Influence of berry ripeness on seed tannins extraction in wine

Pauline Rousserie, Soizic Lacampagne, Sandra Vanbrabant, Amélie Rabot, Laurence Geny-Denis

Version of Record: https://www.sciencedirect.com/science/article/pii/S0308814620301564
Manuscript_a387d1617da3ddfbcd329ec48149a885

Influence of berry ripeness on seed tannins extraction in wine

Pauline ROUSSERIE [a] *, Soizic LACAMPAGNE [a], Sandra VANBRABANT [a], Amélie RABOT [a], Laurence GENY-DENIS [a]

[a] Université de Bordeaux, Unité de recherche Œnologie, EA 4577, USC 1366 INRA, ISVV, 33882 Villenave d'Ornon Cedex, FRANCE

Pauline ROUSSERIE : pauline.rousserie@u-bordeaux.fr - +(33) 6.31.93.57.46

Soizic LACAMPAGNE : soizic.lacampagne@u-bordeaux.fr

Sandra VANBRABANT : sandra.vanbrabant@u-bordeaux.fr

Amélie RABOT: amelie.rabot@u-bordeaux.fr

Laurence GENY-DENIS: laurence.geny-denis@u-bordeaux.fr

Corresponding author: Pauline ROUSSERIE (pauline.rousserie@u-bordeaux.fr)

ABSTRACT

The extraction of seed and skin tannins in wine has been investigated at three different grape maturity stages. For that, the tannins content and composition of seeds and skins at three different maturity stages were characterized. After that, an original approach of nanovinification was conducted. At each maturity stages, three winemaking modalities have been produced: (i) a control modality, (ii) a seed modality made of exclusively with seed and (iii) a skin modality made of exclusively with skins.

The aim of this work is to describe and explain the seed tannins kinetics release in wine but also the impact of grape maturity on seed tannins extractability. For that, the evolution of seed and wine tannins content have been followed during the winemaking, from alcoholic fermentation to post-fermentative maceration.

Keywords: Wine, Tannins, Seeds, Skins, Extractibility, *Vitis vinifera*

1. INTRODUCTION

Tannins are known to be one of the most determinants of the quality of red wine. Their importance in red wine is linked to their participation of wine sensory attributes such as colour, mouthfeel, astringency and bitterness. Condensed tannins also called proanthocyanidins (PAs) are found in both skins and seeds berry (Bordiga et al., 2011; Ćurko et al., 2014; Downey et al., 2003; Lorrain et al., 2011; Mattivi et al., 2009; Monagas et al., 2003; Obreque-Slier et al., 2010, 2012), and in smaller amount, in pulp (Bindon et al 2010, 2014; Mané et al., 2017; Sparrow et al., 2015). The quantity and the structure of PAs differ with their location in grape tissue and with the berry developmental stage (Rousserie et al., 2019). The extraction of grape phenolic compounds in wine during fermentation differs according to their origins (Bindon et al., 2014). Generally, it is well accepted that skins PAs are more easily extracted in the must during winemaking than the seeds one. Indeed, their diffusion is known to be directly linked with the disruption of cell walls which allows vacuoles, containing tannins, to release their content into the must. In other words, the diffusion of skin PAs in wine is essentially a diffusion process. On the other hand, seed PAs extraction in wine require more time contact than skin PAs. If it is true that the scientific community have found an agreement on this point, it is still not clear why extraction takes more time. According to different studies, it can be due to the apparition of ethanol, to the rehydration of seeds, to the seed maturity degree or both (Bindon et al., 2014; Canals et al., 2005; Federico Casassa et al., 2013; Hernandez-Jimenez et al., 2012). Even though the amount of PAs at the beginning of winemaking represents the main variable which will affect the amount of tannins extracted into wine, all researches conducted on seeds PAs extraction have not taken it in account.

The main aim of this work was to study the relation between the tissue origins of berry PAs (skin and seed), the maturity stage of the berry and the composition of extracted tannins in

wine. In this purpose, the present study focuses on Merlot grapes of the vintage 2018 at three different maturity stages (under-ripeness, ripeness and over-ripeness) using °Brix as a maturity indicator. In order to better understand the seed tannins extraction in wine, an original approach of nanovinification has been set up that allows to follow the evolution of tannins concentration in wine, but also in seeds. At each maturity stages, three winemaking modalities have been produced: (i) a control modality, (ii) a seed modality made of exclusively with seed and (iii) a skin modality made of exclusively with skins. After having analysed the tannin composition of fresh seeds, the tannin composition of seeds and wine have been characterized at four points of the winemaking (the middle of the alcoholic fermentation and its end and the middle of the post-fermentative maceration and its end).

2. MATERIAL AND METHODS

2.1. Materials

Standards of (+)-catechin (CAS. 154-23-4, MW 290,28), (-)-epicatechin (CAS. 490-46-0, MW 290,28) and (-)-epicatechin-3-*O*-gallate (CAS. 1257-08-5, MW 442,37) were acquired from Extrasynthese (Genay, France). Phloroglucinol (CAS. 108-73-6, MW 126.11), ascorbic acid (CAS. 50-81-7, MW 176,12) and sodium acetate (CAS. 127-09-3, MW 82,03) were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). Methanol (HPLC grade) and Hydrochloric Acid (HPLC grade) were purchased from VWR (Fontenay sous bois, France), Acetic acid (HPLC grade) was purchased from Fisher Scientific (Illkirch, France). Deionized water was purified with Milli-Q water system (Millipore, Darmstadt, Germany). 0.45-µm pore size syringe filter were acquired from Roth (Lauterbourg, France).

2.2.Grape samples

Grapevine berries of *Vitis vinefera* L. *cv* Merlot were collected using a completely randomized design from a commercial vineyard during the 2018 season. Edge rows and the first two vines in a row were avoided. Clusters were collected from the top, middle and bottom of the vine. Berries were collected at three different maturity stages: under-ripeness, commercial ripeness and over-ripeness. The different maturity stages were determined in function of the harvest date chosen by the vineyard: under-ripeness berries were collected one week before the harvest date and the over-ripeness berries were collected one week after the harvest date.

Approximatively 20 kilograms of berries were collected at these three different maturity stages. Three groups of 100 berries randomly selected, were frozen in liquid nitrogen upon collection in the field and stored at -80°C until analysed. The remaining samples were used to make wine.

For each maturity stages, three groups of twenty fresh berries were weighted. After, seeds and skins were separated and weighted. The number of seeds per berry was noted. In order to estimate the volume of juice for one hundred berries, three groups of one hundred berries were destemmed and crushed. The collected juice was used to estimate the maturity stage of grape. The parameters total soluble solids (TS) (° Brix), potential alcohol content, titrable acidity ($g.L^{-1}$), pH value, tartaric acid ($g.L^{-1}$) and malic acid ($g.L^{-1}$) were measured with a WineScan^{TM} Flex (Foss, Hilleroed, Denmark) coupled to Foss Integrator 2 software (version 2.0.2).

2.3.Winemaking

Wines were elaborated at Plateau de Vinification of the Institut des Sciences de la Vigne et du Vin (University of Bordeaux), according to the method « Specific nanovinification to determinate the origin of wine tannins ».

In order to evaluate the effect of each berry tissue component in wine, three winemaking modalities have been produced in duplicate for each maturity stages: a modality control, a modality seed and a modality skin.

All experiments have been made into 1 L fermenters. Fermentations were performed with a *Saccharomyces cerevisiae* yeast inoculum of strain Actiflore F33 (Laffort, Bordeaux, France) at 10 $g.hL^{-1}$. During fermentation, density at 20°C was measured directly with a digital densimeter (Anton Paar, model DMA 35).

During winemaking four samples were made on each modality (berry, seed and skin wine): one at half alcoholic fermentation (density = 1.030), one at the end of alcoholic fermentation (density = 0,990), one at the middle of the post-fermentative maceration (approximately one week after the end of the alcoholic fermentation) and one at the end of the post-fermentative maceration (approximately two weeks after the end of the alcoholic fermentation). For each

sample, 20 mL of wine were collected. In order to keep the ratio vegetal material:juice stable, at each sample, the correspondent amount of berries, seeds and skin were respectively removed from the control modality, the seed modality and the skin modality.

2.4.Extract of phenolic compounds

After having manually removed seeds from grapes and from fermenters, they were washed four times with distilled water. Seeds were ground into a ball grinder. Phenolic compounds of 500 mg of the resulted powder were extracted twice using 20 mL of methanol/hydrochloric acid (99:1, v/v) each times in a closed Erlenmeyer. After maceration for 3 h at 20 °C in the dark and under mechanical stirring, the extract was subjected to ultrasonic bath during 10 min. The resulting powder was filtered and phenolic compounds were extracted a second time using the same method.

2.5.HPLC Analysis

Analysis were performed on a Thermo Ultimate 3000 HPLC system consisted of an autosampler (WPS-3000 TSL), a pump (LPG 3400 SD), and a diode array detector (DAD-3000) coupled to a Chromeleon data treatment system (version 7.2). Separation was performed on reversed-phase Hichrom ODS C_{18} (4,6 x 250 mm, 5µm) at room temperature. A gradient consisting of water/acetic acid (99:1, v/v) (solvent A) and methanol (solvent B) was applied at a flow rate of 1 $mL.min^{-1}$ as follows: 5-20% B from 0-45 min, 20-32% B from 45-60 min, 32-100% B from 60-62 min, 100 % B from 62-67 min, 100-5% from 67-68 min, with the re-equilibration of the column from 68-72 min under the initial conditions. The absorbance was recorded at 280 nm.

Flavan-3-ols characterization

- *Wine sample*

To determine flavan-3-ols monomers concentration, wine samples were filtered through 0,45 µm PFTE syringe-tip filters into LC vials and directly subjected to reversed-phase chromatographic separation at 20°C using a Hichrom ODS C_{18} column (250 mm x 4.6 mm, 5µm).

- *Seed Extract*

For fresh seeds 600 µl of seed extracts were concentrated to dryness using a rotavapor at 25 °C, for seeds collected during the winemaking 3 ml were concentrated using the same method. In both case the residue was dissolved into 100 µL of MeOH and 400 µL of deionized water. Sample were filtered through 0.45 µm PFTE syringe-tip filters into LC vials and directly subjected to reversed-phase chromatographic separation at 20°C using a Hichrom ODS C_{18} column (250 mm x 4.6 mm, 5µm).

Proanthocyanidins characterization

- *Wine Sample*

Prior to proanthocyanidins analysis, a solid-phase extraction (SPE) step was used in order to remove organic acids, residual sugars and other compounds insoluble in the organic phase (Kennedy & Waterhouse, 2000). Each sample was purified on a LC18 cartridge (Supelco, Saint Quantin, France) previously activated with methanol followed by purified water. Wine was purified and concentrated as follow: 5 mL of wine was vacuum dried (Rotavapor R Buchi) and then diluted in 20 mL of deionized water. The sample was applied on the column and the column was washed with 50 mL of deionized water and eluted with 50 mL of methanol. Then, the methanol fraction was vacuum dried and dissolved in 1 mL of methanol.

- *Seed Extract*

For fresh seeds 500 µl of seed extracts were concentrated to dryness using a rotavapor at 25 °C, for seeds collected during the winemaking 2 ml were concentrated using the same method.

To determine the amount of PAs, the mDP and the percentage of PAs galloylation, phloroglucinolysis was performed based on method described by Kennedy and Jones (Kennedy & Jones, 2001). The phloroglucinolysis reagent solution containing 0.1 N HCl in MeOH, 50 $g.L^{-1}$ of phloroglucinol and 10 $g.L^{-1}$ of acid ascorbic was prepared. One hundred millilitres of wine sample dissolved in MeOH was added to 100 µL of the phloroglucinolysis reagent and the reaction mixture was placed at 50°C for 20 min, then 5 volumes of 10 mM aqueous sodium acetate was added to stop the reaction. The same method was used with seed extracts. The resulted samples were filtered through 0,45 µm PFTE syringe-tip filters into LC vials and subjected to reversed-phase chromatographic separation at 20°C using a Hichrom ODS C_{18} column. Concentration of free monomers and hydrolysed terminal subunits were determined from standard curves preparated with pure standards of (+)-catechin, (-)-epicatechin, and (-)-epicatechin-3-*O*-gallate. The concentration of extension subunits-phloroglucinol adducts was calculated from molar extension coefficients found in literature (Kennedy & Jones, 2001). The mean degree of polymerization (mDP) and the percentage of galloylation were calculated according the method described by Kennedy and Jones (Kennedy & Jones, 2001). All the qualitative and quantitative analyses of phenolic composition were performed in triplicate.

2.6.Yeast cells observations

The protocol of yeast cells observations is adapted from Nguela et al., 2019. To recover yeast cells, at each sample point 1 ml of wine has been centrifugated (5 min at 1500 g). To remove

non-sorbed polyphenol, pellets were washed four times with 2 ml of Phosphate Buffered Saline (PBS). Yeast cells were re-suspended in 1 ml of PBS and submitted to microscopy observations. Imaging was performed with a Nanozoomer 2 OHT (Hamamatsu, Germany) under an FITC filter (λexc = 480 nm, λem = 520 nm).

2.7.Statistics

Statistical data analyses were conducted using the analysis of variance (one-way ANOVA) of XLSTAT V 2019.1.1 software (Addinsoft). Comparison of mean values was performed using Tukey's honestly significant difference when samples were significantly different by ANOVA ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1.Grape composition and winemaking

Physiological characteristics of grape berries are provided in Table 1. As such characteristics are heterogeneous in a vineyard, sample were harvested and sorted out as described above. Ripeness of berry samples was assessed by measurement of berry weight (g/berry), volume of juice for one hundred berry (ml), malic acid content (g/L), °Brix and sugar content (g/L).

The volume of wine at the end of winemaking is the same for each maturity stage. The volume of juice for one hundred berries is decreasing all along the maturity: 112 ml for under-ripeness berries, 80 ml for ripeness berries and 71 for over-ripeness berries (table 1). Thus, the amount of vegetal material needed to produce 900 ml of wine is rising all along the maturity (table 1), meaning that the ratio juice/pomace is also rising for each maturity stages (table 1).

3.2.Berry, Seed and Skin wine: influence of tannin origins on wine tannins content

Figure 1 shows the appearance of tannins into wine according to their origins (skins, seeds, and whole berries), the berry maturity and the vinification step. Depending on tannin origin the emergence is different. Indeed, for skin wine no significant evolution of tannins content is observed all along the winemaking, highlighting the fact that skins have probably released most of their tannins content into the wine from the first step of winemaking. This observation is concordant with a considerable number of previous studies which have shown that the diffusion rate of skins tannins in wine is fast due to their cell location and the ability of skin cells to disrupt (Busse-Valverde et al., 2012; Miller et al., 2019). For seed wine, the tannin appearance into wine pattern during winemaking is different. Wine tannins content is progressively rising all along the winemaking, highlighting here the fact that seed tannins appearance in wine take probably more time than the skin one (Miller et al., 2019). This observation has also been made by previous studies (Amrani-Joutei & Glories, 2014; Sparrow et al., 2015; Sun et al., 1999), even though the explanation of slower seed tannins apparition in wine is still poorly understood (ethanol effect, seed cell hydration, disorganization of the outer lipid layer…). Furthermore, independently from the berry maturity stage, at the end of the post-fermentative maceration the tannins content of seed wine (≈ 0,9 $g.L^{-1}$) is more than double that of skin wine (≈ 0,3 $g.L^{-1}$) pointing out the fact that seeds could bring more tannins in wine than skins. This observation has also been made by Kennedy who has noticed that seed tannins represented more than 60 % of the total tannins content of a Pinot Noir wine (Kennedy, 2008). Finally and logically, the tannin appearance pattern for berry wine is extremely closed to the seed one, meaning that, in our case, the augmentation of tannins content in berry wine is likely due to the release of seed tannins in wine. This observation bring to the fore the importance of seed tannin contribution to wine tannins. By knowing the potential disadvantageous effect of seed tannins on the astringency and bitterness of wine

209 (Pascual et al., 2016; Shang et al., 2013), the influence of berry maturity on seed wine tannins
210 content has been investigated.

211 **3.3.Seed wine: influence of ripeness on wine tannins content**

212 Figure 1 (graph seed wine) represents tannin apparition in seed wine according to berry
213 ripeness level. The finale tannin concentrations of seed wine for the under-ripeness stage, the
214 ripeness stage and the over-ripeness stage are respectively 0.67, 0.70 and 0.88 $g.L^{-1}$, with no
215 significant differences observed. By the way, for the under-ripeness and the ripeness stage, it
216 seems that wine tannins content reached a plateau at the middle of the post-fermentative
217 maceration. Yet, for the over-ripeness stage, the wine tannins content seems to continuously
218 increase until the end of the post-fermentative maceration. Even though the seed tannins
219 content and composition of the three ripeness levels were comparable (table 2), the amount of
220 seeds used to produce seed wine was strongly different. Indeed, when 60 grams of seeds are
221 used to produce 900 ml of wine at the under-ripeness stage, 67 grams are needed for the
222 ripeness stage, and 101 grams are needed for the over-ripeness stage. This difference could
223 explain why the tannins content of seed wine made of over-ripeness seeds is progressively
224 increasing all along the winemaking. Indeed the ratio seed:juice exprimed in grams of seed
225 per liter of wine is 67 $g.L^{-1}$ for the under-ripeness stage, 74 $g.L^{-1}$ for the ripeness stage and
226 112 $g.L^{-1}$ for the over-ripeness stage. This ratio augmentation according to the berry maturity
227 is automatically linked to a diminution of seed surface contact with wine, which could
228 potentially explain differences of tannin apparition pattern in wine according to berry
229 maturity.

230 To sum up the seed tannins content and composition of seeds at the three different ripeness
231 levels are comparable and wine tannins content of wines produced with these seeds is also
232 comparable. Yet, the amount of seeds used for the over-ripeness wine is strongly higher than

233 the other wines. This observation can mostly be explained by two hypotheses: (i) the seed
234 surface contact of over-ripeness seeds during the winemaking was weaker than those of the
235 other wines or, (ii) tannins of over-ripeness seeds are less extractable than tannin of the under-
236 ripeness and ripeness stages.

237 So, in order to better understand the seed tannin release mechanisms in wine, and the potential
238 differences of extractability according to the ripeness level, seed tannins content has been
239 characterized at the four different winemaking stages for the three ripeness levels.

240 **3.4.A focus on seed tannins**

241 **3.4.1 Seed tannins content: influence of ripeness on tannins content**

242 Table 2 shows the concentration and composition of seed tannins (monomeric and polymeric
243 fractions) at the three different ripeness levels. The total monomers tannins content
244 significantly increases from under-ripeness to ripeness and decreases from ripeness to over-
245 ripeness. By the way, (-)-epicatechin is the monomer the most present in seeds followed by (-
246)-epicatechin-gallate and (+)-catechin which is in concordance with previous studies (Rinaldi
247 et al., 2014; Chira et al., 2011; Mattivi et al., 2009). Even though differences are observed for
248 the monomers content, berry ripeness stage has no significant impact on the total tannins
249 content nor on the tannin percentage of galloylation. This observation is not in agreement with
250 other studies which show a drastic and continuous decrease of tannins content during ripening
251 linked to oxidative processes (Obreque-Slier et al., 2010, 2012; Kennedy et al., 2000a, 2000b,
252 Romeyer et al., 1985). Nevertheless, in our case the time delay between the under-ripeness
253 and the over-ripeness stages time is 21 days corresponding to a variation of 3°Brix, to our
254 knowledge no publications have investigated the difference of seed tannins content in such a
255 short space of time at the end of berry ripening. In agreement with this hypothesis, Garrido-
256 Banuelos et al*.,* have found no significant differences in berry tannins content with grape

presenting a variation of 3°Brix, with no specific regards on seed tannins content (Garrido-Bañuelos et al., 2019). The supposition that oxidative processes have already been in an advanced stage when the under-ripeness condition has been collected could explain the absence of differences. Furthermore the mean degree of polymerisation (mDP) of over-ripeness seed tannin is significantly higher than those of other maturity stages. Still, even if this difference is significantly different, no important evolution can be observed. Indeed, when the mDP of under-ripeness seed is 5.7, the mDP of over-ripeness seed is 6.2. This observation is concordant with other works on Merlot seeds (Obreque-Slier et al., 2012). Finally, the same pattern of extension and terminal subunits of tannin is observed for each maturity stage. The (-)-epicatechin is the favorite extension and terminal unit followed in both case by the (-)-epicatechin gallate and the catechin.

To sum up, even though some differences of mDP are observed for the three different maturity stages, it seems that, in our case, 21 days of harvest delay have only a minimal impact on seed total tannins content and composition. To go further, it is known that the evolution of seed tannins is related to evolution of cell wall during berry ripening. Indeed, the solidification of cells rich in tannins resulting from the lignification of the inner layers and outer integument could affect their aptitude to release their compounds in wine. This is why, the reduction of seed tannins release observed at the end of ripeness is mostly attributed to the reduction of their extractability potential (Cadot et al, 2006; Ristic & Iland, 2005; Pratt, 1971). That finding raises immediately a question: If no difference of tannin composition is observed, is there a difference of seed extractable tannins according to the berry maturity?

3.4.2 Evolution of seed tannins content at different winemaking stages

With the aim of characterised possible differences of extractable tannins according to the vinification step and the berry maturity, the content of seed tannins has been studied at each

281 vinification step (table 3). Even though the mean degree of polymerisation is comparable
282 between the three maturity stages at the first step of winemaking, as vinification progresses,
283 differences are observed. Indeed, for each maturity stages, at the end of the vinification, the
284 mDP of seed tannins is significantly higher than the one found for fresh seeds. Furthermore,
285 there are no significant differences of galloylation percentage between the fresh seed tannins
286 and the seed tannin at the end post-fermentative maceration for each maturity stage.
287 Independently from the berry maturity stage, these results suggests that more seed tannins are
288 polymerized, less they are extractable. Interestingly, the same observation has been made by
289 Fournand et al., about skin tannins, suggesting molecules sizes is one of the important
290 parameters which influence tannins extractability (Fournand et al., 2006).

291 To go further, by knowing the precise amount of seed present in each fermenter, and the
292 average seed tannins content per grams of seed at each winemaking step, the percentage of
293 seed tannin release has been calculated (figure 2).

294 The part on non-extractable seed tannins seems to be influenced by berry maturity. Indeed,
295 when at under-ripeness stage 25 % of seed tannins are not extracted in wine, 18 % are not
296 extracted at ripeness stage and only 10 % are not extracted at over-ripeness stage.
297 Furthermore, according to the vinification steps and maturity stages, differences are observed.
298 Indeed, during alcoholic fermentation, 72 % of tannins are extracted for the under-ripeness
299 seeds, 77 % for the ripeness seeds and 89 % for the over-ripeness seeds. Consequently, the
300 percentage of extracted tannins during maceration decrease according to the maturity degree:
301 only 1 % of tannins are extracted during post-fermentative maceration for the over-ripeness
302 seeds. These differences highlight that riper are berries, faster seed tannins are extracted.

303 To sum up, in our case, berry maturity stage seems to have no impact on total seed tannins
304 content. Yet, a considerable difference of seed tannin release rate is observed according to the

winemaking step and the berry maturity. This observation suggesting that the difference of seed tannin extractability is more linked to the difference of wine matrix composition which differs with berry maturity stages than the seed tannins content and composition. The link between pulp sugar content and skin tannins extractability has already been explored with no successful outcomes (Fournand et al., 2006). To our knowledge no experiments have been conducted on the impact of sugar content and seed tannins extraction. Now in order to compare seed tannin release kinetics in wine and seed tannin apparition in wine, the comparison between wine tannins content and seed tannins content at each winemaking point has been done.

3.4.3 Comparison between wine tannins content and seed tannins content at the four winemaking point

With the aim of characterizing the seed tannin release in wine, the evolution curve of the tannins content of seed wine have been overlapped on the evolution curve of seed tannins content (figure 3). For that the tannins content of seed wine and seeds used for the seed wine have been characterized at each winemaking step. The same pattern is observed for each berry maturity stage: while at half of the alcoholic fermentation seeds have already released most of their tannins content, the tannins content of seed wine is progressively increasing all along the vinification. These results suggest that seed tannin are trapped in the vine matrix and release gradually in wine.

The impact of yeast strain on phenolic wine compound (extraction and retention) has been widely studied. As an example, Carew et al., demonstrate that yeast strain choice affect both the concentration and composition of Pinot noir wine (Carew et al., 2013). In the same vein, after having let a commercial red wine macerate with different yeast strains, Gonzales-Royo et al., have observed a decrease of wine tannins content (González-Royo et al., 2017). By the

329 way, Nguela et al., have confirmed by confocal and electronic microscopy that interactions
330 between yeast strain and tannin occur in the cell wall, but also in the cell cytoplasm (Mekoue
331 Nguela et al., 2019). Nevertheless, the effect of yeast on seed tannin extraction during
332 fermentation has, to our knowledge, not been investigated. Regarding these results, the
333 question of the exact part playing by yeast strains on seed tannin extraction kinetics on wine
334 needs has to be highlighted. Observations of yeast cells at each point of the winemaking are
335 presented in supplementary data. A progressive diminution of yeast cells fluorescence is
336 observed all along the winemaking, suggesting that yeast cells could act as a tannins trap
337 during vinification. Indeed, yeast cells could have the ability to trap tannins at the beginning
338 of the fermentation and release it progressively during the winemaking, explaining results
339 shown in figure 3.

340 ## 4. CONCLUSION

341 In this study, we have investigated the effect of berry maturity stage on seed tannin extraction
342 according to the winemaking step of Merlot grapes. It has been shown that seed tannins
343 release in wine appears to be more linked to the composition of wine matrix than the
344 composition of seeds. Results have also highlighted that most of seed tannins are extracted
345 during the beginning of winemaking and progressively released in wine. This finding suggests
346 that seeds tannins are trapped in wine matrix during the first step of vinification. The yeast
347 diversify could appear as a winemaking tool to modulate red wine tannin composition, and
348 thus the style of finished wines. In conclusion, even if this work needs to be confirmed on
349 other grape varieties and other vintages, the results observed raise new perspectives on the
350 management of winemaking practices.

ABBREVIATIONS

% G: Percentage of galloylation

C: (+)-catechin

EAF: End of Alcoholic Fermentation

EC: (-)-epicatechin

ECG: (-)-epicatechin gallate

EM: End of post-fermentative Maceration

HAF: Half of Alcoholic Fermentation

HM: Half of post-fermentative Maceration

mDP: Mean Degree of Polymerization

PAs: Proanthocyanidins

PBS: Phosphate Buffered Saline

ACKNOWLEDGEMENT

We thank the CIVB (Conseil Interprofessionnel des Vins de Bordeaux) for its financial support.

REFERENCES

Amrani Joutei, K., & Glories, Y. (1994). Etude en conditions modèles de l'extractibilité des composés phénoliques des pellicules et des pépins de raisins rouges. OENO One, 28(4), 303-317. https://doi.org/10.20870/oeno-one.1994.28.4.1134

Bindon, K. A., Kassara, S., Cynkar, W. U., Robinson, E. M. C., Scrimgeour, N., & Smith, P. A. (2014). Comparison of extraction protocols to determine differences in wine-extractable tannin and anthocyanin in Vitis vinifera L. cv. Shiraz and Cabernet Sauvignon grapes. Journal of Agricultural and Food Chemistry, 62(20), 4558-4570. https://doi.org/10.1021/jf5002777

Bindon, K. A., Smith, P. A., Holt, H., & Kennedy, J. A. (2010). Interaction between grape-derived proanthocyanidins and cell wall material. 2. Implications for vinification. Journal of Agricultural and Food Chemistry, 58(19), 10736-10746. https://doi.org/10.1021/jf1022274

Bordiga, M., Travaglia, F., Locatelli, M., Coïsson, J. D., & Arlorio, M. (2011). Characterisation of polymeric skin and seed proanthocyanidins during ripening in six Vitis vinifera L. cv. Food Chemistry, 127(1), 180-187. https://doi.org/10.1016/j.foodchem.2010.12.141

Busse-Valverde, N., Bautista-Ortín, A. B., Gómez-Plaza, E., Fernández-Fernández, J. I., & Gil-Muñoz, R. (2012). Influence of skin maceration time on the proanthocyanidin content of red wines. European Food Research and Technology, 235(6), 1117-1123. https://doi.org/10.1007/s00217-012-1842-4

Cadot, Y., Miñana-Castelló, M. T., & Chevalier, M. (2006). Anatomical, histological, and histochemical changes in grape seeds from Vitis vinifera L. cv Cabernet franc during fruit development. Journal of Agricultural and Food Chemistry, 54(24), 9206-9215. https://doi.org/10.1021/jf061326f

Canals, R., Llaudy, M. C., Valls, J., Canals, J. M., & Zamora, F. (2005). Influence of ethanol concentration on the extraction of color and phenolic compounds from the skin and the seeds of Tempranillo grapes at different stages of ripening. Journal of Agricultural and Food Chemistry, 53(10), 4019-4025. https://doi.org/10.1021/jf047872v

Carew, A. L., Smith, P., Close, D. C., Curtin, C., & Dambergs, R. G. (2013). Yeast Effects on Pinot noir Wine Phenolics, Color, and Tannin Composition. Journal of Agricultural and Food Chemistry, 61(41), 9892-9898. https://doi.org/10.1021/jf4018806

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. https://doi.org/10.3390/molecules16021519

Ćurko, N., Kovačević Ganić, K., Gracin, L., Đapić, M., Jourdes, M., & Teissedre, P. L. (2014). Characterization of seed and skin polyphenolic extracts of two red grape cultivars grown in Croatia and their sensory perception in a wine model medium. Food Chemistry, 145, 15-22. https://doi.org/10.1016/j.foodchem.2013.07.131

Downey, M. O., Harvey, J. S., & Robinson, S. P. (2003). Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. Australian Journal of Grape and Wine Research, 9(1), 15-27. https://doi.org/10.1111/j.1755-0238.2003.tb00228.x

Federico Casassa, L., Beaver, C. W., Mireles, M. S., & Harbertson, J. F. (2013). Effect of extended maceration and ethanol concentration on the extraction and evolution of phenolics, colour components and sensory attributes of Merlot wines : Extended maceration and ethanol concentration. Australian Journal of Grape and Wine Research, 19(1), 25-39. https://doi.org/10.1111/ajgw.12009

413 Fournand, D., Vicens, A., Sidhoum, L., Souquet, J.-M., Moutounet, M., & Cheynier, V.
414 (2006). Accumulation and extractability of grape skin tannins and anthocyanins at different
415 advanced physiological stages. Journal of Agricultural and Food Chemistry, 54(19),
416 7331-7338. https://doi.org/10.1021/jf061467h

417 Garrido-Bañuelos, G., Buica, A., Schückel, J., Zietsman, A. J. J., Willats, W. G. T., Moore, J.
418 P., & Du Toit, W. J. (2019). Investigating the relationship between grape cell wall
419 polysaccharide composition and the extractability of phenolic compounds into Shiraz wines.
420 Part I : Vintage and ripeness effects. Food Chemistry, 278, 36-46.
421 https://doi.org/10.1016/j.foodchem.2018.10.134

422 González-Royo, E., Esteruelas, M., Kontoudakis, N., Fort, F., Canals, J. M., & Zamora, F.
423 (2017). The effect of supplementation with three commercial inactive dry yeasts on the
424 colour, phenolic compounds, polysaccharides and astringency of a model wine solution and
425 red wine : The effect of supplementation with three commercial inactive dry yeasts. Journal of
426 the Science of Food and Agriculture, 97(1), 172-181. https://doi.org/10.1002/jsfa.7706

427 Hernandez-Jimenez, A., Kennedy, J. A., Bautista-Ortin, A. B., & Gomez-Plaza, E. (2012).
428 Effect of ethanol on grape seed proanthocyanidin extraction. American Journal of Enology
429 and Viticulture, 63(1), 57-61. https://doi.org/10.5344/ajev.2011.11053

430 Kennedy, J. A. (2008). Grape and wine phenolics : Observations and recent findings. Ciencia
431 e Investigación Agraria, 35(2), 107-120. https://doi.org/10.4067/S0718-16202008000200001

432 Kennedy, J. A., & Jones, G. P. (2001). Analysis of Proanthocyanidin Cleavage Products
433 Following Acid-Catalysis in the Presence of Excess Phloroglucinol. Journal of Agricultural
434 and Food Chemistry, 49(4), 1740-1746. https://doi.org/10.1021/jf001030o

435 Kennedy, J. A., Matthews, M. A., & Waterhouse, A. L. (2000). Changes in grape seed
436 polyphenols during fruit ripening. Phytochemistry, 55(1), 77-85.

437 Kennedy, J. A., Troup, G. J., Pilbrow, J. R., Hutton, D. R., Hewitt, D., Hunter, C. R., Jones,
438 G. P. (2000a). Development of seed polyphenols in berries from Vitis vinifera L. cv. Shiraz.
439 Australian Journal of Grape and Wine Research, 6(3), 244-254.
440 https://doi.org/10.1111/j.1755-0238.2000.tb00185.x

441 Kennedy, J. A., & Waterhouse, A. L. (2000b). Analysis of pigmented high-molecular-mass
442 grape phenolics using ion-pair, normal-phase high-performance liquid chromatography.
443 Journal of Chromatography A, 866(1), 25-34. https://doi.org/10.1016/S0021-9673(99)01038-
444 9

445 Lorrain, B., Chira, K., & Teissedre, P. L. (2011). Phenolic composition of Merlot and
446 Cabernet-Sauvignon grapes from Bordeaux vineyard for the 2009-vintage : Comparison to
447 2006, 2007 and 2008 vintages. Food Chemistry, 126(4), 1991-1999.
448 https://doi.org/10.1016/j.foodchem.2010.12.062

449 Mané, C., Souquet, J. M., Ollé, D., Verriés, C., Véran, F., Mazerolles, G., Cheynier, V.,
450 Fulcrand, H. (2007). Optimization of Simultaneous Flavanol, Phenolic Acid, and Anthocyanin
451 Extraction from Grapes Using an Experimental Design : Application to the Characterization
452 of Champagne Grape Varieties. Journal of Agricultural and Food Chemistry, 55(18),
453 7224-7233. https://doi.org/10.1021/jf071301w

454 Mattivi, F., Vrhovsek, U., Masuero, D., & Trainotti, D. (2009). Differences in the amount and
455 structure of extractable skin and seed tannins amongst red grape varieties. Australian Journal
456 of Grape and Wine Research, 15(1), 27-35. https://doi.org/10.1111/j.1755-0238.2008.00027.x

457 Mekoue Nguela, J., Vernhet, A., Julien-Ortiz, A., Sieczkowski, N., & Mouret, J.-R. (2019).
458 Effect of grape must polyphenols on yeast metabolism during alcoholic fermentation. Food
459 Research International, 121, 161-175. https://doi.org/10.1016/j.foodres.2019.03.038

460 Miller, K., Noguera, R., Beaver, J., Medina-Plaza, C., Oberholster, A., & Block, D. (2019). A
461 Mechanistic Model for the Extraction of Phenolics from Grapes During Red Wine
462 Fermentation. Molecules, 24(7), 1275. https://doi.org/10.3390/molecules24071275

463 Monagas, M., Gómez-Cordovés, C., Bartolomé, B., Laureano, O., & Ricardo da Silva, J. M.
464 (2003). Monomeric, oligomeric, and polymeric flavan-3-ol composition of wines and grapes
465 from Vitis vinifera L. Cv. Graciano, Tempranillo, and Cabernet Sauvignon. Journal of
466 Agricultural and Food Chemistry, 51(22), 6475-6481. https://doi.org/10.1021/jf030325+

467 Obreque-Slier, E., López-Solís, R., Castro-Ulloa, L., Romero-Díaz, C., & Peña-Neira, Á.
468 (2012). Phenolic composition and physicochemical parameters of Carménère, Cabernet
469 Sauvignon, Merlot and Cabernet Franc grape seeds (Vitis vinifera L.) during ripening. LWT -
470 Food Science and Technology, 48(1), 134-141. https://doi.org/10.1016/j.lwt.2012.02.007

471 Obreque-Slier, E., Peña-Neira, Á., López-Solís, R., Zamora-Marín, F., Ricardo-da Silva, J.
472 M., & Laureano, O. (2010). Comparative study of the phenolic composition of seeds and
473 skins from Carménère and Cabernet Sauvignon grape varieties (Vitis vinifera L.) during
474 ripening. Journal of Agricultural and Food Chemistry, 58(6), 3591-3599.
475 https://doi.org/10.1021/jf904314u

476 Pascual, O., González-Royo, E., Gil, M., Gómez-Alonso, S., García-Romero, E., Canals, J.
477 M., … Zamora, F. (2016). Influence of Grape Seeds and Stems on Wine Composition and
478 Astringency. Journal of Agricultural and Food Chemistry, 64(34), 6555-6566.
479 https://doi.org/10.1021/acs.jafc.6b01806

480 Pratt, C. (1971). Reproductive Anatomy of cultivated grapesâ A review. American Journal
481 of Enology and Viticulture, 22(2), 92-109.

482 Rinaldi, A., Jourdes, M., Teissedre, P. L., & Moio, L. (2014). A preliminary characterization
483 of Aglianico (Vitis vinifera L. cv.) grape proanthocyanidins and evaluation of their reactivity
484 towards salivary proteins. Food Chemistry, 164, 142-149.
485 https://doi.org/10.1016/j.foodchem.2014.05.050

486 Ristic, R., & Iland, P. G. (2005). Relationships between seed and berry development of Vitis
487 Vinifera L. cv Shiraz : Developmental changes in seed morphology and phenolic composition.
488 Australian Journal of Grape and Wine Research, 11(1), 43-58. https://doi.org/10.1111/j.1755-
489 0238.2005.tb00278.x

490 Romeyer, F. M., Macheix, J. J., & Sapis, J. C. (1985). Changes and importance of oligomeric
491 procyanidins during maturation of grape seeds. Phytochemistry, 25(1), 219-221.
492 https://doi.org/10.1016/S0031-9422(00)94532-1

493 Rousserie, P., Rabot, A., & Geny-Denis, L. (2019). From flavanols biosynthesis to wine
494 tannins : What place for grape seeds? Journal of Agricultural and Food Chemistry. Consulté à
495 l'adresse http://pubs.acs.org/doi/10.1021/acs.jafc.8b05768

496 Shang, T. Y., Zhang, Y. B., & Liu, Y. (2013). Effect of Adding Seeds During Maceration on
497 Quality of Wine From Vitis Vinifera CV. Cabernet Sauvignon. Advanced Materials Research,
498 709, 867-870. https://doi.org/10.4028/www.scientific.net/AMR.709.867

499 Sparrow, A. M., Dambergs, R. G., Bindon, K. A., Smith, P. A., & Close, D. C. (2015).
500 Interaction of grape skin, seed, and pulp on tannin and anthocyanin extraction in Pinot noir
501 wines. American Journal of Enology and Viticulture, 66(4), 472-481.
502 https://doi.org/10.5344/ajev.2015.15022

503 Sun, B. S., Pinto, T., Leandro, M. C., Ricardo-Da-Silva, J. M., & Spranger, M. I. (1999).
504 Transfer of Catechins and Proanthocyanidins From Solid Parts of the Grape Cluster Into
505 Wine. American Journal of Enology and Viticulture, 50(2), 179.

506

TABLES CAPTIONS

Table 1: Physiological parameters of Merlot berries (vintage 2018) according to the berry maturity stage: under-ripeness, ripeness, over-ripeness.

Table 2: Tannin concentrations and composition of Merlot seeds according to the berry maturity stage (Total monomers content and total tannins content in mg/g of seed ; C: (+)-Catechin ; EC: (-)-Epicatechin ; ECG: (-)-epicatechin gallate ; mDP : mean degree of polymerization ; %G : percentage of galloylation)

ANOVA to compare data, values with different letters within each row are significantly different (Tukey's test, $p < 0.05$)

Table 3: Tannin concentrations and composition of Merlot seeds according to the berry maturity stage and vinification step (Total monomers content and total tannins content in mg/g of seed ; C: (+)-Catechin ; EC: (-)-Epicatechin ; ECG: (-)-epicatechin gallate ; mDP : mean degree of polymerization ; %G : percentage of galloylation ; % : percentage of extracted tannins ; HAF : Half of Alcoholic Fermentation ; EAF : End of Alcoholic Fermentation ; HM : Half of Maceration ; EM : End of Maceration)

ANOVA to compare data, values with different letters within each row are significantly different (Tukey's test, $p < 0.05$)

FIGURE CAPTIONS

Figure 1: Tannins apparition in wine according to berry origin and berry maturity. (HAF : Half of Alcoholic Fermentation ; EAF : End of Alcoholic Fermentation ; HM : Half of Maceration ; EM : End of Maceration).

Figure 2: Percentage of seed tannin extraction according to the vinification step and the berry maturity. (A: Overview ; B: Detailled ; HAF : Half of Alcoholic Fermentation ; EAF : End of Alcoholic Fermentation ; HM : Half of Maceration ; EM : End of Maceration).

Figure 3: Comparison of seed tannins content in wine and in seed according to the berry maturity. (HAF : Half of Alcoholic Fermentation ; EAF : End of Alcoholic Fermentation ; HM : Half of Maceration ; EM : End of Maceration).

SUPPLEMENTARY DATA

Supplementary data: Microscopic observations of yeast cells under FITC filter at HAF (A), EAF (B), HM (C) and EM (D).

DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence work reported in this paper.

TABLES

Table 1: Physiological parameters of Merlot berries (vintage 2018) according to the berry maturity stage: under-ripeness, ripeness, over-ripeness

Physiological parameters	Date of harvest		
	Under-ripeness 12/09	Ripeness 25/09	Over-ripeness 03/09
Berry weight (g)	2.20	1.76	1.53
Seed weight (mg)	42.4	35.6	43.1
Skin weight (g)	0.29	0.23	0.40
Number of seeds per berry	1.8	1.65	1.85
Volume of juice for 100 berries (ml)	112	80	71
°Brix	18.3	19.2	20.1
Sugar content (g/L)	199.9	209.2	220.1
Potential alcohol	11.9	12.4	13.1
pH	3.3	3.4	3.5
Total acidity (g/L)	3.9	3.1	2.9
Malic Acid (g/L)	2.06	1.75	1.70

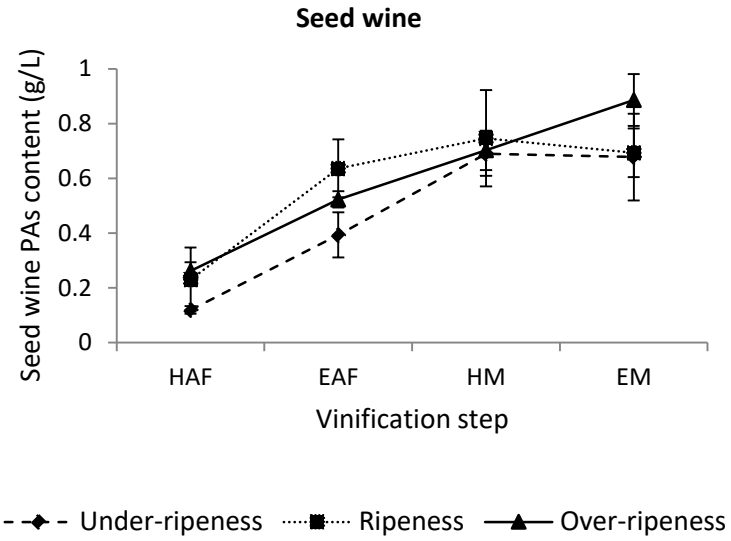
Amount of vegetal material corresponding to 900 ml of juice	Date of harvest		
	Under-ripeness 12/09	Ripeness 25/09	Over-ripeness 03/09
Berry weight (g)	293	326	608
Ratio berry :juice (g/L)	*325*	*362*	*675*
Seed weight (g)	60	67	101
Ratio seed :juice (g/L)	*67*	*74*	*112*
Skin weight (g)	233	259	507
Ratio skin :juice (g/L)	*259*	*288*	*563*

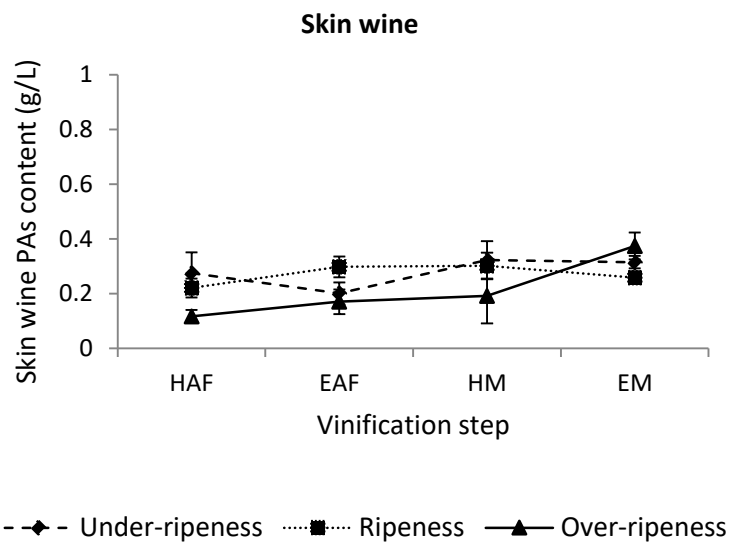
Table 2: Tannin concentrations and composition of Merlot seeds according to the berry maturity stage (Total monomer content and total tannin content in mg/g of seed ; C: (+)-Catechin ; EC: (-)-Epicatechin ; ECG: (-)-epicatechin gallate ; mDP : mean degree of polymerization ; %G : percentage of galloylation)
ANOVA to compare data, values with different letters within each row are significantly different (Tukey's test, $p < 0.05$)

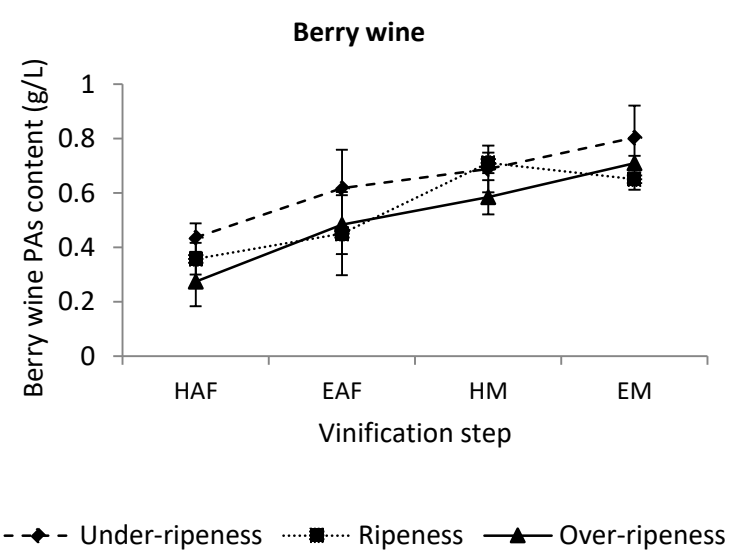
		Under-ripeness	Ripeness	Over-ripeness
		Monomeric fraction		
	Total monomers content	2.37 ± 0.017 a	2.68 ± 0.070 b	1.96 ± 0.098 c
	C	0.36 ± 0.006 a	0.37 ± 0.010 a	0.30 ± 0.009 b
	EC	1.48 ± 0.017 a	1.70 ± 0.044 b	1.25 ± 0.074 c
	ECG	0.54 ± 0.007 a	0.61 ± 0.026 b	0.42 ± 0.018 c
		Polymeric fraction		
	Total tannins content	56.77 ± 1.999 a	60.11 ± 1.478 a	57.55 ± 1.405 a
	mDP	5.7 ± 0.07 a	5.6 ± 0.07 a	6.3 ± 0.10 b
	%G	36.0 ± 0.37 a	36.3 ± 0.31 a	35.3 ± 0.52 a
Extension subunits (%)	(+)-Catechin	7.3 ± 0.1 a	5.9 ± 0.1 b	6.9 ± 0.1 c
	(-)-Epicatechin	58.8 ± 0.4 a	59.8 ± 0.4 a	59.2 ± 0.5 a
	(-)-Epicatechin gallate	33.9 ±0.4 a	34.3 ± 0.5 a	33.8 ± 0.6 a
Terminal subunits (%)	(+)-Catechin	3.8 ± 0.1 a	3.9 ± 0.4 a	4.3 ± 0.3 a
	(-)-Epicatechin	50.8 ± 1.1 a	50.9 ± 0.1 a	53.2 ± 0.3 b
	(-)-Epicatechin gallate	45.3 ± 1.2 a	45.2 ± 0.4 a	42.6 ± 0.1 b

Table 3: Tannin concentrations and composition of Merlot seeds according to the berry maturity stage and the vinification step (Total monomer content and total tannin content in mg/g of seed ; C: (+)-Catechin ; EC: (-)-Epicatechin ; ECG: (-)-epicatechin gallate ; mDP : mean degree of polymerization ; %G : percentage of galloylation ; % : percentage of extracted tannins ; HAF : Half of Alcoholic Fermentation ; EAF : End of Alcoholic Fermentation ; HM : Half of Maceration ; EM : End of Maceration)
ANOVA to compare data, values with different letters within each row are significantly different (Tukey's test, $p < 0.05$)

		Fresh	HAF	EAF	HM	EM
			Monomeric fraction			
Under-ripeness	Total Monomers	2.37 ± 0.017 a	0.92 ± 0.116 b	0.60 ± 0.058 c	0.54 ± 0.060 c	0.39 ± 0.089 d
	C	0.36 ± 0.006 a	0.32 ± 0.054 a	0.21 ± 0.016 b	0.22 ± 0.009 b	0.18 ± 0.024 b
	EC	1.48 ± 0.017 a	0.45 ± 0.035 b	0.29 ± 0.028 c	0.26 ± 0.039 c	0.16 ± 0.056 d
	ECG	0.54 ± 0.007 a	0.16 ± 0.028 b	0.09 ± 0.015 c	0.07 ± 0.012 cd	0.05 ± 0.011 d
Ripeness	Total Monomers	2.68 ± 0.070 a	0.77 ± 0.015 b	0.37 ± 0.111 c	0.26 ± 0.067 c	0.22 ± 0.017 c
	C	0.37 ± 0.010 a	0.24 ± 0.029 b	0.12 ± 0.039 c	0.12 ± 0.033 c	0.12 ± 0.006 c
	EC	1.70 ± 0.044 a	0.41 ± 0.008 b	0.19 ± 0.029 bc	0.11 ± 0.029 c	0.08 ± 0.014 c
	ECG	0.61 ± 0.026 a	0.10 ± 0.004 b	0.05 ± 0.005 bc	0.03 ± 0.005 c	0.02 ± 0.006 c
Over-ripeness	Total Monomers	1.96 ± 0.098 a	0.59 ± 0.003 b	0.17 ± 0.003 c	0.16 ± 0.018 c	0.15 ± 0.040 c
	C	0.30 ± 0.009 a	0.28 ± 0.001 a	0.11 ± 0.008 b	0.10 ± 0.011 b	0.11 ± 0.016 b
	EC	1.25 ± 0.074 a	0.25 ± 0.001 b	0.05 ± 0.004 c	0.05 ± 0.005 c	0.02 ± 0.021 c
	ECG	0.42 ± 0.018 a	0.07 ± 0.002 b	0.02 ± 0.003 c	0.01 ± 0.002 c	0.01 ± 0.005 c
			Polymeric fraction			
Under-ripeness	Total tannins	56.77 ± 1.999 a	21.05 ± 0.885 b	15.99 ± 1.032 c	17.47 ± 4.162 bc	14.11 ± 1.477 c
	mDP	5.7 ± 0.07 ab	5.5 ± 0.09 b	6.0 ± 0.23 a	6.6 ± 0.25 c	6.9 ± 0.19 c
	%G	36.0 ± 0.37 ab	37.5 ± 0.64 ac	37.7 ± 0.71 c	35.9 ± 0.87 b	35.7 ± 1.03 b
Ripeness	Total tannins	60.11 ± 1.478 a	15.05 ± 1.542 b	13.61 ± 2.953 bc	11.57 ± 0.899 c	10.92 ± 1.793 c
	mDP	5.6 ± 0.07 a	6.0 ± 0.25 ab	7.0 ± 0.51 b	8.5 ± 1.10 c	8.2 ± 0.15 c
	%G	36.3 ± 0.31 a	32.7 ± 3.22 a	34.3 ± 1.86 a	35.7 ± 1.03 a	35.4 ± 1.21 a
Over-ripeness	Total tannins	57.55 ± 1.405 a	14.40 ± 1.151 b	6.47 ± 0.606 c	5.64 ± 0.978 c	5.42 ±0.758 c
	mDP	6.3 ± 0.10 a	6.2 ± 0.28 a	8.6 ± 0.36 b	9.6 ± 0.68 c	8.8 ± 0.52 b
	%G	35.3 ± 0.52 a	34.5 ± 1.45 a	33.8 ± 1.40 a	33.3 ± 2.40 a	32.9 ± 3.11 a


Seed wine
Seed wine PAs content (g/L)
1
0.8
0.6
0.4
0.2
0
HAF
EAF
HM
EM
Vinification step

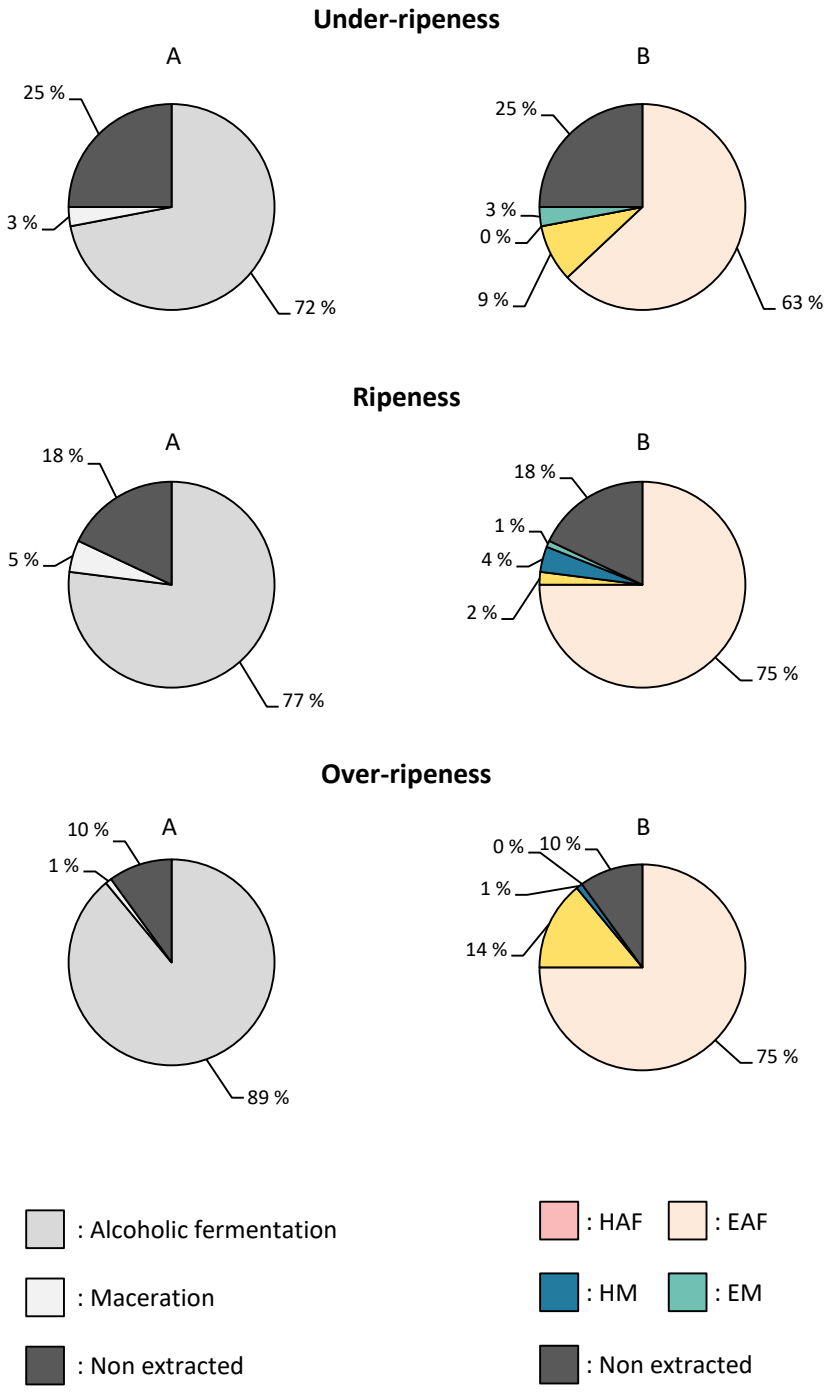

Skin wine
Skin wine PAs content (g/L)
1
0.8
0.6
0.4
0.2
0
HAF
EAF
HM
EM
Vinification step

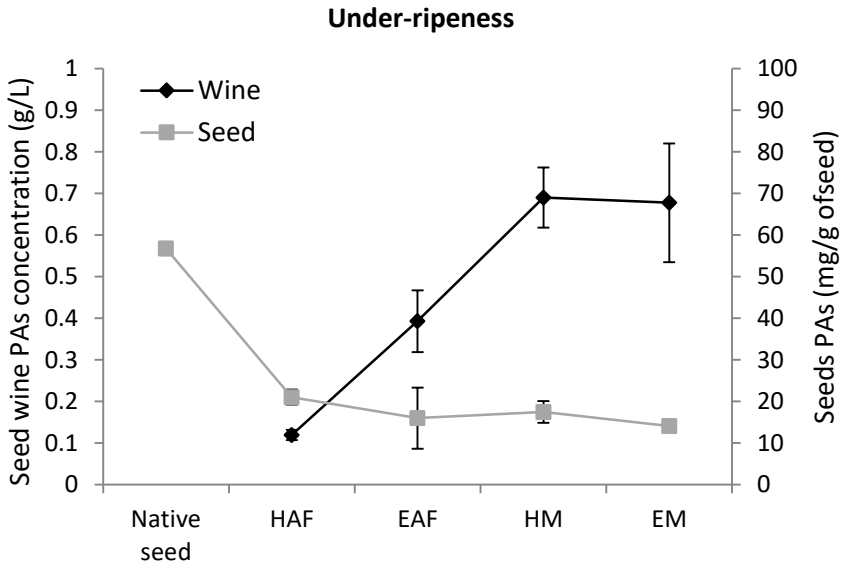

Berry wine
Berry wine PAs content (g/L)
1
0.8
0.6
0.4
0.2
0
HAF
EAF
HM
EM
Vinification step

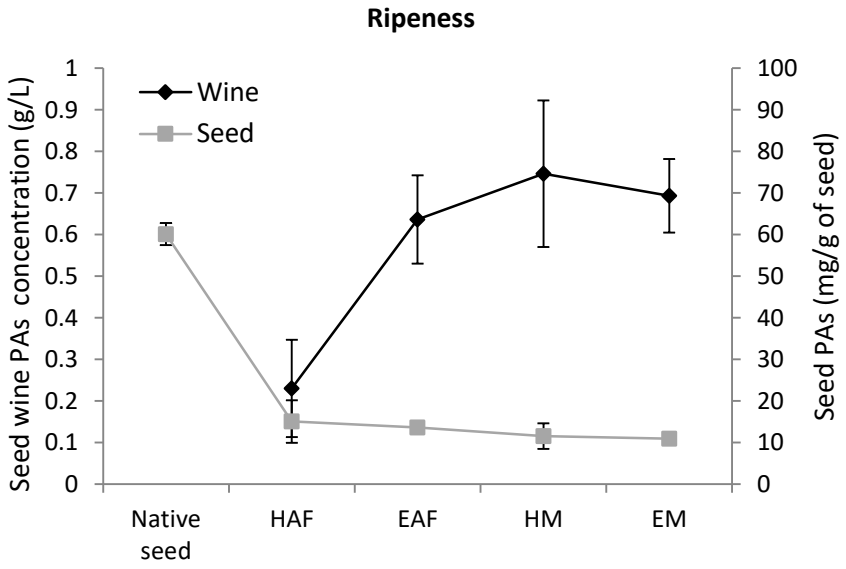
Under-ripeness
Ripeness
Over-ripeness

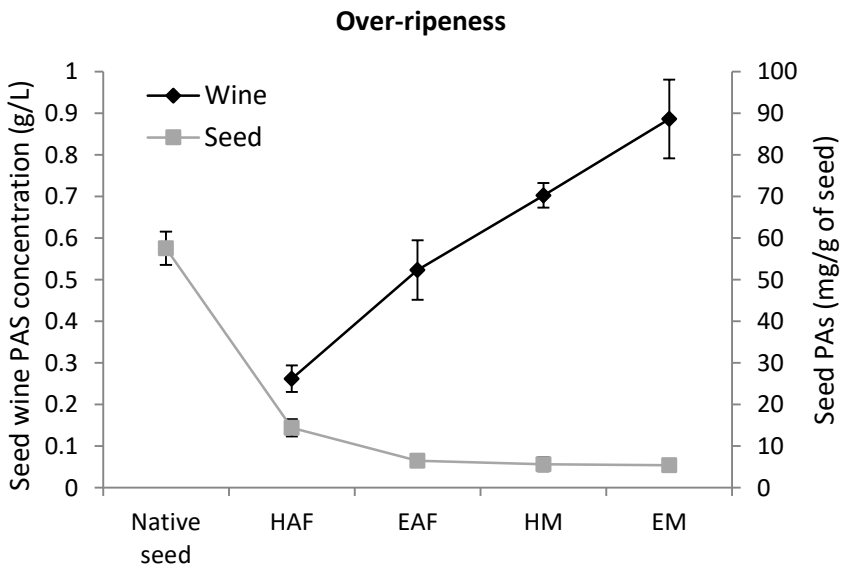
Under-ripeness
Ripeness
Over-ripeness

Under-ripeness
Ripeness
Over-ripeness


Under-ripeness
A
25 %
3 %
72 %
B
25 %
3 %
0 %
9 %
63 %
Ripeness
A
18 %
5 %
77 %
B
18 %
1 %
4 %
2 %
75 %
Over-ripeness
A
10 %
1 %
89 %
B
0 %
10 %
1 %
14 %
75 %
: Alcoholic fermentation
: Maceration
: Non extracted
: HAF
: EAF
: HM
: EM
: Non extracted


Under-ripeness
Wine
Seed
Seed wine PAs concentration (g/L)
Seeds PAs (mg/g ofseed)
Native seed
HAF
EAF
HM
EM


Ripeness
Wine
Seed
Seed wine PAs concentration (g/L)
Seed PAs (mg/g of seed)
Native seed
HAF
EAF
HM
EM


Over-ripeness
Wine
Seed
Seed wine PAS concentration (g/L)
Seed PAs (mg/g of seed)
Native seed
HAF
EAF
HM
EM