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Analysis of commercial vegetable tanning agents by reversed-phase liquid chromatography–electrospray ionization–tandem mass spectrometry and its application to wastewater

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Abstract

Commercial vegetable tanning agents that are derived from plants and consist of condensed or hydrolyzable tannins were analyzed by electrospray ionization–tandem mass spectrometry (ESI–MS/MS) to identify their major constituents and to study their collision-induced dissociation. In the condensed tannin wattle a series of proanthocyanidin dimers to tetramers was identified together with the flavonoid monomers catechin and gallocatechin. The composition of the hydrolyzable tannin chestnut was more heterogenous. Besides the monomers ellagic and gallic acid a variety of gallotannins were detected, namely mono-, di- and trigalloylglucose, and a variety of ellagitannins. Reversed-phase HPLC–ESI–MS/MS methods were developed to detect condensed and hydrolyzable tannins in tannery wastewaters by multiple reaction monitoring (MRM). The methods proved suitable even for highly loaded wastewaters. However, the detected amount of wattle tanning agent in spent retanning baths was about two orders of magnitude below the amount used for the retanning. This suggests that the condensed tannins of polyphenolic structure are rapidly transformed during the tanning process to yet unknown products.

Keywords: Water analysis; Wattle; Chestnut; Tannins; Vegetable tannins; Polyphenols; Flavonoids

1. Introduction

Vegetable tannins are natural polyphenols distributed ubiquitously in plants [1–4] and they do, thus, also occur in a variety of food, such as vegetables, fruits, seeds and plant-derived beverages. Vegetable tannins have gained considerable attention not only due to their nutritional value, their astringency and taste, but also due to their chemical properties such as their ability to bind proteins and metals. Furthermore, beneficial effects for human health have been ascribed to tannins based on their anti-carcinogenic, anti-microbial and anti-oxidative properties [5–7]. Correspondingly, various methods have been developed to detect vegetable tannins from plant extracts and in food and beverages [8–10] and reversed-phase HPLC with UV-detection was most frequently used [11–15]. For structural elucidations mass spectrometry and NMR-spectroscopy have been employed [1,16,18], but only a few articles deal with commercial vegetable tannin extracts used for leather production [19]. The application of mass spectrometry to plant polyphenols was reviewed recently [20] and includes the use of ESI–MS [17,21–25], APCI–MS [26] and FAB–MS [16,18].

Most of the recent analytical studies were directed towards condensed tannins (e.g. [16,18,19,21–

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23,26]). This compound class, also denoted as proanthocyanidins, consists of oligomeric and polymeric chains based on polyhydroxyflavan units. Typical monomers are the stereoisomeric compounds (+)/(−)-catechin and (+)/(−)-epicatechin that differ in their stereochemistry in positions 2 and 3. Another important monomer is gallocatechin, which bears one additional hydroxy-group, and from which also the stereoisomers (+)/(−)-gallocatechin and (+)/(−)-epigallocatechin exist. The monomeric units are typically linked through C4–C8 interflavonoid bonds, but linkage through C4–C6 does also occur (Fig. 1).

A second important class of vegetable tannins are the so-called hydrolyzable tannins, which are polyesters of sugar derivatives. Recent analytical methods for the detection of hydrolyzable tannins are less thoroughly developed. Only a few articles on the determination of hydrolyzable tannins by mass spectrometry are available [17–19,24,25]. In hydrolyzable tannins, mainly D-glucose is esterified by hydroxyphenolic acids, predominantly by gallic or ellagic acid, yielding either gallo- or ellagitannins (Fig. 2). Even more structural diversity for both classes of tannins is provided by further esterification of side chains and oxidative coupling of additional groups [1–4].

According to their name, tannins have since long been used for the tanning of hides to convert them into leather. Extracts of various tannin-rich plant materials, especially of barks and wood of trees, are used for this purpose and it has been shown that polymers of a molecular mass range of M_r=500–

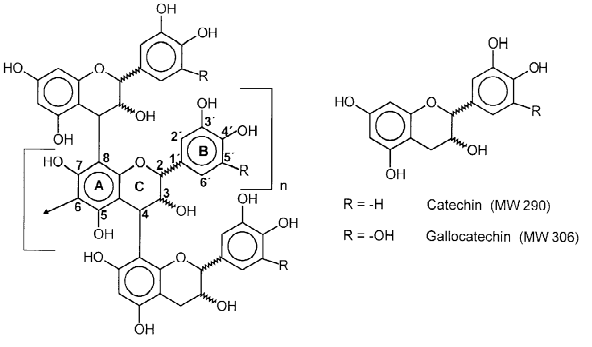


Fig. 1. Oligomeric structure of condensed tannins (proanthocyanidins) and their corresponding monomers (catechin and gallocatechin) with n=1 trimers, n=2 tetramers etc.

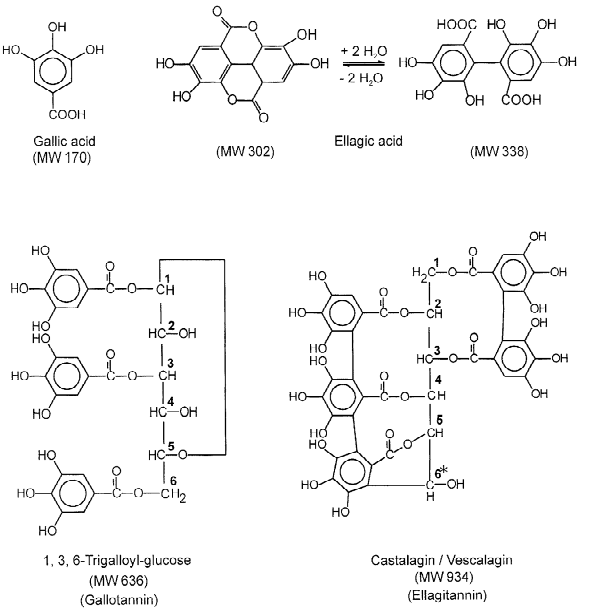


Fig. 2. Structures of monomers of hydrolyzable tannins, gallic and ellagic acid and of a gallotannin (1,3,6-trigalloyl-glucose), and of an ellagitannin (vescalagin/castalagin).

3000 g/mol are most effective [27–29]. Generally commercial tanning agents are mixtures of several compounds, which are of complex and polymeric structure.

The use of vegetable tannins in the leather industry is supposed to cause several problems, such as elevated dissolved organic carbon content (DOC) in biologically treated effluents and difficulties in the precipitation of chromium. Concerning vegetable tannins in wastewater, some laboratory studies on the transformation and on inhibitory effects of vegetable tannins in anaerobic processes are available [30,31]. The fate of vegetable tanning agents during tannery wastewater treatment, however, has not been studied yet.

The objective of this work was, thus, twofold: firstly, to establish an analytical method for the separation and selective detection of commercially available vegetable tanning agents of either class, and secondly to apply this method to real wastewaters of tanneries in order to study the occurrence and fate of vegetable tannins in these wastewaters. Reversed-phase high-performance liquid chromatography is well established for the chromatographic

separation of vegetable tannins. The selective detection and identification of vegetable tannins in complex wastewater samples was accomplished by using electrospray ionization–tandem mass spectrometry.

2. Experimental

2.1. Chemicals and materials

Vegetable tannins (powder extracts) wattle and chestnut as well as tannery wastewater samples were kindly provided by a German tannery. Standard materials (+)-catechin, (−)-epicatechin, gallic acid and ellagic acid were of HPLC grade and purchased from Fluka (Deisenhofen, Germany). Methanol (gradient grade) and formic acid (analytical reagent grade) were purchased from Merck (Darmstadt, Germany). An Elga maxima ultra pure water system (Ubstadt-Weiher, Germany) was used for the further purification of deionised water.

2.2. Sample preparation

Stock solutions (c=100 mg l^{-1}) of the vegetable tannins wattle and chestnut were freshly prepared in ultra pure water before use, including 1% formic acid for increasing sensitivity and suppressing adduct formation. Tannery wastewater samples were filtered over 0.45 μm cellulose-nitrate membrane filters (Macherey-Nagel, Düren, Germany).

2.3. HPLC–MS equipment

Chromatographic separation was performed using a HP 1100 series liquid chromatograph (Hewlett-Packard, Waldbronn, Germany), consisting of a vacuum solvent degassing unit, a binary high pressure gradient pump, an automated sample injector and a column thermostat. For control, UV-detection was performed by a HP 1100 diode array detection (DAD) system.

Mass spectrometry was performed using a Quattro-LC triple-stage-quadrupole mass spectrometer with the orthogonal Z-spray-electrospray interface (Micromass, Manchester, UK). Nitrogen was used as drying gas as well as nebulizing gas, generated from pressurized air in a Whatman Model 75-72 nitrogen generator (Whatman, Haverhill, USA). Collision gas was argon 5.0 (Messer, Berlin, Germany) for MS/MS-mode. Flow injection was carried out with a Model 11 single syringe pump (Harvard, Holliston, USA) directly connected to the interface.

2.4. HPLC–MS conditions

HPLC analyses were performed on a 125×3 mm Hypersil ODS reversed-phase column with a particle size of 3 μm (Knauer, Berlin, Germany) at a column temperature of 40 °C with a flow-rate of 0.5 ml min^{-1}. Chromatographic separation was performed using a binary gradient consisting of (A) water and (B) methanol, both with 0.1% formic acid (v/v). The elution profile for the vegetable tannins was: 0–4 min 5% B, 12 min 35% B, 14 min 90% B, 16 min 90% B, 17 min 5% B with 7 min for equilibration. Sample volumes of 20 μl were injected. UV spectra were recorded in the range of 200–600 nm.

Mass spectrometric detection was performed in the negative ion mode after electrospray ionization. For HPLC–MS analyses, the mass spectrometer was set to the following optimised tune parameters: interface temperature 120 °C, source temperature 250 °C, nebulizer gas flow ~85 l h^{-1}, desolvation gas flow of 850–950 l h^{-1} and a capillary voltage of 3.0 kV. The cone voltage had to be optimized for each of the monomers and each of the oligomers by using LC–MS instead of infusion. Care has to be taken to avoid fragmentation of oligomers in the interface due to excess cone voltage. Otherwise, fragmentation of oligomers in the interface leads to the formation of anions that correspond to the respective monomer and would bias the selective detection of the monomers. For operating in the MRM-mode the LM/HM resolution was set to 9.0 for MS 1 and 14.0 for MS 2. Collision-induced dissociation (CID) was performed with a collision gas pressure of 1.1–1.3× 10^{-3} mbar in the collision cell. In addition to HPLC, direct infusion experiments were carried out at a flow-rate of 0.5 ml h^{-1}.

3. Results and discussion

3.1. Molecular anions and fragmentation

The detection and fragmentation of the tannins by the electrospray–MS was studied with wattle as a typical condensed tannin and chestnut as a representative of hydrolyzable tannins. Both vegetable tannins belong to the most important extracts used for leather production. Negative ionization was used since complex adduct formation occurred in the positive ion mode.

3.1.1. Condensed tannin wattle

A negative ionization mass spectrum of the condensed tannin wattle obtained after infusion into the electrospray source is given in Fig. 3. As outlined above, condensed tannins consist of polyhydroxyflavan subunits with interflavonoid C–C-linkages. Correspondingly, an oligomeric series of procyanidin anions formed from catechin is detected, with dimers $[(290+288)-H]^-$, trimers $[(578+288)-H]^-$ and tetramers $[(866+288)-H]^-$. Even a peak at m/z 1441 that corresponds to the procyanidin pentamer, can be found, but only at very low intensity. This oligomeric pattern is often described in the literature [16,19,22]. Moreover, the monomeric building blocks catechin and gallocatechin can be detected at m/z 289 and m/z 305, respectively. Since mass spectrometry cannot distinguish between stereoisomers like (+)/(−)-catechin/epicatechin or (+)/(−)-gallocatechin/epigallocatechin the names catechin and gallocatechin for all isomers are used.

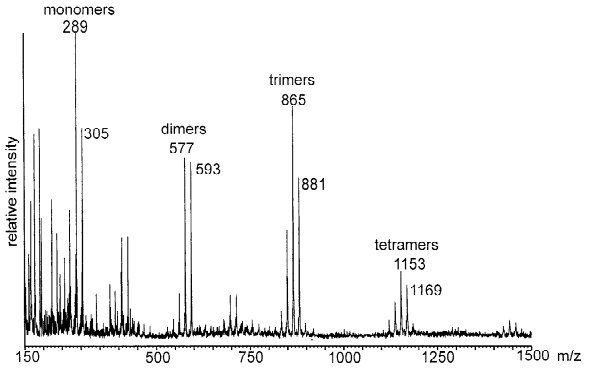


Fig. 3. Mass spectrum of vegetable tannin wattle ($c=100$ mg l^{-1}) obtained by infusion with ESI–MS in the negative ion mode.

However, no single oligomeric signals are found (Fig. 3), but a group of signals with a mass difference of 16 amu. These signals correspond to oligomers with more or less hydroxy groups than in the catechins. For example, m/z 305 corresponds to the monomer gallocatechin that bears one additional hydroxy group in comparison to catechin [23,32], and the molecular anion series of m/z 593, 881 and 1169 corresponds to oligomers with one such gallocatechin, each. Moreover, the molecular anion series of m/z 561, 849 and 1137 corresponds to oligomer series with one hydroxy group less than in catechin in one of its monomers.

The MS/MS-experiments confirm these structural suggestions. Daughter ion spectra of the molecular anions of dimers (m/z 577 and 593) and trimers (m/z 865 and 881) of wattle are displayed in Fig. 4. The fragmentation reflects the oligomeric composition and the major fragment ions are due to the cleavage of the interflavonoid C–C-linkages with losses of catechin units (288 amu). For the molecular anions m/z 577 and m/z 865 these sequences end at the monomer catechin (m/z 289) (Fig. 4b and d), whereas the members of the oligomeric series with an additional hydroxy-group, further on denoted as gallocatechin oligomers, (m/z 593 and m/z 881) end with a gallocatechin fragment (m/z 305 in Fig. 4a and c).

Another series of fragments evolves from a Retro-Diels-Alder (RDA) fission of the flavonoid nucleus [16,26] giving rise to a fragment of m/z 425 from both anions, m/z 593 and m/z 577 (Fig. 4a and b). This product (m/z 425) eliminates water, probably

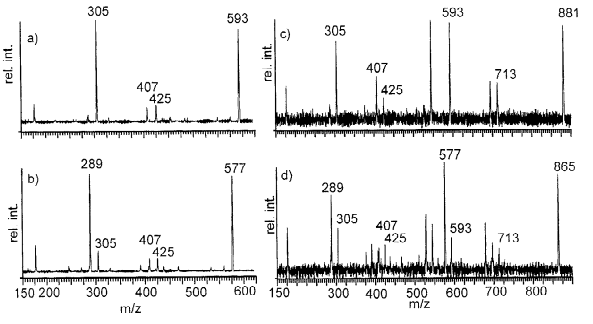


Fig. 4. Daughter ion spectra of wattle components: (a) gallocatechin dimer (m/z 593), (b) catechin dimer (m/z 577), (c) gallocatechin trimer (m/z 881) and (d) catechin trimer (m/z 865).

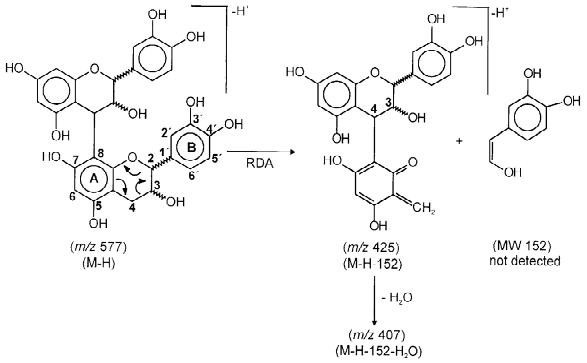


Fig. 5. Retro-Diels-Alder (RDA) fission leading to fragment m/z 425 and m/z 407.

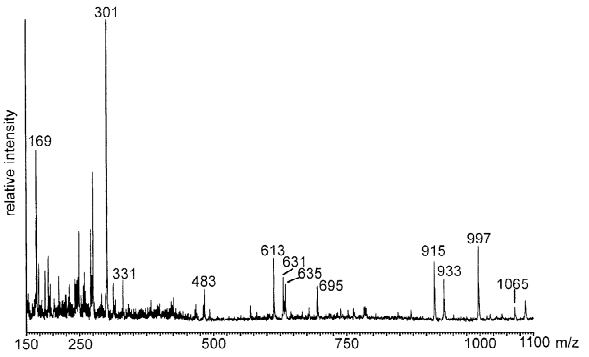


Fig. 6. Mass spectrum of the vegetable tannin chestnut ($c = 100$ mg l^{-1}) obtained by infusion with ESI–MS in the negative ion mode.

from ring C in position C_3/C_4, resulting in a fragment ion of m/z 407. A pathway of the RDA fragmentation is given in Fig. 5. A similar series of RDA fissions was observed for the trimers (m/z 881 and m/z 865) (Fig. 4c and d) yielding the products m/z 713/695 and m/z 425/407. Obviously, the trihydroxylated aromatic ring of the gallocatechin oligomers is preferentially split off. The signal intensity of higher oligomers was too weak to perform MS/MS experiments.

RDA fission of the monomers catechin (m/z 289) and gallocatechin (m/z 305) results in a fragment ion of m/z 137. Moreover, a major fragment ion of m/z 125 occurs (data not shown). According to the literature [23] the fragment ion m/z 125 is formed from ring A of catechins or gallocatechins with a structure corresponding to 1,3,5-trihydroxybenzene.

3.1.2. Hydrolyzable tannin chestnut

The ESI–mass spectrum of the commercial chestnut extract is less clearly structured (Fig. 6). Chestnut is a hydrolyzable tannin and, thus, anions of gallic acid (m/z 169) and also of ellagic acid (m/z 301) can be found. The signal m/z 933 points at the compounds castalagin and vescalagin, that differ only in their stereochemistry in position C_6 of the glucose core (Fig. 2) and that are characteristic components of chestnut tannins [14,15]. The molecular anion of m/z 631 corresponds to castalin and vescalin, that show structures similar to castalagin and vescalagin, but without ellagic acid esterified in position C_1 and C_3 of the glucose core [18,19]. All these polyhydroxylated ellagitannins tend to eliminate water to form m/z 915 from castalagin or vescalagin as well as m/z 613 from castalin or vescalin. The peak at m/z 1065 may originate from grandinin or roburin E [25], which have also been reported as important constituents of chestnut tannins [14,15].

Although chestnut extracts are classified as elagitannins they may, nevertheless, contain gallotannins (Fig. 2), because ellagitannins are biologically formed from pentagalloyl-glucose (gallotannin) [1,2,4]. The series of signals (m/z 331, 483 and 635) indicates the presence of mono- to trigalloyl-glucose esters in the chestnut extract. The intensive peaks at m/z 997 and m/z 695 remain unidentified, but they may be derived from castalagin/vescalagin (m/z 933) and castalin/vescalin (m/z 631), from which they differ by 64 amu.

Again, daughter ion spectra were recorded to confirm the identity of the suggested molecules and to detect the transition reactions suited for MRM-detection of the hydrolyzable tannin chestnut. Daughter ion spectra of the galloyl-glucose esters (m/z 635, 483 and 331) are shown in Fig. 7. The trigalloyl-glucose anion (m/z 635) eliminates gallic acid (m/z 483) and water (m/z 465) from where a second galloyl moiety is eliminated (m/z 313) (Fig. 7a). Finally, from this dehydrated monogalloyl-glucose anion (m/z 313) the glucose is eliminated, leaving a gallic acid anion (m/z 169) behind. Elimination of a galloyl ester group (m/z 331), a subsequent loss of water (m/z 313) and the occurrence of gallic acid (m/z 169) are also observed for the

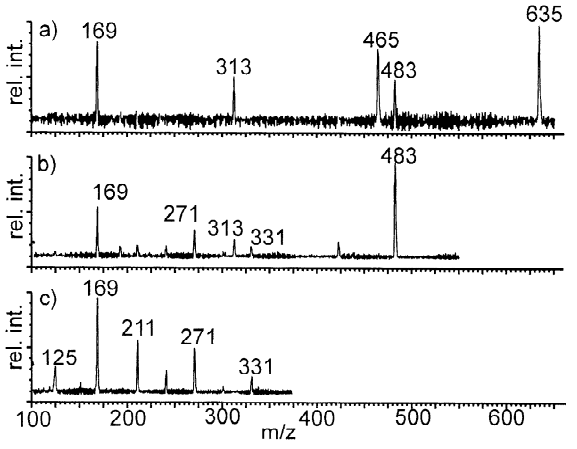
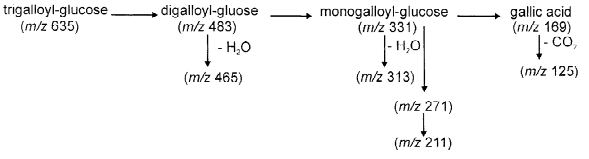


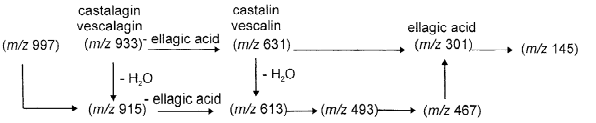
Fig. 7. Daughter ion spectra of chestnut components: (a) trigalloyl-glucose (m/z 635), (b) digalloyl-glucose (m/z 483) and (c) monogalloyl-glucose (m/z 331).

digalloyl-glucose anion (m/z 483) (Fig. 7b). Correspondingly, gallic acid (m/z 169) is detected as the major fragment of monogalloyl-glucose (m/z 331), which then decarboxylates to m/z 125 (Fig. 7c). The same fragment is also formed from the gallic acid anion (m/z 169) (data not shown here). The daughter ions m/z 271 and m/z 211, that are formed during the fragmentation of di- and monogalloyl-glucose (m/z 483 and m/z 331) (Fig. 7b and c), could not be interpreted, but have already been described elsewhere [25]. This fragmentation sequence is summarized in Scheme 1.

As mentioned above, chestnut belongs to the ellagitannins and therefore several other compounds bearing ellagic acid were detected and their daughter ion spectra were recorded in the MS/MS mode (data not shown). The molecular anion at m/z 997 gives an intensive peak at m/z 915, corresponding to dehydrated vescalagin/castalagin, but also the transition to ellagic acid (m/z 301) is observed. For vescalagin/castalagin (m/z 933) a neutral loss of ellagic



Scheme 1. Suggested fragmentation pathway of gallotannins observed by collision-induced dissociation.



Scheme 2. Suggested fragmentation pathway of ellagitannins observed by collision-induced dissociation.

acid to vescalin/castalin (m/z 631) occurs, followed by a fragmentation to ellagic acid (m/z 301). Moreover, the molecular anions m/z 915 and m/z 613, corresponding to dehydrated vescalagin/castalagin and vescalin/castalin, showed hardly any fragments except ellagic acid (m/z 301) and two unknown fragments at m/z 493 and m/z 467 of poor intensity. Ellagic acid itself (m/z 301) generates an intensive daughter ion at m/z 145. Scheme 2 shows the decomposition pathway of molecular anions containing ellagitannins.

3.2. HPLC–MS/MS

Reversed-phase high-performance liquid chromatography using an acidic water–methanol gradient elution system is very common for the separation of both condensed and hydrolyzable tannins [11,13]. Based on the MS results discussed above, we used RP-HPLC–ESI–MS/MS with MRM for the selective detection of the tannin components.

3.2.1. Condensed tannin wattle

For the detection of wattle tannin the most intensive transitions of the oligomers of the catechin and the gallocatechin series and of the monomers were chosen as summarized in Table 1. Excess cone

Table 1
The six most intensive MRM-transitions of the vegetable tannin wattle and the corresponding cone voltages (CV) and collision energies (CE)

MRM m/z	Compound	CV (V)	CE (eV)
881>305	Gallocatechin trimer	55	35
865>289	Procyanidin trimer	55	35
593>305	Gallocatechin dimer	45	30
577>289	Procyanidin dimer	40	25
305>125	Gallocatechin	30	25
289>125	Catechin	30	25

voltage must be avoided in order to prevent fragmentation of the oligomers in the interface and their appearance as monomers.

The MRM-chromatograms of a wattle tannin extract are shown in Fig. 8. The catechin homologues are not chromatographically resolved but largely coelute with the gallocatechin series. As this series bears one additional hydroxy-group (m/z 881, 593, 305), it exhibits shorter elution times than the procyanidins (m/z 865, 577, 289). Due to the large number of stereoisomers of one homologue, no complete separation is achieved within one mass trace; more than four peaks are observed for most transitions. The lack of chromatographic separation is fully compensated by the selectivity of the MRM-detection. The mass chromatograms of the monomers (Fig. 8e and f) show no interferences from higher oligomers.

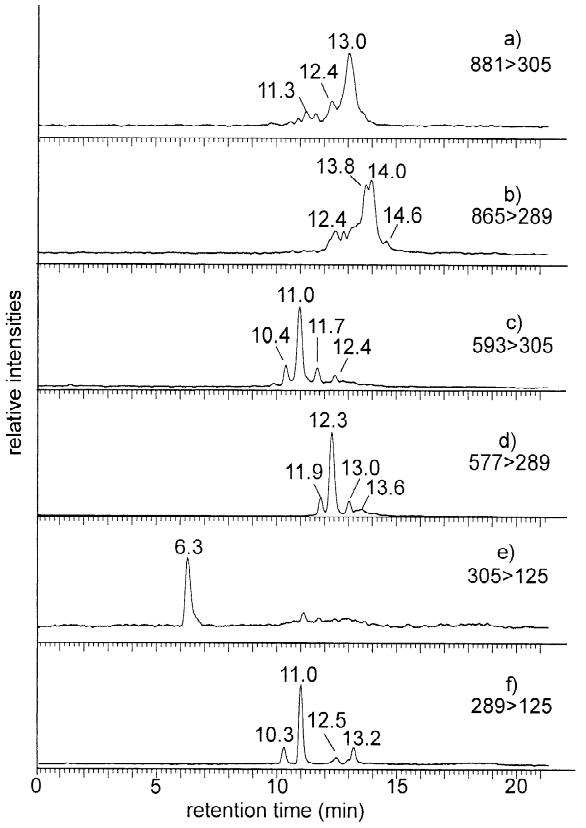


Fig. 8. MRM-chromatograms of the vegetable tannin wattle (c = 100 mg l^{-1}): (a) gallocatechin trimer, (b) catechin trimer, (c) gallocatechin dimer, (d) catechin dimer, and the monomers (e) gallocatechin and (f) catechins (RT 11.0 min (+)-catechin, RT 13.2 min (−)-epicatechin).

Only one chromatographic signal is obtained in the transition selected for gallocatechin (m/z 305> 125; Fig. 8e) and it is, thus, likely that this signal (RT 6.3 min) represents one of the gallocatechins. Four stereoisomers of the catechin type exist, being (+)/(−)-catechin and (+)/(−)-epicatechin. With the transition m/z 289>125 four signals in the wattle tannin were detected (Fig. 8f). The identity of two of these peaks was confirmed by comparison with reference compounds: (+)-catechin (RT 11.0 min) and (−)-epicatechin (RT 13.2 min). It is, thus, likely that the two remaining peaks at 10.3 min and 12.5 min are due to the other two stereoisomers.

3.2.2. Hydrolyzable tannin chestnut

Six constituents of the chestnut extract were selected for detection by MRM via their most intensive transitions as listed in Table 2. The respective chromatograms obtained by RP-HPLC–ESI–MS/MS of a commercial chestnut tannin are displayed in Fig. 9. In comparison to the condensed tannin wattle the hydrolyzable tannin chestnut contains more polar compounds, leading to considerably shorter retention times. The large difference in the retention time of gallic acid (3.1 min, Fig. 9f) and ellagic acid (16.3 min, Fig. 9e) illustrates the large polarity difference between these two acidic components of hydrolyzable tannins. As for the condensed tannins, fragmentation of oligomers in the source can be avoided by using appropriate cone voltages. Then, the oligomers do not interfere with the detection of the monomers.

The mono-, di- and trigalloylated glucose shows

Table 2
The six most intensive MRM-transitions of the vegetable tannin chestnut and the corresponding cone voltages (CV) and collision energies (CE)

MRM m/z	Compound	CV (V)	CE (eV)
997>915	Unknown compound	50	30
635>169	Trigalloyl-glucose	50	50
483>169	Digalloyl-glucose	35	30
331>169	Monogalloyl-glucose	30	20
301>145	Ellagic acid	55	35
169>125	Gallic acid	25	15

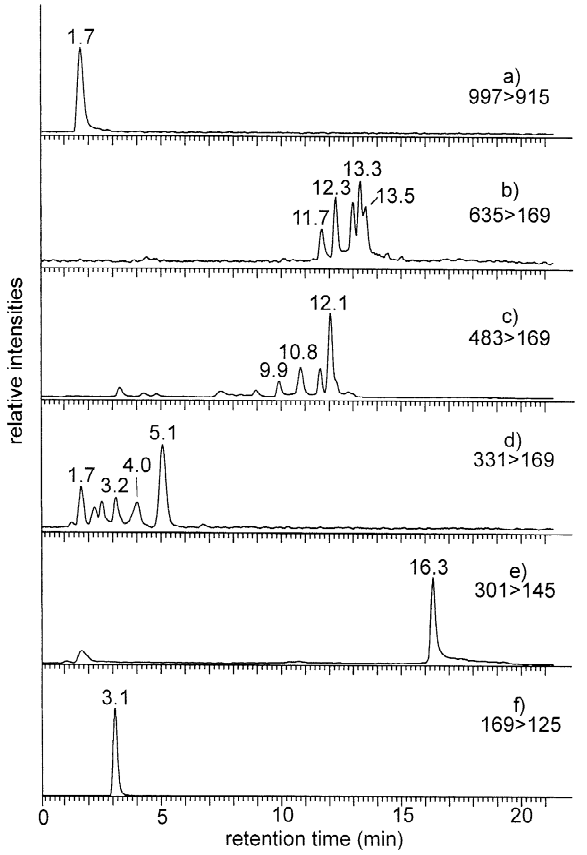


Fig. 9. MRM-chromatograms of the vegetable tannin chestnut ($c = 100$ mg l^{-1}): (a) unknown compound, (b) trigalloyl-glucose, (c) digalloyl-glucose, (d) monogalloyl-glucose, and the monomers (e) ellagic acid and (f) gallic acid.

increasing retention, but the di- and trigalloyl-glucose isomers partly overlap. A much shallower gradient and longer run times would have been needed for a complete resolution [17]. Within one mass trace, the respective galloyl-glucose isomers appear to be largely separated. These oligomers are structurally less diverse than the flavonoids of the condensed tannins, but positional isomers and stereoisomers appear.

Remarkably, only one chromatographic peak is detected in the m/z 997>915 trace. As previously mentioned the daughter ion spectrum of this unknown component (m/z 997) exhibited fragments that correspond to dehydrated castalagin/vescalagin and ellagic acid. Indeed the castalagin/vescalagin components do not show positional isomers according to the literature [25] and are as well quite polar as compared to ellagic acid. They may even elute before gallic acid [25]. Thus, it is believed that this compound (m/z 997) belongs to the group of ellagitannins.

3.3. Application on tannery wastewater

This RP-HPLC–ESI–MS/MS method was applied to four samples of spent retanning baths (Fig. 10). The MRM-chromatograms clearly reflect those obtained for the wattle tannin in all oligomeric mass traces (Fig. 8a–d) and no interference from other compounds are seen. Thus, the RP-HPLC–ESI–MS/MS method can be applied to detect the condensed tanning agent wattle from highly loaded wastewaters

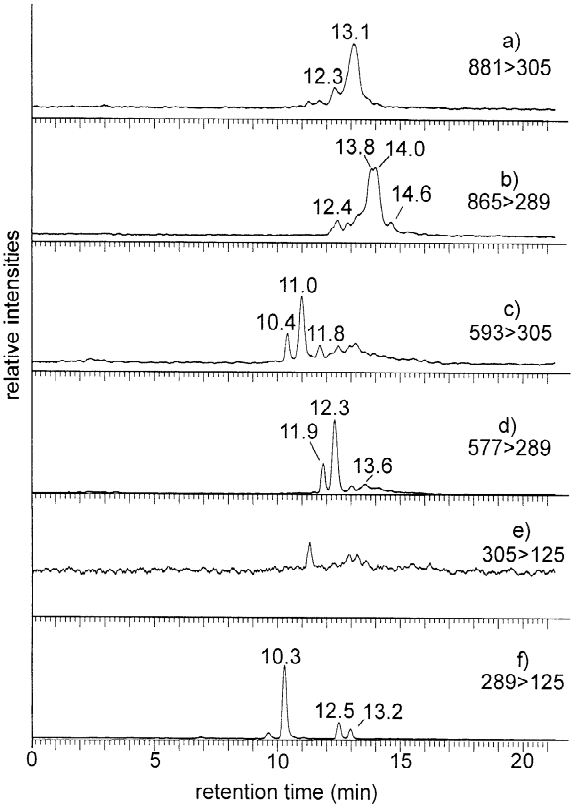


Fig. 10. MRM-chromatograms of a spent retanning bath (for details refer to Fig. 8).

with dissolved organic carbon contents (DOC) of up to 5000 mg l^{-1}.

Differences are visible for the monomers as no gallocatechin (m/z 305>125; Fig. 10e) was found in the spent retanning baths and the pattern of the catechin and epicatechin stereoisomers (m/z 289> 125; Fig. 10f) also changed. Here, (+)-catechin (RT 11.0 min) was largely removed, whereas the signals of two non-ascribed stereoisomers (RT 10.3 min and 12.5 min) are now more prominent.

A quantitative assessment was performed via external calibration in the range 10–100 mg l^{-1} with wattle tannin in pure water. According to that calibration the total amount of dimers (m/z 577, 593) and trimers (m/z 865, 881) in the four samples was in the range of what was found in a 100 mg l^{-1} aqueous solution of wattle. This corresponds to only 2% of the initial concentration of about 17 g l^{-1} of wattle in the retanning process. Concerning that the DOC contribution of the initial wattle concentration was about 8.5 g l^{-1}, and that the DOC of the spent bath was around 4.6 g l^{-1}, a 98% removal of the tannin seems highly unlikely.

In order to clarify, whether precipitation and complexation may have diminished the amount of dissolved wattle in the bath, wattle tannin was added to the samples. The recovery of wattle was in the range of 70%. Further on, neither the addition of chromium(3+), nor calcium(2+) had a measurable influence on the detection of wattle by the RP-HPLC–ESI–MS/MS method developed here. It is, thus, unlikely that the large difference between the initial concentration of wattle in the tanning bath and the detected concentration in the spent baths is due to analytical problems. These results confirm previous findings from independent analysis using RP-HPLC with UV- and EC-detection [33].

Therefore, it is believed that the wattle tannins with their large number of hydroxy-groups were subjected to chemical alterations during the retanning process. Subsequent laboratory experiments proved that neither wattle nor chestnut were stable at pH 7, since the compounds detected by the RP-HPLC–MS/MS methods vanished within a few days. This instability of the vegetable tannins wattle and chestnut increases with increasing pH-values. However, the present information on the structures of oxidation products of flavonoids is still contradictory [34,35] and additional investigations would be required to identify the products.

4. Conclusions

A method for the analysis of tanning agents by RP-HPLC coupled to electrospray ionization–tandem mass spectrometry was developed, that allows the analysis of condensed as well as hydrolyzable tannin components under the same chromatographic conditions.

A wide variety of tannin components were detected and their structure was ascribed by daughter ion spectra obtained after collision-induced dissociation. In the condensed tannin wattle a series of proanthocyanidin oligomers (up to the pentamers) could be detected together with the monomeric building blocks catechin and gallocatechin. Four stereoisomers of catechin were found in the tanning extract, two of them could be identified as (+)-catechin and (−)-epicatechin. A variety of ellagic acid glucose esters (ellagitannins) was determined in the hydrolyzable tannin chestnut, besides gallotannins such as mono-, di- and trigalloyl-glucose and the acidic components gallic and ellagic acid. Components of higher molecular mass (>1000 amu), which were expected to be present in the tanning agents, could not be detected.

Using optimized cone voltages, multiple reaction monitoring proved suitable for the detection of monomers, dimers and trimers of tanning agents also in highly loaded wastewaters. Quantitation of oligomeric tannin components, however, is hampered by the lack of pure standard material concentrations. First applications of this method to spent retanning baths suggest that the condensed tannin wattle is not stable but is rapidly transformed to unknown products in the wastewater.

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