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Identification of oenological tannins extracted from oak wood

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This study deals with the determination of the origin of oenological tannins using the modified OIV procedure. The method is based on HPLC analyses of proanthocyanidin contents (catechin, epicatechin, epigallocatechin, epicatechin-3-O-gallate and epigallocatechin-3-O-gallate) after thiolytic cleavage of the flavonol intermonomer linkages in proanthocyanidins under heat in an acid medium (thioacidolysis). The purpose of the study was to differentiate oenological tannins gained from quebracho bark 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

Identifizierung von in der Kellerwirtschaft verwendeten Tanninen aus Eichenholz. *Ziel dieser Arbeit war die Bestimmung der Herkunft von bei der Weinbereitung verwendeten Tanninen mittels einer modifizierten OIV-Methode. Diese basiert auf der HPLC-Analyse der Gehalte an Proanthocyaniden (Catechin, Epicatechin, Epigallocatechin, Epicatechin-3-O-gallat und Epigallocatechin-3-O-gallat) nach einer thiolytischen Aufspaltung der intermonomeren Flavonolbindungen unter Hitze in einem sauren Medium (Thioacidolyse). Ziel der Untersuchng war die Differenzierung von bei der Weinbereitung verwendeten Tanninen aus Quebracho-Rinde und Eichenholz. Es wurde festgestellt, dass beide Tanningruppen nach der Thioacidolyse keine Flavan-3-ole bilden, aber sie zeigen spezifische Peaks, welche die Bestimmung ihrer Herkunft erlauben.*
Schlagwörter: Tannine, Thioacidolyse, Eichenholz, Quebracho, HPLC

L'identification des tannins provenant du bois de chêne, utilisés dans la vinification. *Le but du présent travail était de déterminer l'origine des tannins utilisés dans la vinification au moyen d'une méthode OIV modifiée. Cette dernière se base sur une analyse HPLC des teneurs en proanthocyanides (catéchine, épicatéchine, épigallocatéchine, épicatéchine-3-O-gallate et épigallocatéchine-3-O-gallate) après une fission thiolytique des liaisons flavonoliques intermonomères sous la chaleur dans un milieu acide (thioacidolyse). Le but de l'examen était de différencier les tannins utilisés dans la vinification, provenant de l'écorce de quebracho et du bois de chêne. On a constaté que les deux groupes de tannins ne forment pas de flavan-3-ols après la thioacidolyse, mais qu'ils présentent des pics spécifiques permettant de déterminer leur origine.*
Mots clés : tannins, thioacidolyse, bois de chêne, quebracho, HPLC

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 a good balance and complexity of the wine. The International Organization of Vine and Wine (OIV) approved the use of oenological tannins as fining agents for white wines. However, oenological 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, whereas red wines that are made with periods of skin contact ranging from a few days to several weeks can have considerable tannin concentrations (Harbertson et al., 2008).

In chemical terms, tannins are relatively bulky phenol molecules, produced by the polymerization of monomeric 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). A traditional source of hydrolysable tannins in wine are 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, which requires the analytical characterisation of the oenological tannins (Laghi et al., 2010). A wide spectrum of oenological tannins is now available on the market, classified mainly according to their 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 between commercial tannins and to verify the information presented by suppliers (Obreque-Sliér et al., 2009). In this study our objective is the identification and distinction between quebracho and oak wood tannins.

Materials and methods

Tannin samples

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 added into 10 ml methanol. After 24 hours, the solutions were filtered and analysed using thioacidolysis and HPLC. Thereafter 21 preparations of oenological tannins from five suppliers available on the Austrian market were analysed and their chromatograms were compared with chromatograms of oak wood and quebracho extracts.

Chemicals

The following chemicals were used: HPLC grade methanol (J.T.Baker, Deventer, Netherlands), distilled water, MilliQ water (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), (-)-epigallocatechin, (-)-epicatechin gallate (Extrasynthese, Genay, France), epigallocatechin gallate (Roth, Karlsruhe, Germany), gallic acid and ellagic acid (Sigma-Aldrich).

For the preparation of tannin-methanol solutions with a concentration of 1 g/l, 10 mg tannin were added into 10 ml methanol.

Thioacidolysis

Thioacidolysis of tannin preparations was performed according to modified O.I.V. method for the differentiation of proanthocyanidin tannins by HPLC (O.I.V., 2010).
Thioacidolysis is a selective acidic depolymerisation method using a thiol as a nucleophilic agent for gaining monomeric composition and discrimination of polymeric proanthocyanidins. 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 added 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 to 60 °C for 10 minutes. The tube was then cooled with air. After cooling 1 ml distilled water was added. The mixture was then 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 using a LiChrospher 100, RP-18, 250 x 4 mm, 5 µm column (Merck, Darmstadt, Germany). The mobile phase was different to the OIV method; 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 achieved at 40 °C in 55 minutes by the following modified gradient: concentration of B solution started at 5 %, then it was lead from 5 % to 10 % in 14 minutes, from 10 % to 30 % in 20 minutes, from 30 % to 90 % in 6 minutes, then held at 90 % for 10 minutes, and finally returned back to 5 % in 5 minutes. The post-runtime was 15 minutes. The flow rate was constant and the same as in the original method, namely 1 ml/min. Samples were measured at wavelength 280 nm and the injection volume was 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. Beside the peaks of the standard substances two additional peaks (Fig. 1) appeared on the chromatogram as benzyl-thioether compounds and reagent residue, after thioacidolysis.

We could not identify every peak on the samples' chromatograms, because we used a HPLC system without Mass Spectrometry. In spite of this disadvantage we could recognize the origin of tannin preparations according to the presence or absence of single peaks and overall features of the chromatogram, since some peaks are specific only for a 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 grape tannins do. The presented analysis showed that tannin samples from quebracho bark gave a typical chromatogram (Fig. 2), without any proanthocyanidin or prodelphinidin compounds but with another specific peak at retention time between the 38th and 39th minute.
Tannins extracted from oak wood presented similar features as quebracho tannins, they did not contain any proanthocyanidin as well. A difference was noticed in the presence of gallic and ellagic acid. In addition, tannins obtained from toasted oak wood show a specific double peak at retention time 44 minutes (Fig. 3). For not toasted and medium toasted oak wood a distinctive initial peak is typical.
With the established HPLC method 20 samples of tannin preparations available on the Austrian market were analysed. They were marked by the suppliers as oak wood tannin, quebracho tannin, mixture of specific tannins without an origin given and samples without any information about origin or chemical composition of the tannin preparation (Table 1).
According to the resulting chromatograms with typical peaks (Fig. 4), we noticed, that eleven samples (T1 to T11) were obtained from not toasted oak wood, samples T12 to T14 from toasted oak wood and samples T15 and T16 were extracted from quebracho wood. We assume that samples T17 to T20 were prepared as a mixture of quebracho and oak wood, because of the presence of gallic and ellagic acid and the distinctive initial peak specific for not-toasted oak wood. There was no accordance between labelling and analytical data for T19 which was labelled catechinic

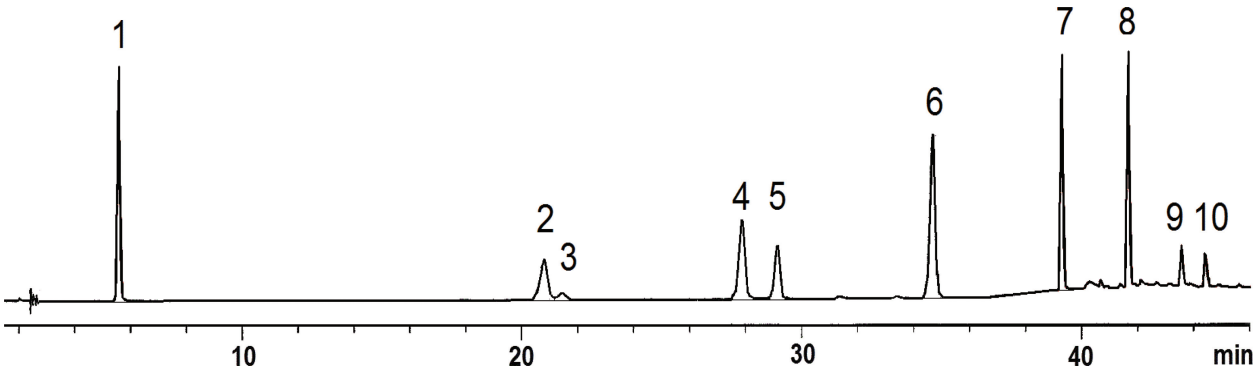

Fig. 1: Standard solution after thioacidolysis: 1-gallic acid, 2-catechin, 3-epigallocatechin, 4-epigallocatechin gallate, 5-epica-techin, 6-epicatechin gallate, 7-ellagic acid, 8 and 10 benzyl-thioether compounds, 9-reagent residue

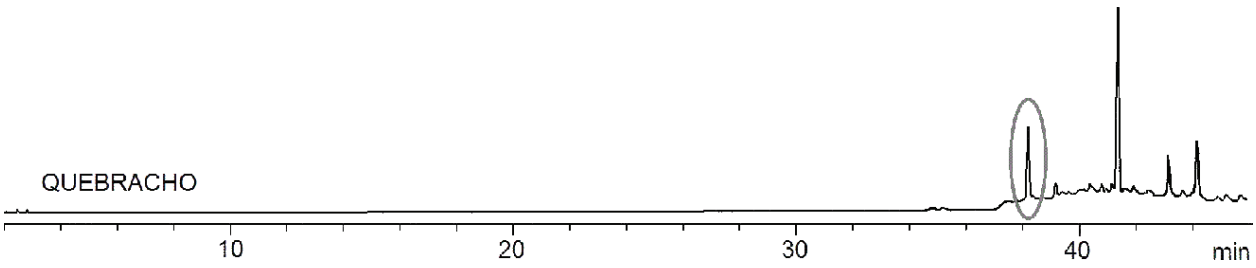

Fig. 2: Chromatogram of quebracho tannin with specific peak

tannin, whereas no peak of catechin was found in the chromatogram of the sample. Typical chromatograms of catechinic tannins were found for sample T17 and T20, which confirms that they were made from grape parts (proanthocianidic tannins).

Conclusion

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

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

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

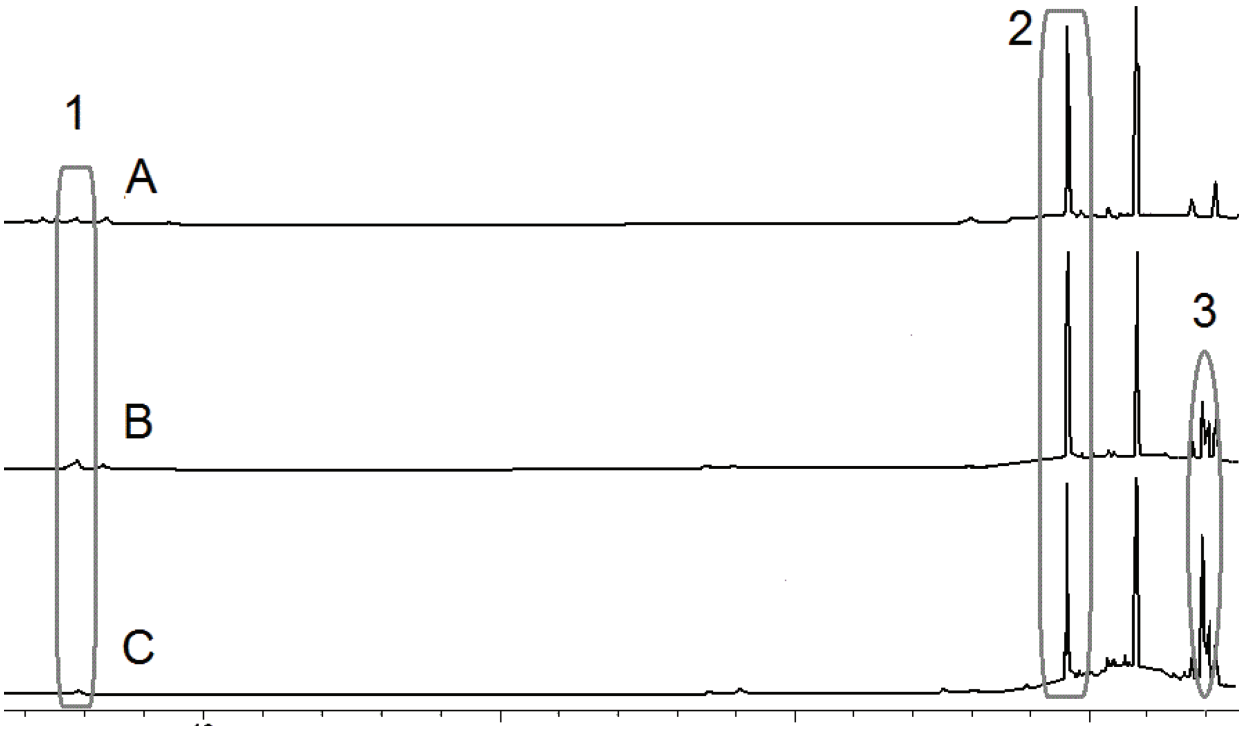

Fig. 3: Chromatograms of oak wood tannins, with gallic acid (1), ellagic acid (2) peaks and typical double peak (3) with toasted oak samples; A-not toasted oak wood, B-oak wood medium toasted, C-oak wood heavily toasted

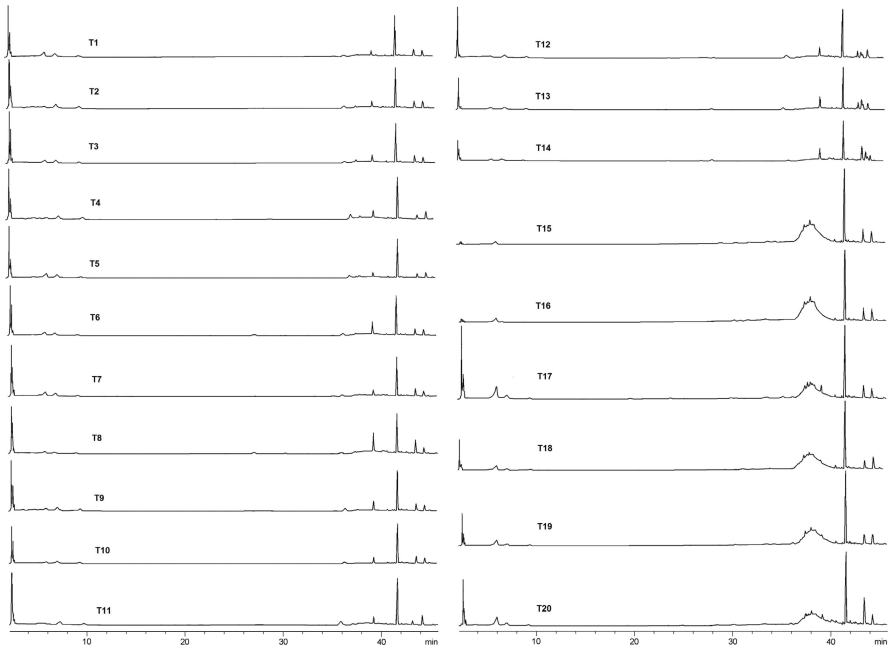

Fig. 4: Chromatograms of tannin samples; not toasted oak tannins (T1-T11), toasted oak tannins (T12-T14), quebracho tannins (T15, T16), tannin mixture (T17-T20)

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