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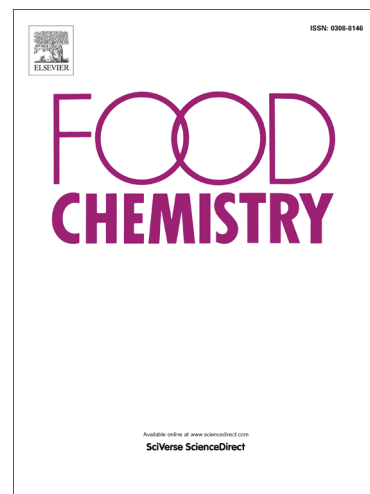
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New insight into the unresolved HPLC broad peak of Cabernet Sauvignon grape seed polymeric tannins by combining CPC and Q-ToF approaches

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Abstract

Polymeric tannins from grapes have always been reported as an unresolved broad peak in HPLC chromatograms, and this has severely limited their identification to date. This study aimed to disassemble this broad peak and explore the polymeric tannin molecules inside. By applying centrifugal partition chromatography (CPC), an efficient separation approach was developed to split the broad peak of grape seed tannins into fractions. Then, the fractions were analyzed by Q-ToF (quadrupole time-of-flight mass spectrometry) to determine the corresponding structures of the tannins. The results suggest that grape seed polymeric tannins were eluted consecutively according to their degree of polymerization (DP). Condensed tannins identified in wine grape seed have a range of DP and degree of galloylation (DG) up to 20 and 11, respectively. The molecular mass of the largest molecule detected was 6067. To our knowledge, this is the first report to offer an insight into the broad peak of polymeric tannins found with HPLC and to characterize the tannins with a DP up to 20 as shown by HRMS and MS/MS data.

Keywords: condensed tannins; grape; CPC; Q-ToF; polymer

1. Introduction

Condensed tannins are oligomeric and polymeric forms of flavan-3-ol monomer units that are also known as proanthocyanidins in the domain of phytochemistry. Owing to their considerable contribution to wine sensory perception (W. Ma, Guo, Zhang, Wang, Liu, & Li, 2014) and other bioactivities (Rasines-Perea & Teissedre, 2017), the condensed tannins derived from grape and wine have attracted the attention of wine chemists for decades. As the unique source of condensed tannins in wine (J.A. Kennedy, Saucier, & Glories, 2006), grape is easier to study than wine since grape tannins occur in their initial forms prior to any polymerization or oxidation reactions during vinification or wine aging. Until now, numerous studies have investigated the oligomeric condensed tannins in grape (Ge, Zhu, Kazuma, Wei, Yoshimatsu, & Komatsu, 2016; Lin, Sun, Chen, Monagas, & Harnly, 2014; Wen Ma, Waffo-Teguo, Jourdes, Li, & Teissedre, 2016). While polymeric tannins are much more abundant in grape than oligomers (K. Chira, Schmauch, Saucier, Fabre, & Teissedre, 2009), little is known about their chemical structure or composition. The characterization of grape

polymeric tannins remains a challenge owing to the lack of an efficient fractioning approach and the limit of detection of direct mass spectrometry.

A number of strategies have been applied to investigate tannin polymers. Depolymerization of polymerized tannins is one of the most popular approaches, which involves both thiolysis methodology (Rigaud, Perez-Illarbe, Da Silva, & Cheynier, 1991) and phloroglucinolysis methodology (James A Kennedy & Taylor, 2003). Based on the mechanism of acid-catalyzed cleavage of interflavan linkages, it provides information on the mean degree of polymerization and constitutive flavanol units. Nevertheless, neither the polymer distribution nor the structures of the polymerized tannins can be characterized via this approach since all the constitutive units are cleaved. Reverse-phase HPLC-UV-MS is generally used to identify tannin molecules but it is effective only for the analysis of monomers and oligomers rather than polymeric tannins. Usually, polymeric tannins have to be removed in the sample preparation process, because their reverse-phase HPLC-UV profile always shows a distinct broad peak distributed across the chromatogram, i.e. the so-called “unsolved hump” (K. Chira, Schmauch, Saucier, Fabre, & Teissedre, 2009; T. Esatbeyoglu & Winterhalter, 2010; Habib, Platat, Meudec, Cheynier, & Ibrahim, 2014; Kohler, Wray, & Winterhalter, 2008; Ky & Teissedre, 2015; Tarascou, Souquet, Mazauric, Carrillo, Coq, Canon, et al., 2010; Travaglia, Bordiga, Locatelli, Coisson, & Arlorio, 2011). This unresolved broad peak has been attributed to the diversification of their DP, subunits, linkages regio- and stereoisomers of grape seed polymeric tannins. In recent years, attempts have been made to apply normal phase/HILIC HPLC to explore the higher polymerized tannin molecules (Tuba Esatbeyoglu, Wray, & Winterhalter, 2015; Wen Ma, Waffo-Teguo, Jourdes, Li, & Teissedre, 2016; R. J. Robbins, Leonczak, Johnson, Li, Kwik-Urbe, Prior, et al., 2009; Rebecca J. Robbins, Leonczak, Li, Johnson, Collins, Kwik-Urbe, et al., 2012). Unfortunately, an unresolved broad peak was again present in the end of the chromatograms for grape tannins, so the composition and structure of the hump remain elusive.

Mass spectroscopy (MS) is widely used for chemical analysis in viticulture and oenology. Coupled to liquid chromatography (LC) or gas chromatography (GC), it can efficiently identify complex compounds in grape and wine such as polyphenols, aromas and even pesticides (Flamini & TRALDI, 2010). For the analysis of polydisperse polymeric tannins without LC separation, matrix-assisted laser desorption/ionization–time-of-flight (MALDI-TOF) mass spectrometry is a popular approach owing to its advantages of producing only a singly charged molecular ion, reducing fragmentation and allowing detection of high mass with precision (De Marchi, Seraglia, Molin, Traldi, De Rosso, Panighel, et al., 2015; Monagas, Quintanilla-Lopez, Gomez-Cordoves, Bartolome, & Lebron-Aguilar, 2010; Yang & Chien, 2000). Molecular weight distribution of tannin fractions could also be assessed through MALDI-TOF MS analysis of protein-tannin complexes (Mane, Sommerer, Yalcin, Cheynier, Cole, & Fulcrand, 2007). Alternatively, ESI-MS/MS (electrospray ionization tandem mass spectrometry) has the advantages to identify tannins with more information of both their molecular ions and MS/MS fragment ions (Lin, Sun, Chen, Monagas, & Harnly, 2014). However, present together with oligomeric tannins in grape and wine samples, polymers are difficult to detect properly owing to not only ionization suppression in ESI-MS/MS but also low molar percentage for each isomeric compound (Fulcrand, Mané, Preys, Mazerolles, Bouchut, Mazauric, et al., 2008). Online two-dimensional LC (HILIC \times RP) coupled to ESI-Q-ToF offered an efficient solution to well isolate polymeric tannins before MS analysis (Kalili, Vestner, Stander, & de Villiers, 2013). Nevertheless, there remains a challenge for high MW tannins due to not sufficient absolute concentration polymers injected in the second dimensional LC.

Centrifugal partition chromatography (CPC) is a one type of countercurrent separation apparatus, providing chemists with efficient ways to work with complex matrixes. Nowadays, it has been widely used in the separation or purification of various natural products (Bisson, Brunel, Badoc, Da Costa, Richard, Merillon, et al., 2016; Slaghenaufi, Marchand-Marion, Richard, Waffo-Teguo, Bisson, Monti, et al., 2013). With the suitable system, it easily gets the target fraction or compound with a preparative scale. The aim of the present work was to develop an original CPC fractionation methodology to disentangle the unresolved HPLC hump and enrich the targeted polymeric tannins. Furthermore, the target compounds were available to be characterized by UHPLC-ESI-Q-TOF.

2. Methods

2.1 Reagents and materials

All the organic solvents (acetonitrile, methanol, glacial acetic acid, chloroform, ethyl acetate, ethanol and acetone) for extraction and separation were of analytical grade (Prolabo-VWR, Fontenays/Bois. France). Deionized water was purified with a Milli-Q water system (Millipore. Bedford. MA. USA). All the solvents for UHPLC-Q-TOF analysis were obtained from Fisher Scientific (Geel. Belgium). They were water (Optimal® LC/MS), methanol (Optimal® LC/MS) acetonitrile (Optimal® LC/MS) and formic acid (Optimal® LC/MS).

The grape sample was harvested in 2014 at the stage of ripening from the vineyard of appellation Saint-Emilion located in the Bordeaux vine growing region France. The grape variety was *Vitis vinifera* L. cv. Cabernet Sauvignon.

2.2 Preparation of polymeric tannins from grape seeds

Grape seeds were first removed from grape berries and lyophilized. Next, the samples ground in the grinder. 10 g sample powder was loaded into an ASE 350 accelerated solvent extraction system (Dionex Corporation. Sunnyvale. CA) with the cell of 34 mL. It was extracted with eight consecutive solid/liquid extractions (acetone/water = 80:20, v/v). Then, the extract was solubilized in 250 mL of water/ethanol (95:5, v/v) and extracted three times with chloroform ($v = 250$ mL) to remove lipophilic material. Finally, the aqueous phase was extracted three times with ethyl acetate ($v = 250$ mL) to obtain two phases. The organic and water fractions represented the oligomeric and polymeric tannins, respectively (Kleopatra Chira, Lorrain, Ky, & Teissedre, 2011). The crude extract used in the present study was the grape seed polymeric tannins located in the water fraction.

2.3 Purification of target compound

2.3.1 CPC apparatus

The CPC apparatus was an FCPC 200 provided by Kromaton Technologies (Saintes-Gemmes-sur-Loire, France). It consisted of a rotor (20 circular partition disks; total column capacity of 204 mL; 1320 partition cells), a binary high-pressure gradient pump (Gilson 321-H1), a high-pressure injection valve (20 mL sample loop, Rheodyne) and a Kromaton UV-Vis detector. Fractions were collected by a Gilson 204 fraction collector.

2.3.2 CPC solvent system selection

A small quantity of each CPC solvent candidate was prepared. For each candidate, 2 mg of polymeric tannins were dissolved in 1 mL of each of the two phases and vortexed. Then, an aliquot (200 μ L) of the upper and lower phase was taken individually, evaporated, dissolved in 1 mL of H₂O/MeOH (50:50, v/v) and analyzed by UHPLC-ESI-Q-TOF. The partition coefficient K_D was calculated by the ratio of peak area between the two phases of extracted ion chromatograms.

2.3.3 CPC separation conditions and procedures

Polymeric tannins were separated by the two-phase system ethyl acetate: acetone: water (2:2:1, v/v/v). For each injection, 500 mg of extract were dissolved in 10 mL of the upper and lower phases (50:50, v/v) of the system and 0.45 μ m filtered. Experiments were carried out in descending mode at 1000 rpm with a flow rate of 3 mL/min. The fraction collector was set to 3 min/tube. An aliquot (200 μ L) from the targeted tubes was taken, evaporated, dissolved in 1 mL of H₂O/MeOH (50:50, v/v) and analyzed by UHPLC-ESI-Q-TOF. When grouping the tubes, samples presenting the similar HPLC profiles were pooled together, evaporated in *vacuo*, suspended in water and freeze-dried. Nine fractions were obtained.

2.4 Identification of polymeric condensed tannins by high-resolution quadrupole time-of-flight mass spectrometry

The UHPLC analyses of K_D calculation and CPC fractions were carried out on a C18 UHPLC column (2.1 \times 50 mm, 1.8 μ m, Agilent Zorbax Eclipse plus, France) at a column temperature of 25 °C and a flow rate of 0.4 mL/min. The UHPLC-HRMS system used was an Agilent 1290 Infinity equipped with an ESI-Q-TOF mass spectrometer (Agilent 6530 Accurate Mass). The mobile phases were water (Eluent A) and acetonitrile (Eluent B), both containing 0.1% formic acid. The gradient of solvent B was as follows: 7% for 0.15 min; 7 to 14% for 1 min; 14 to 35% for 1.85 min; 35 to 50% for 0.3 min; 50 to 100% for 0.1 min and 100% for 0.5 min. The UHPLC column was equilibrated for 3 min using the initial condition before the next injection. UV detection was carried out at 280 nm.

The UHPLC analysis of polymeric tannins was performed on a C18 UHPLC column (2.1 \times 150 mm, 1.8 μ m, Agilent Zorbax Eclipse plus, France) at a column temperature of 25 °C. The mobile phases were water (Eluent A) and acetonitrile (Eluent B), both containing 0.1% formic acid. The flow rate was at 0.3 mL/min. The gradient of solvent B started at 4%, then went from 4 to 20% for 30 min, from 20 to 35% for 15 min, and finally reached 100% at 46 min and lasted for 3 min. The UHPLC column was equilibrated for 3 min using the initial condition before the next injection.

This UHPLC system was coupled to an ESI-Q-ToF-MS with an electrospray ion source with Agilent Jet Stream Technology. The mass spectrometer was operated in the extended dynamic range of 2 GHz (m/z 3200 Th). The nebulizer pressure and flow rate were set at 25 psi and 9 L/min, respectively. Drying gas temperature was 300°C. The sheath gas flow and temperature were set at 11 L/min and 350°C. The fragmentation, skimmer, OCT and capillary voltage were at 150 V, 65 V, 750 V and 4000 V, respectively. All analyses were performed in negative mode. The collision energies used for MS/MS analysis were 25 V, 30 V or 35 V for the different compounds. Instrument calibration was achieved by infusion of a TOF ESI Tune Mix solution (standard mix G1969-85000, Supelco Inc.) containing compounds having the following m/z values for the negative ionization mode: m/z 112.985587, 301.998139, 601.978977, 1033.988109, 1333.968947, 1633.949786, 1933.930624, 2233.911463, 2533.892301 and 2833.873139. Mass calibration had residual error for the expected masses between \pm 0.3 ppm. The data analysis was performed on Mass Hunter Qualitative Analysis software.

3. Results and discussion

3.1 Fractionation of polymeric tannins by CPC

According to our previous study (Wen Ma, Waffo-Teguo, Jourdes, Li, & Teissedre, 2016), the CPC solvent system ethyl acetate/ethanol/water (6:1:5, v/v/v) fractionates grape seed oligomeric tannins well according to their degree of polymerization (DP). In the case of polymeric tannins, a higher polarity index of the upper phase was required. Unfortunately, by increasing the proportion of ethanol in the ethyl acetate/ethanol/water mixture, we were unable to keep the two phases separate for CPC, so acetone was used instead. Several separation systems based on ethyl acetate/acetone/water (5:2:6; 3:1:3; 2:1:1; 2:2:1, v/v/v) were tested. The solvent ethyl acetate/acetone/water (2:2:1, v/v/v) was first excluded since this system was miscible. The partition coefficient K_D , calculated as the ratio of the compounds extracted ion chromatogram area in each phase is listed in Table S1. Since the ideal K_D ranges from 0.5 to 2, ethyl acetate/acetone/water (2:1:1, v/v/v) was chosen to be the CPC system in the present study. Since the targeted compounds were polymeric tannins with high polarity index, the descending CPC mode was used to fractionate the tannin products with the decrease in their DP.

Hence, polymeric tannins were subjected to CPC in descending mode using the separation solvent system ethyl acetate/acetone/water (2:1:1, v/v/v), and 44 tubes were collected. The application of CPC for polymeric tannin fractionation achieved a good yield, was not time-consuming and gave a high recovery thanks to the non-solid static phase. The composition in each tube was analyzed by UHPLC-Q-ToF and quantified with the extracted ion chromatogram area of each compound. The results are shown in Fig. 1. Nonamers were eluted first. Then, with the decrease in DP, octamers, heptamers, hexamers, pentamers, tetramers, trimers and dimers were eluted consecutively. As shown in Fig. 1B, monomers were eluted finally. The relative extracted ion chromatographic area of each compound in each tube indicated the ratio of chromatographic area in the present tube to the area of the most abundant tube. The relative areas of trimers and tetramers, with two peaks in tubes 3, 9 and tube 4, 6, respectively, are likely due to the chemical characteristics of their isomers.

As shown in Fig. 2., the reverse-phase UHPLC-UV profile of grape seed polymeric tannins (Fig. 2A) always showed a broad peak distributed across the chromatogram (retention time 2.4 mins-3.6 mins) as described in previous studies (T. Esatbeyoglu & Winterhalter, 2010; Kohler, Wray, & Winterhalter, 2008; Travaglia, Bordiga, Locatelli, Coisson, & Arlorio, 2011). Based on their UHPLC-UV chromatographic profiles, nine fractions were obtained by grouping some tubes together. After fractionation, sharp peaks were found in fraction 9 (Fig. 2B), fraction 8 (Fig. 2C), fraction 7 (Fig. 2D), fraction 6 (Fig. 2E) and fraction 5 (Fig. 2F). Monomers, dimers and trimers were mainly present in fraction 9, fraction 8, fraction 7, fraction 6 and fraction 5 (Table S2). Fraction 4 and fraction 3 were principally comprised of tetramers, pentamers and hexamers. Their UHPLC-UV chromatographic profiles proved to be obscure (Fig. 2G, Fig. 3H), perhaps owing to the diversities of polymeric tannins, involving isomers, subunits and subunit linkages. A broad peak around 3.7 mins appeared in the UV chromatograms of fraction 2 (Fig. 2I) and fraction 1 (Fig. 2K). The main products of fraction 2 (105.9 mg, 32.09%) were hexamers, heptamers, octamers, trimers and their galloylated derivatives. The first fraction was comprised of nonamers, decamers, undecamers and their galloylated derivatives, which represented most of the products (206.3 mg, 62.52%). Briefly, the CPC separation method

successfully separated grape seed polymeric tannins according to their DP and nine fractions were obtained.

3.2 Mass spectroscopic analysis of condensed tannins

Mass spectrometry is widely used to identify compounds by the ions of their molecules and fragments. High-resolution mass spectrometry (HRMS) provides more accurate information about the mass. The molar mass of condensed tannins derived from grape and wine can vary with the degree of polymerization, galloylation, glycosylation, B-ring trihydroxylation and linkages between flavan-3-ols (Fig. 3) (K. Chira, Schmauch, Saucier, Fabre, & Teissedre, 2009; Delcambre & Saucier, 2012). Their isomeric structures also vary with both the regioisomeric (order of linkage for the flavan-3-ols) and stereoisomeric (stereostructure of each flavan-3-ols subunit) forms.

As described in equation (1), the molecular mass (M) and molecular formula of condensed tannins can be calculated according to their DP (n), number of galloyl groups (a), number of glycosyl groups (b), number of (epi)galocatechins (c) and number of A-type bonds (d). The following equation could be widely used for condensed tannins derived from grape and wine. In the present study, only the tannin molecules with diversified DP, galloyl groups and linkages were discovered in cabernet sauvignon seeds.

$$M_{\text{tannins}} = n \times M_{\text{catechin}} - 2 \times (n-1) \times M_{\text{hydrogen}} + a \times M_{\text{galloyl group}} + b \times M_{\text{glycosyl group}} + c \times M_{\text{oxygen}} - 2 \times d \times M_{\text{hydrogen}} \quad (1)$$

$$\text{Molecular formula} = C_{15n+7a+6b} H_{12n+4a+10b-2d+2} O_{6n+4a+5d+c} \quad (2)$$

Results showed that with the increase in the DP of tannins, the peak signals of single-charged deprotonated ions ($[M-H]^-$) usually weakened and finally became mixed with the background noise in the mass. Nevertheless, more double- and triple-charged polymer ions ($[M-2H]^{2-}$, $[M-3H]^{3-}$) were found in the mass spectra. For tannins with even values of DP (such as 4, 6, 8, 10 etc.), it has been reported that the peak signal of double-charged tannin ions ($[M-2H]^{2-}$) and single-charged tannin ions with half of the DP ($[M/2-H]^-$) are usually unsolved owing to overlapping by adjacent intense $[M-2H]^{2-}$ ions (Hayasaka, Waters, Cheynier, Herderich, & Vidal, 2003). As shown in Fig. 4 (A), the ion peak at m/z 577.1329 corresponds to a single-charged B-type dimer (B-type-DP2, $[M-H]^-$). Another nearby ion peak at m/z 576.1243 was found to be a double-charged B-type tetramer (B-type-DP4, $[M-2H]^{2-}$). By examining the distance between the ^{12}C and ^{13}C isotope ions, the single/double-charged deprotonated molecular ion peaks were determined. Normally, $[M-H]^-$ have a distance of 1.00 amu between their isotopic ions, while a distance of 0.50 amu indicates the presence of $[M-2H]^{2-}$ ion. The ion peak at m/z 578.1371 and m/z 576.6241 was consistent with the isotopic single-charged B-type dimer (ISO-B-type-DP2, $[M-H]^-$) and the double-charged B-type tetramer (ISO-B-type-DP4, $[M-2H]^{2-}$), respectively. Another ion peak at m/z 575.1201 was consistent with the molecular mass of the proposed A-type dimer (A-type-DP2, $[M-H]^-$, error =1 ppm), which was located around 2 amu from the B-type dimer. Its isotopic ion peak overlapped with the double-charged B-type tetramer (B-type-DP4, $[M-2H]^{2-}$). Moreover, a weaker ion peak signal at m/z 579.1526 is proposed to correspond to two monomers with a single charge ($[2M-H]^-$). Hence, in the range between m/z 575 and 580, four compounds including B-type-DP2, B-type-DP4, A-type-DP2 and B-type-DP4 were determined.

Similarly, an example of tannin molecules with both even DP and even DG is shown in Fig. 4 (B). The ions of the molecules A-type-DP2-2G, B-type-DP4-4G, B-type-DP2-2G and $2 \times$ (B-type-DP1-G) were identified. Furthermore, attributed to the isotopic ion peak signal at m/z 879.6745, the ion peak at m/z 879.1737 is proposed to correspond not only to the single-charged A-type-DP2-2G but also to the double-charged A-type-DP4-4G. In addition, Fig. 4 (C), Fig. 4 (D) and Fig. 4 (E) show the corresponding molecules with their even (6, 8 and 10) and half values of DP (3, 4 and 5) in different states of charge.

In agreement with a previous report (Hummer & Schreier, 2008), most of the polymeric tannin molecules were assigned as multiple charged ions in the present study. Multiply charged ions offered us the possibility to explore the condensed tannins with a higher DP. Therefore, the negative ionization mode was chosen in this study since it produces more multiply charged ions than the positive mode (Mouls, Mazauric, Sommerer, Fulcrand, & Mazerolles, 2011). As illustrated in Fig. S1, multiply charged ions (m/z 1296.7842 and 864.1895) of nonamers were observed. The doubly charged and triply charged ions were diagnosed by carbon isotope ion spaces of 0.5 amu (Fig. S1C) and 0.33 amu (S Fig. S1B), respectively.

After molecular ion identification, the condensed tannins were further characterized by assigning their MS/MS fragments. Quinone methide fission (QM), retro-Diels–Alder fission (RDA, -152 Da) and heterocyclic ring fission (HRF, -126 Da) are three main fragmentation pathways of proanthocyanins (Li & Deinzer, 2007). Depending on the ion lost from the extension unit (-288 Da) or the terminal unit (-290 Da), quinone methide fission can be classified as QMe or QMt, respectively. The condensed tannin with DP14+5G may be considered as an example, its MS/MS fragment spectrum with the precursor m/z 1597.9748 ($[M-3H]^3$) being demonstrated in Fig 5. Several common fragments of condensed tannin were observed. QM cleavage led to the MS/MS fragment ions at m/z 287.0604 (DP1), 575.1171 (DP2), 729.1480 (DP2+G), 863.1814 (DP3), 1015.1728 (DP3+G), 1151.2510 (DP4) and 1303.2646 (DP4+G). RDA was another important fragmentation pathway of tannins, like m/z 423.0658 corresponding to the RDA fragment of the dimer. Nevertheless, since the loss of mass of both RDA and the galloyl group were m/z 152, some QM could be interpreted as the loss of fragments of QM and RDA, such as m/z 575.1171 (DP2+G), 863.1814 (DP3+G), 1151.2510 (DP4+G) and 1303.2646 (DP4+2G). The ions of m/z 413.0941 and 449.0888 corresponded to the HRF fission of the dimer. Furthermore, m/z 125.0231 often appeared in the fragments of tannins in negative ion mode, which corresponds to phloroglucinol formed from the fission.

3.3 Identification of polymeric tannins by UHPLC-ESI-Q-ToF

A better resolution of ion peaks in mass spectra was attributed to both the enrichment of the targeted molecules by CPC fractionation and the accuracy of the high-resolution mass spectrometry. The first fraction of CPC was injected into UHPLC-ESI-Q-ToF and the result was presented in Table 1. Molecular mass and formula were calculated by equation 1 and equation 2, respectively. The molecular identification was achieved by the “find compounds by formula” tool in the Agilent mass hunter qualitative analysis software.

All of the detected polymeric tannin molecular ion peaks were multiply charged (doubly, triply or quadruply), the monocharged ions were hardly observed. By applying ESI-Q-ToF, it was accessible to detect the multiple individual isotopic ions (Fig. 5). However, the most abundant ions were not always the monoisotopic ones. The recorded mass was referred to the isotopic mass detected with

the lowest mass value. The ppm differences between the measured and theoretical isotopic masses were under 9, which was higher than the oligomeric tannins we studied before.

The MS/MS fragment ions were recorded and assigned to various fragmentation pathways in Table 1. The space between QMt and QMe fragments was always 2 amu, as with the fragments of monomer (m/z 289 and 287), galloylated monomer (m/z 441 and 439), dimer (m/z 577 and 575), single galloylated dimer (m/z 729 and 727); trimer (m/z 865 and 863); single galloylated trimer (m/z 1017 and 1015) and tetramer (m/z 1153 and 1151). Meanwhile, RDA fission and neutral loss of molecule H_2O (18 Da) often occurred with QM, which led to fragments like m/z 425 [M-H-152]⁻, 423 [(M-H-152)⁻], 407 [M-H-152- H_2O]⁻, 405 [M-H-152- H_2O]⁻, 695 [M-H-152- H_2O]⁻, 1151 [M-H-152]⁻, 1015 [M-H-152]⁻, 1303 [M-H-152]⁻. Similarly, HRF fission and the neutral loss of molecule H_2O (18 Da) interpreted the fragment ions of 451 [M-H-126]⁻, 449 [M-H-126]⁻, 433 [M-H-126- H_2O]⁻, 431 [M-H-126- H_2O]⁻, 415 [M-H-126-2 × H_2O]⁻, 413 [M-H-126-2 × H_2O]⁻, 739 [M-H-126]⁻, 737 [M-H-126]⁻. The fragment ions at 437 [M-H-122- H_2O]⁻, 417 [M-H-122-2 × H_2O]⁻, 419 [M-H-122-2 × H_2O]⁻, 1013 [M-H-122- H_2O]⁻, 1029 [M-H-122- H_2O]⁻, 1605 [M-H-122- H_2O]⁻ were attributed to benzofuran-forming (BFF) fission with the loss of m/z 122. Eventually, 50 molecules were identified with molecular mass ranging from 1730 to 6067. Their DP and DG ranged from 6 to 20 and from 0 to 11, respectively.

4. Conclusions

The broad peak of grape seed polymeric tannins routinely found with HPLC-UV was dissembled by an original CPC separation methodology with the solvent system of ethyl acetate/acetone/water (2:2:1, v/v/v). The polymeric tannins from *Vitis vinifera* L. cv. Cabernet Sauvignon were fractionated according to their DP. Thanks to polymer enrichment in the CPC fraction, polymeric tannins were characterized by UHPLC-Q-ToF. The condensed tannins present in Cabernet Sauvignon seed extract have a broad range of DP and DG up to 20 and 11, respectively. The largest molecule mass reached 6067. To our knowledge, this is the first report to offer an insight into the broad peak found with grape seed polymeric tannins on HPLC and to characterize the grape tannins on the basis of HRMS and MS/MS data.

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References

- Bisson, J., Brunel, M., Badoc, A., Da Costa, G., Richard, T., Merillon, J. M., & Waffo-Teguo, P. (2016). Hyphenating Centrifugal Partition Chromatography with Nuclear Magnetic Resonance through Automated Solid Phase Extraction. *Analytical Chemistry*, 88(20), 9941-9948.
- Chira, K., Lorrain, B., Ky, I., & Teissedre, P.-L. (2011). Tannin Composition of Cabernet-Sauvignon and Merlot Grapes from the Bordeaux Area for Different Vintages (2006 to 2009) and Comparison to Tannin Profile of Five 2009 Vintage Mediterranean Grapes Varieties. *Molecules*, 16(2), 1519-1532.
- Chira, K., Schmauch, G., Saucier, C., Fabre, S., & Teissedre, P. L. (2009). Grape variety effect on proanthocyanidin composition and sensory perception of skin and seed tannin extracts from

- Bordeaux wine grapes (cabernet Sauvignon and merlot) for two consecutive vintages (2006 and 2007). *Journal of Agricultural and Food Chemistry*, 57(2), 545-553.
- De Marchi, F., Seraglia, R., Molin, L., Traldi, P., De Rosso, M., Panighel, A., Dalla Vedova, A., Gardiman, M., Giust, M., & Carraro, R. (2015). Characterization of seed proanthocyanidins of thirty-two red and white hybrid grape varieties. *VITIS-Journal of Grapevine Research*, 54(3), 121-128.
- Delcambre, A., & Saucier, C. (2012). Identification of new flavan-3-ol monoglycosides by UHPLC-ESI-Q-TOF in grapes and wine. *Journal of Mass Spectrometry*, 47(6), 727-736.
- Esatbeyoglu, T., & Winterhalter, P. (2010). Preparation of dimeric procyanidins B1, B2, B5, and B7 from a polymeric procyanidin fraction of black chokeberry (*Aronia melanocarpa*). *Journal of Agricultural and Food Chemistry*, 58(8), 5147-5153.
- Esatbeyoglu, T., Wray, V., & Winterhalter, P. (2015). Isolation of Dimeric, Trimeric, Tetrameric and Pentameric Procyanidins from Unroasted Cocoa Beans (*Theobroma cacao* L.) Using Countercurrent Chromatography. *Food Chemistry*, 179, 278-289.
- Flamini, R., & TRALDI, P. (2010). *Mass spectrometry in grape and wine chemistry*. (Vol. 22).
- Fulcrand, H., Mané, C., Preys, S., Mazerolles, G., Bouchut, C., Mazauric, J. P., Souquet, J. M., Meudec, E., Li, Y., Cole, R. B., & Cheynier, V. (2008). Direct mass spectrometry approaches to characterize polyphenol composition of complex samples. *Phytochemistry*, 69(18), 3131-3138.
- Ge, Y. W., Zhu, S., Kazuma, K., Wei, S. L., Yoshimatsu, K., & Komatsu, K. (2016). Molecular ion index assisted comprehensive profiling of B-type oligomeric proanthocyanidins in rhubarb by high performance liquid chromatography-tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, 408(13), 3555-3570.
- Habib, H. M., Platat, C., Meudec, E., Cheynier, V., & Ibrahim, W. H. (2014). Polyphenolic compounds in date fruit seed (*Phoenix dactylifera*): Characterisation and quantification by using UPLC-DAD-ESI-MS. *Journal of the Science of Food and Agriculture*, 94(6), 1084-1089.
- Hayasaka, Y., Waters, E. J., Cheynier, V., Herderich, M. J., & Vidal, S. (2003). Characterization of proanthocyanidins in grape seeds using electrospray mass spectrometry. *Rapid Communications in Mass Spectrometry*, 17(1), 9-16.
- Hummer, W., & Schreier, P. (2008). Analysis of proanthocyanidins. *Molecular Nutrition and Food Research*, 52(12), 1381-1398.
- Kalili, K. M., Vestner, J., Stander, M. A., & de Villiers, A. (2013). Toward unraveling grape tannin composition: application of online hydrophilic interaction chromatography x reversed-phase liquid chromatography-time-of-flight mass spectrometry for grape seed analysis. *Analytical Chemistry*, 85(19), 9107-9115.
- Kennedy, J. A., Saucier, C., & Glories, Y. (2006). Grape and wine phenolics: history and perspective. *American Journal of Enology and Viticulture*, 57(3), 239-248.
- Kennedy, J. A., & Taylor, A. W. (2003). Analysis of proanthocyanidins by high-performance gel permeation chromatography. *Journal of Chromatography A*, 995(1), 99-107.
- Kohler, N., Wray, V., & Winterhalter, P. (2008). Preparative isolation of procyanidins from grape seed extracts by high-speed counter-current chromatography. *Journal of Chromatography A*, 1177(1), 114-125.
- Ky, I., & Teissedre, P. L. (2015). Characterisation of Mediterranean grape pomace seed and skin extracts: polyphenolic content and antioxidant activity. *Molecules*, 20(2), 2190-2207.
- Li, H.-J., & Deinzer, M. L. (2007). Tandem mass spectrometry for sequencing proanthocyanidins. *Analytical Chemistry*, 79(4), 1739-1748.
- Lin, L. Z., Sun, J., Chen, P., Monagas, M. J., & Harnly, J. M. (2014). UHPLC-PDA-ESI/HRMSn profiling method to identify and quantify oligomeric proanthocyanidins in plant products. *Journal of Agricultural and Food Chemistry*, 62(39), 9387-9400.
- Ma, W., Guo, A., Zhang, Y., Wang, H., Liu, Y., & Li, H. (2014). A review on astringency and bitterness perception of tannins in wine. *Trends in Food Science and Technology*, 40(1), 6-19.

- Ma, W., Waffo-Teguo, P., Jourdes, M., Li, H., & Teissedre, P.-L. (2016). Chemical Affinity between Tannin Size and Salivary Protein Binding Abilities: Implications for Wine Astringency. *PLoS ONE*, 11(8), e0161095.
- Mane, C., Sommerer, N., Yalcin, T., Cheynier, V., Cole, R. B., & Fulcrand, H. (2007). Assessment of the molecular weight distribution of tannin fractions through MALDI-TOF MS analysis of protein-tannin complexes. *Analytical Chemistry*, 79(6), 2239-2248.
- Monagas, M., Quintanilla-Lopez, J. E., Gomez-Cordoves, C., Bartolome, B., & Lebron-Aguilar, R. (2010). MALDI-TOF MS analysis of plant proanthocyanidins. *Journal of Pharmaceutical and Biomedical Analysis*, 51(2), 358-372.
- Mouls, L., Mazauric, J. P., Sommerer, N., Fulcrand, H., & Mazerolles, G. (2011). Comprehensive study of condensed tannins by ESI mass spectrometry: average degree of polymerisation and polymer distribution determination from mass spectra. *Analytical and Bioanalytical Chemistry*, 400(2), 613-623.
- Rasines-Perea, Z., & Teissedre, P.-L. (2017). Grape Polyphenols' Effects in Human Cardiovascular Diseases and Diabetes. *Molecules*, 22(1), 68.
- Rigaud, J., Perez-Illarbe, J., Da Silva, J. M. R., & Cheynier, V. (1991). Micro method for the identification of proanthocyanidin using thiolysis monitored by high-performance liquid chromatography. *Journal of Chromatography A*, 540(C), 401-405.
- Robbins, R. J., Leonczak, J., Johnson, J. C., Li, J., Kwik-Urbe, C., Prior, R. L., & Gu, L. (2009). Method performance and multi-laboratory assessment of a normal phase high pressure liquid chromatography-fluorescence detection method for the quantitation of flavanols and procyanidins in cocoa and chocolate containing samples. *Journal of Chromatography A*, 1216(24), 4831-4840.
- Robbins, R. J., Leonczak, J., Li, J., Johnson, J. C., Collins, T., Kwik-Urbe, C., & Schmitz, H. H. (2012). Determination of Flavanol and Procyanidin (by Degree of Polymerization 1–10) Content of Chocolate, Cocoa Liquors, Powder(s), and Cocoa Flavanol Extracts by Normal Phase High-Performance Liquid Chromatography: Collaborative Study. *Journal of AOAC International*, 95(4), 1153-1160.
- Slaghenaufi, D., Marchand-Marion, S., Richard, T., Waffo-Teguo, P., Bisson, J., Monti, J. P., Merillon, J. M., & De Revel, G. (2013). Centrifugal partition chromatography applied to the isolation of oak wood aroma precursors. *Food Chemistry*, 141(3), 2238-2245.
- Tarascou, I., Souquet, J. M., Mazauric, J. P., Carrillo, S., Coq, S., Canon, F., Fulcrand, H., Cheynier, V., & . (2010). The hidden face of food phenolic composition. *Archives of Biochemistry and Biophysics*, 501(1), 16-22.
- Travaglia, F., Bordiga, M., Locatelli, M., Coisson, J. D., & Arlorio, M. (2011). Polymeric proanthocyanidins in skins and seeds of 37 *Vitis vinifera* L. cultivars: a methodological comparative study. *Journal of Food Science*, 76(5), C742-749.
- Yang, Y., & Chien, M. J. (2000). Characterization of grape procyanidins using high-performance liquid chromatography/mass spectrometry and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Journal of Agricultural and Food Chemistry*, 48(9), 3990-3996.

Figure captions

Fig. 1. Relative extracted ion chromatographic areas of condensed tannins in CPC collector tubes (* Relative extracted ion chromatographic area of each compound is percentage ratio of chromatographic area in present tube to the area of the most abundant tube.)

Fig. 2. HPLC-UV chromatograms of grape seed polymeric tannins and its fractions separated by CPC (Absorbance: 280 nm)

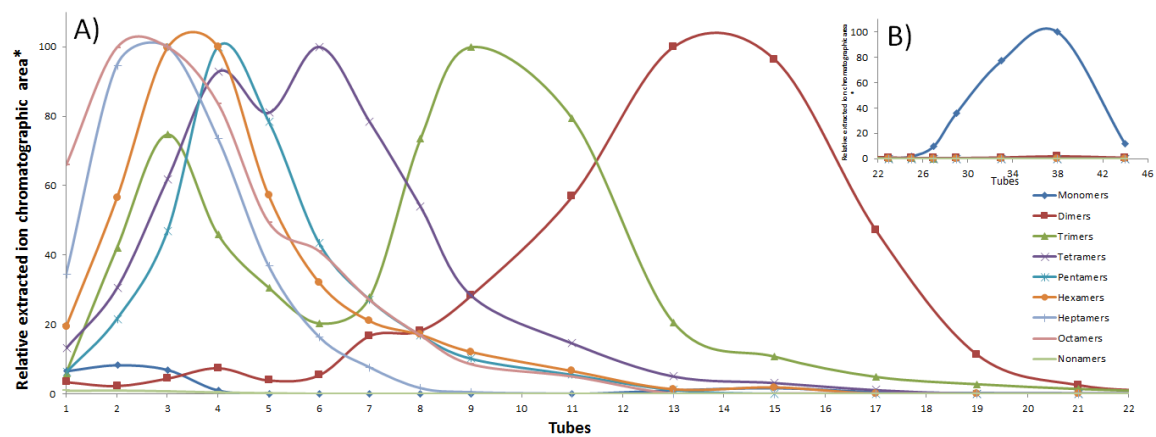
Fig. 3. Structures of dimeric condensed tannins present in grape and wine (R1 = H/OH; R2 = H/glycosyl group/galloyl group)

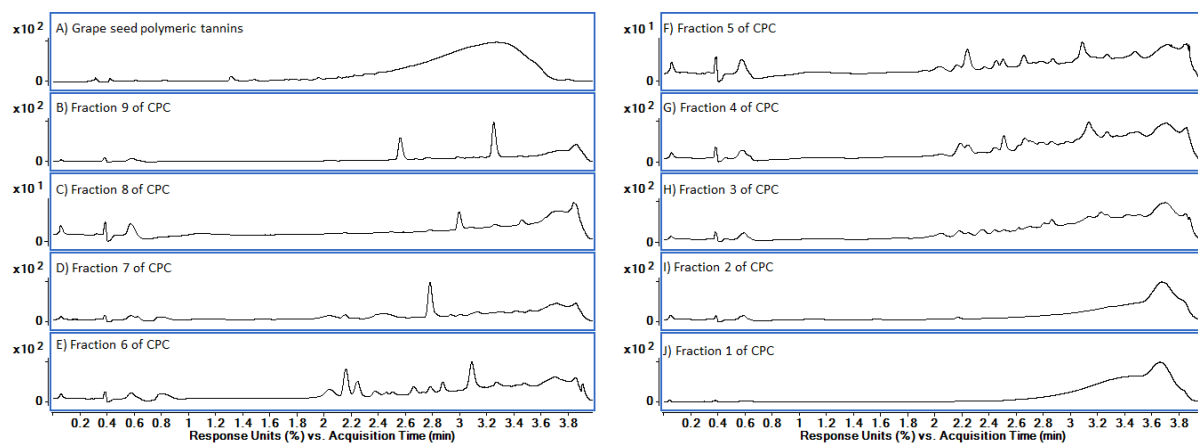
Fig. 4. Mass spectra to illustrate A/B type, isotopic and ion charge state distributions for (A) dimers and tetramers, (B) dimer-*O*-gallate, tetramer-di-*O*-gallate and monomers-*O*-gallate, (C) trimers and hexamers, (D) tetramers and octamers, (E) pentamers and decamers

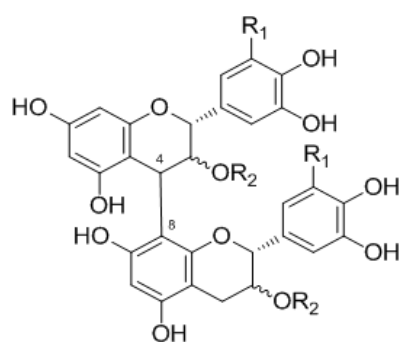
Fig 5 MS/MS fragment spectrum of polymeric tannin with DP14 and 5G

Table 1 HRMS data for polymeric condensed tannins found in first CPC fraction

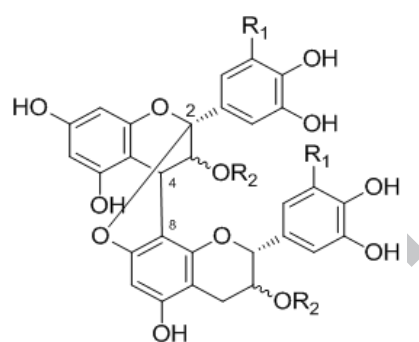
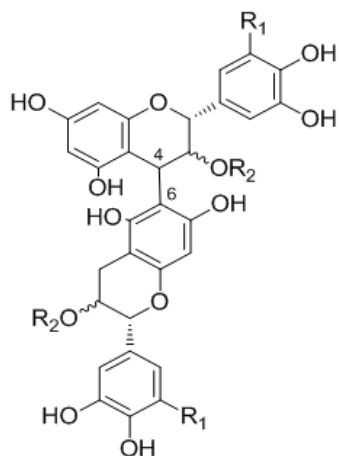
(Mass values in bold represent precursor of MS/MS analysis; *MW was calculated by referring to equation 1. Accurate masses of carbon, hydrogen and oxygen were 12.0000, 1.0078 and 15.9949, respectively; ** Doubly charged ions)



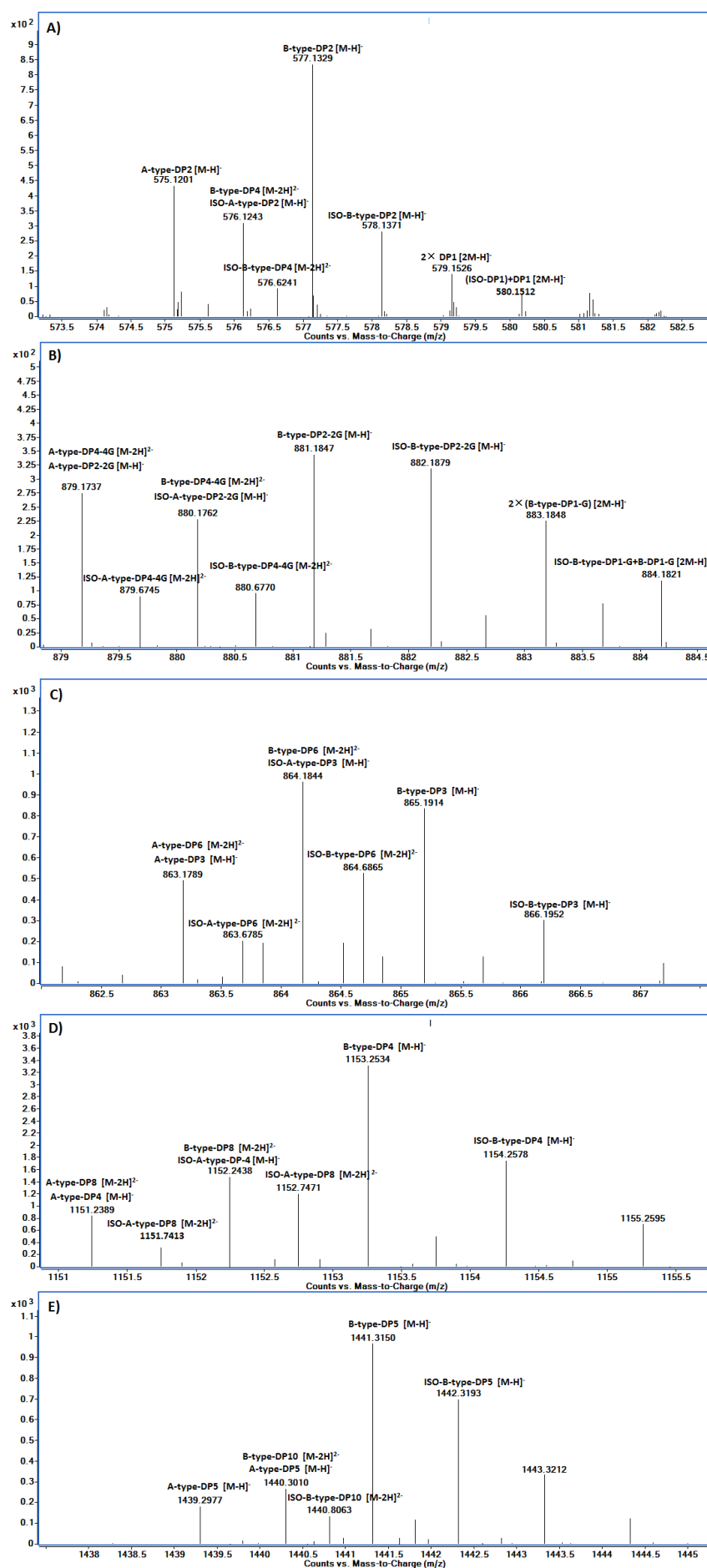




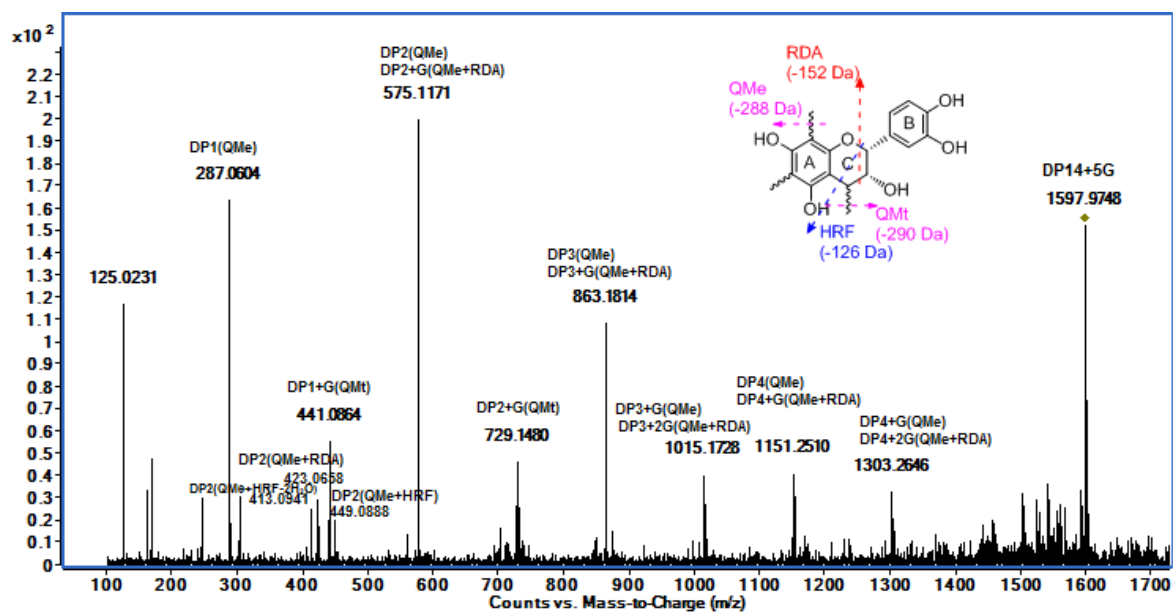
B-type dimeric condensed tannin



A-type dimeric condensed tannin



ACCEPTED MANUSCRIPT



D P	D G	Formula	M W*	RT	(M- 2H) ⁻²	(M- 3H) ⁻³	(M- 4H) ⁻⁴	Identification of MS/MS fragments				
					(m ns)	(ppm)	(ppm)	QMt/QMt+ RDA	QMe/QMe+R DA	QMt+HRF	QMe+ HRF	BF F
6	0	C90H74O 36	17 30	21. 4	864.1 926	nd	nd	289;407;425 ;	287;405;575; 863	451	413;4 49	12 5
					(2.17)			577;695				
6	1	C97H78O 40	18 82	31. 2	940.6 956	nd	nd	577		575;727;1151	413	
					(-2.39)							
6	2	C104H82 O44	20 34	28. 6	1016. 1996	nd	nd	289;407;577	287;405;439; 575	449		
					(-1.85)							
6	3	C111H86 O48	21 86	27. 5	1092. 2076	nd	nd	289;407;407		287;405;423; 439;575	449	41 7
					(0.39)							
7	0	C105H86 O42	20 18	27. 0	1008. 7206	nd	nd	289;415		287;575;863**	413;4 37	28 7
					(-3.44)							
7	1	C112H90 O46	21 70	18. 1	1084. 7292	nd	nd	289;407;425 ;	287;405;423; 575;	413;449	287	
					(-0.32)			577;1017	863;1015			
7	2	C119H94 O50	23 22	22. 4	1160. 2321	773.1 507	nd	289;407;577 ;	287;405;575; 727;	449;413	125	
					(-1.13)	(-3.14)		1017;425	439;423;863;			
7	3	C126H98 O54	24 74	22. 7	1236. 241	nd	nd	289;407;425 ;	287;405;575; 727;	413		
					(1.72)			577;1017	863;1015			
7	4	C133H10 O58	26 26	24. 7	1312. 2617	874.5 008	nd	289;425;577 ;865	287;439;575; 863;	413		
					(13.26)	(7.96)			1303			
8	0	C120H98 O48	23 07	18. 1	1152. 2525	nd	nd	289;425;577 ;865	287;405;423; 575;	413;449	287	
					(-1.36)				863			
8	1	C127H10 O52	24 59	25. 6	1228. 2558	818.5 08	nd	289;425;577 ;	287;405;423; 439;	451	413;4 49	12 5
					(-3.05)	(4.99)		865;1152**	575;1015			
8	2	C134H10	26	22.	1304.	869.1	nd	407;577;865	287;575;423;	413;449	287	

		6056	11	1	2647	712		;1017	727;			
					(-0.26)	(-3.57)			863;1015			
8	4	C148H11 4064	29 15	31. 2	1456. 2737	970.8 512	nd	407;441;425 ;	287;423;575; 727;	451		
					(-1.61)	(1.89)		577;1016**	863;693			
8	8	C176H13 0080	35 23	34. 8	1762. 8559	1173. 3052	nd	289;407;577 ;	287;405;575; 863	451	413;4 49	28 7
					(0.55)	(-7.81)		885;729				
9	0	C135H11 0054	25 95	27. 1	1296. 2779	863.8 542	nd	289;407;425 ;577;	287;405;423; 575;	413;449	287	
					(-6.06)	(-0.71)		865;1153		863;1151;1439		
9	1	C142H11 4058	27 47	18. 8	1372. 2908	914.5 247	nd	865;711;137 2	287;727;863; 1151	413		
					(-0.37)	(-0.38)						
9	2	C149H11 8062	28 99	29. 9	1448. 2883	965.5 303	nd	577;1153		287;423;439;	1025	
					(-5.84)	(0.48)				575;863;1151		
9	3	C156H12 2066	30 51	26. 1	1524. 304	1015. 8689	nd	289;1008**; 1092**	287;439;575	413;4 49	43 7	
					(1.13)	(3.15)						
9	4	C163H12 6070	32 03	30. 5	1600. 305	1066. 5308	nd	289;441;577 ;864**;	287;575;423; 1151			
					(-0.95)	(-4.95)		1153;1305				
9	5	C170H13 0074	33 55	34. 8	1676. 2981	1117. 2092	nd	289;577;661 ;	287;575;727	413		
					(-8.98)	(2.54)		1016**				
1 0	1	C157H12 6064	30 35	34. 3	1516. 1010. 544	8274	nd	289;407;441 ;	287;423;575; 863			
					(1.84)	(-2.19)		577;713				
1 0	2	C164H13 0068	31 87	30. 2	1592. 1061. 2087	8302	nd	289;407; 729;840	287;575;439	1023		
					(0.06)	(-7.36)						
1 0	3	C171H13 4072	33 39	29. 3	1669. 1111. 8892	3315	nd	289	287;405;	41 3		
					(-3.4)	(2.07)			575;1015			
1 0	5	C185H14 2080	36 43	27. 8	1821. 1213. 2283	3531	nd	289;407;425 ;	287;405; 439;575	413		
					(2.7)	(0.67)		713;1016				

1	0	C165H13	31	31.	1584.	1055.	nd	289;407	287;405;	41
1		4066	71	8	8537	8927			575;863	3
					(1.78)	(4.08)				
1	4	C193H15	37	35.	1888.	1258.	nd	407;441;577	287;423; 575;	413
1		0082	79	6	3571	9194		;	439;863;1015	
					(-7.4)	(5.3)		729;1017;88		
								1		
1	5	C200H15	39	33.	nd	1309.	nd	289;577;729	287;575;	
1		4086	31	1		2512		;865	423;863	
						(1.95)				
1	0	C180H14	34	29.	nd	1151.	nd	289;407;865	287;405;423;	449
2		6072	59	8		909			575;863	
						(-7.91)				
1	1	C187H15	36	36.	nd	1202.	nd	289;407	287;405;575	41
2		0076	11	1		5828				3
						(-4.76)				
1	5	C215H16	42	35.	2108.	1405.	nd	289;407;577	287;575;863	449
2		6092	19	8	9215	2614		;425		43
					(5.49)	(-5.93)				7
1	2	C209H16	40	32.	nd	1349.	nd	289;577;729	287;405;575;	413;449;1329
3		6086	51	9		2746		;	727;863;	
						(-3.96)		865	1015;1151;13	
									03	
1	3	C216H17	42	32.	nd	1399.	nd	289;407;577	287;423;439;	413;449
3		0090	03	4		9454		;	575;	287
						(-3.44)		865	863;1015;115	
									1;1303	
1	5	C230H17	45	33.	nd	1501.	nd	289;407;425	287;405;423;	413;449;737
3		8098	07	1		9678		;	439;	
						(5.34)		441;577;729	575;863;1015	
									;1439	
1	6	C237H18	46	30.	nd	1552.	nd	441;577;865	287;439;575;863;	1161
3		20102	59	4		6327			1015;1151	41
						(1.68)				3
1	5	C245H19	47	35.	nd	1597.	nd	441;577;729	287;423;575;727;	413;4
4		00104	95	4		3073		;		49
						(2.95)		865;1017	863;1015;1151;130	57
									3	5
1	5	C260H20	50	36.	nd	1693.	nd	289;729	287;575;863	
5		20110	83	4		3292				
						(-2.36)				

1	8	C281H21	55	30.	nd	1845.	nd	577;864**	287;423;575;	
5		4O122	39	8		3434			863;1151	
						(-0.4)				
1	6	C282H21	55	34.	nd	1840.	nd	729	287;575;	16
6		8O120	23	6		0384			863;1015	05
						(7.51)				
1	1	C310H23	61	36.	nd	nd	1531.	441;865	287;423; 439;575;	
6	0	4O136	31	9		7886			918***;1303	
						(7.21)				
1	6	C297H23	58	35.	nd	1936.	1452.	289;577;729	287;405;575;	449
7		0O126	11	8		3668	0225	;	727; 863	
						(-6.94)	(-5.36)	865		
1	7	C304H23	59	31.	nd	nd	1489.	289;407;577	287;423;439;	41
7		4O130	63	9		7773		;	575;727;863;1151	3
						(-5.28)	865			
1	8	C311H23	61	33.	nd	nd	1527.	289;425;577	287;439;575;	41
7		8O134	15	3		7938		;	727;863	3
						(3.85)	865			
1	9	C318H24	62	37.	nd	nd	1565.	289;729;115	287;423;575;	449
7		2O138	67	0		2981	2**	863	1013	
						(3.63)				
1	1	C347H26	68	36.	nd	nd	1715.	577	287;575;727;	41
8	1	2O152	59	7		3144			863;1151	3
						(-1.4)				
1	1	C292H23	56	38.	nd	1875.	nd	425	287;575;709;863;	41
9		4O118	27	1		4005			1015;1710	9;
						(-2.54)				10
										29
1	5	C320H25	62	33.	nd	nd	1558.	289;577;729	287;575;863;	44
9		0O134	35	7		0494		;	1015;1151	9
						(8.27)	865;1017;11			
							53			
1	7	C334H25	65	36.	nd	nd	1633.	441;577;965	863	
9		8O142	39	9		8198				
						(1.75)				
1	9	C348H26	68	33.	nd	nd	1709.	289;577;	439;575;863;	431
9		6O150	43	1		8162		864**;1152	1015;1555	
						(-3.6)	**			
2	2	C314H25	60	39.	nd	2021.	1516.	729**	297;439;575;863;	413
0		0O128	67	3		433	8144			

Highlights

- A fractionation method was developed to disentangle the HPLC hump of seed tannins.
- UHPLC-Q-ToF was used to characterize polymeric tannins by HRMS and MS/MS data.
- Identified grape seed tannins have a range of polymerization degree up to 20.
- Identified grape seed tannins have a range of galloylation degree up to 11.