, vol. 2. Chichester : Wiley, 2006. 441 p. ISBN-13: 978-0-470-01037-2.

VIVAS, N. 1997. Composition et propriétés des préparations commerciales detanins à usage oenologique. In *La Revue des Oenologues*, vol. 84, 1997, p. 15-21.

VIVAS, N., NONIER, M. F., VIVAS DE GAULEJAC, N., ABSALON, CH., BERTRAND, A., MIRABEL, M. 2004. Differentiation of proanthocyanidin tannins from seeds, skins and stems of grapes (Vitis vinifera) and heartwood of Quebracho (Schinopsis balansae) by matrix-assisted laser desorption/ionization time-of-flight/MS and thioacidolysis /LC/electrospray ionization MS. In *Analytica Chimica Acta*, vol. 513, 2004, no.1, p. 247-256.

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