Impact of grape variety, berry maturity and size on the extractability of skin polyphenols during model wine-like maceration experiments

Elissa Abi-Habib, Céline Poncet-Legrand, Stéphanie Roi, Stéphanie Carrillo, Thierry Doco, Aude Vernhet

Poncet-Legrand Céline (Orcid ID: 0000-0001-8459-1415)
Vernhet Aude (Orcid ID: 0000-0001-7407-0448)

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Elissa ABI-HABIB, Céline PONCET-LEGRAND, Stéphanie ROI, Stéphanie CARRILLO, Thierry DOCO, Aude VERNHET*

UMR-SPO, Institut Agro - Montpellier SupAgro, INRAE, Univ Montpellier, Montpellier, France

*Corresponding author:

Phone: 33 (0)4 99 61 27 58

Fax: 33 (0)4 99 61 28 57

UMR SPO - Bat 28 – 2, place Pierre Viala – 34060 Montpellier Cedex 2 - France

Email address: aude.vernhet@supagro.fr

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Abstract

Background

Skin cell walls modulate anthocyanin and tannin extraction from grape skins. However, relationships between the composition of alcohol insoluble cell wall solids (AIS) and extraction are still unclear. Our objectives were to characterize the impact of variety, berry size and ripeness on skin AIS composition (polysaccharides, proteins) and polyphenol extraction during maceration. **Results.** The grape skin composition and its impact on polyphenol extraction was compared for two varieties, Carignan and Grenache, with skins of berries sorted according to their size and density. Extractions were performed in model wine-like maceration conditions. Fresh skins had similar contents in polymeric tannins, but strongly differed by their anthocyanin contents (higher in Carignan and in the ripest berries) and composition (higher proportions in coumaroylated anthocyanins in Carignan). Anthocyanin extraction was proportionally much higher in Grenache, which was not just related to the Carignan's higher levels in coumaroylated anthocyanins. Chemical reactions decreased anthocyanin concentrations in solution for both varieties. Tannin extraction for Grenache was slightly higher and faster than for Carignan. Skin AISs differed slightly between the two varieties by their carbohydrate composition and protein content, but not between modalities. Polyphenol analyses in the precipitates evidenced at the end of the maceration and in residual skins highlighted differences between the two varieties and between berries with different ripeness.

Conclusion. Structural information on the cell wall network and on its changes during maceration, along with a better understanding of the chemical reactions of anthocyanins and tannins is needed to better relate grape and wine polyphenol composition.

Keywords: grape skins, alcohol insoluble solids, extraction, anthocyanins, tannins, chemical reactivity.

1. INTRODUCTION

Anthocyanins and condensed tannins (flavan-3-ol polymers, also referred to as proanthocyanidins) are responsible for the colour and mouthfeel of red wines and play an important part in their quality. Anthocyanins and tannins in skin cells are mostly located in vacuoles, tannins being also found associated with the cell walls[1,2]. Tannins are also extracted from seeds, although in lower extent than from skins. Tannin and anthocyanin extraction from the skins/seeds of grapes during winemaking is not total and several studies have shown that there is no direct relationship between their contents in grapes from different varieties and those found in the corresponding wines[3,4]. The latter depend on the extraction conditions (ethanol content, temperature, maceration length, …), on the berry size (skin surface to juice ratio[5] and possibly skin composition [6]), and on the cap-management practices [7,8] during winemaking, as well as on their ability to cross the barriers represented by cell structure and in particular cell walls[1,9]. Other factors that modulate anthocyanin and tannin concentrations in wines are: (i) their adsorption on insoluble pulp debris (flesh cell walls) [10] and on yeasts [11]; (ii) their interactions with soluble grape compounds (polysaccharides, proteins) extracted during maceration, leading to precipitations [12]; iii) their chemical reactivity. Indeed, once extracted, anthocyanins and tannins undergo several chemical reactions that profoundly change their composition throughout the process [13,14]. Not all the compounds formed can be identified and quantified, which may contribute to the lack of relationship observed between the polyphenol composition of grapes and that of red wines at the end of the maceration. This relationship, related to several factors, is difficult to model, even under constant process conditions and even though this knowledge would be of great interest for the selection of new grape varieties or for the adaptation of winemaking techniques to different raw materials. Experiments under model conditions, associated with the characterisation of grapes and wines are therefore necessary in order to compare the varieties, dissociate the various phenomena and highlight those factors likely to play a predominant role in the polyphenolic composition of wines.

When dealing with polyphenol extraction from skins, cell walls are considered as one of the main factor that control their final composition in wines [1,10]. Tannin extraction especially is limited by their interactions with skin insoluble cell wall constituents, i.e. polysaccharides and proteins [15]. Interactions between tannins and cell wall polysaccharides were proven by means of adsorption experiments using purified cell walls and proanthocyanidins [10]. Both pectins and hemicelluloses play an important part in these interactions [16,17]. When in solution, the degree of methylation of pectins increases their interactions with proanthocyanidins [18]. Although they are minor components compared to polysaccharides, proteins have a much stronger affinity for tannins [19]. They account for about 10% of the insoluble cell wall components and are mainly structural proteins rich in hydroxyproline, proline and glycine [20,21]. Their role in the extractability of tannins, less studied, has been mentioned by several authors [22, 23]. Changes in skin/pulp cell wall composition occur during berry ripening, leading to a loosening of cell walls and fruit softening. Among these changes, a decrease of pectins, related to their solubilization, has been found [21,23]. These structural modifications have been hypothesized to induce changes in skin cell wall structure (porosity, accessibility to interaction sites) and rigidity that may modulate tannin [24] or anthocyanin extraction [25]. It has also been suggested that anthocyanins may influence tannin extraction/solubility and that high anthocyanin/tannin ratios induce higher tannin concentration in wines, regardless of the initial tannin concentration in fruits [26].

The objectives of this work were to: (i) characterize the impact of grape variety, berry size and ripeness on the extraction and evolution of anthocyanins and tannins during skin maceration; (ii) examine links between extraction and skin composition in insoluble materials; iii) identify the main mechanisms involved. Experiments were performed in wine-like model conditions for two contrasted varieties in terms of anthocyanin/tannin ratios (Carignan and Grenache), in the absence of pulp components (to avoid the impact of adsorption/precipitation events not related to skin composition) and fermentation (to avoid chemical changes related to reactions with yeast metabolites and adsorption by yeast cells). Most of the previous studies were performed on varieties harvested through

time at different degree of ripeness [1,9,24,27]. Grape heterogeneity in terms of berry size and ripeness was considered here at technological maturity.

2. MATERIALS AND METHODS

2.1. Chemicals

Acetonitrile, methanol, ethanol, acetic acid and formic acid were HPLC grade from VWR. KOH Titrisol 1M was purchased from Merck. Acetone, D (+) galacturonic acid hydrated were provided by Fluka. Sodium chloride, tartaric acid, epicatechin, epigallocatechin gallate, lithium chloride, N,N-dimethylformamide, trifluoroacetic acid, myo-inositol, allose, m-hydroxydiphenyl (MHDP), alcohol oxidase from *Pichia pastoris*, norleucine, the 18 amino-acid standard kit and hydrochloric acid 37 % were provided by Sigma-Aldrich, sulphuric acid by Roth. Sodium hydroxide 1M was obtained from Fisher. The lithium citrate loading buffer was obtained from Biochrom. Flavanol dimer B2, flavanol trimer C1 and Malvidin-3-O-glucoside chloride were purchased from Extrasynthese (Genay, France). Ultra-pure water was obtained from a Milli-Q Advantage A10 system (Millipore).

2.2. Grape sampling

Two *Vitis vinifera* grape varieties (Carignan and Grenache) were harvested at an average potential alcohol of 12 % vol. in the vineyard of the Pech Rouge experimental unit (INRAE, Gruissan, France). The berries were sorted according to their natural heterogeneity in terms volume (vol) and density (degree of maturity: deg). This heterogeneity was determined on 1000 berries the day before the harvest by measuring their diameter and estimating their density by flotation in different salt solutions, corresponding to total soluble solid difference between two successive baths of 1 % vol potential alcohol. Berries were recovered by cutting them at the pedicel level and sorted first as a function of their size using a grading machine (vol^-, vol^+) and then as a function of their density (deg^-, deg^+) using an aqueous solution of concentrated rectified grape must at the adequate density (**Fig.**

1A). The cut-off for berry sizes and sugar concentration were defined based on the median population of these 1000 berries. The oenological characteristics of the four batches (vol^-deg^-, vol^-deg^+, vol^+deg^- and vol^+deg^+) obtained for the Carignan and Grenache varieties are presented in supplementary data **S1**. Samples of 180 berries of each modality were recovered. Grape skins were separated from the berries with a scalpel and immediately used for diffusion experiments or frozen in liquid nitrogen and stored at -80°C for later analysis of their composition.

2.3 Preparation of skin alcohol insoluble cell wall material (AIS)

Frozen skins of each variety and modality (from 30 berries, triplicates) were ground in liquid nitrogen. The alcohol insoluble cell wall solids (AISs) were then isolated from the powders using the procedure described in Apolinar–Valiente *et al.* [28], with slight modifications. AISs were prepared in triplicate and analysed separately. Skin powder (5 g) was suspended in 15 ml boiling water for 5 min and homogenized. One part of the homogenized material was purified with two parts of 96% ethanol for 30 min at 40°C in an ultrasound bath. The alcohol insoluble solids (AIS) were separated by centrifugation and extracted again with 70% ethanol for 30 min at 40 °C. A sample from the liquid phase was taken for soluble sugar assay, done with the sulphuric phenol method. When no more sugar was detected, AISs were further washed twice with 96% ethanol and once with acetone. After being dried with an air flux overnight, they were weighed and used for the following analyses.

2.4 Carbohydrates composition of AISs

The neutral sugar composition of the AISs was determined by gas chromatography after polysaccharide hydrolysis with 72% sulphuric acid at 100°C for 3h [29] and conversion of neutral sugars into volatile alditol acetates [30]. Inositol and allose were used as internal standards. The alditol acetates were quantified by gas chromatography with flame ionization detection (GC-FID) (GC 2010 Plus Shimadzu) using a DB225 (30 m × 0.25 mm ID, 0.25 µm film) capillary column and hydrogen 5.6 B50 as the carrier gas. Calibration was done with commercial monosaccharides. Uronic acids were determined colorimetrically in triplicates by the *m*-hydroxydiphenyl method [31]. The AISs were first

submitted to pre-hydrolyse by the action of sulfuric acid, as described by Ahmed and Labavitch [32]. A calibration curve was built using pure galacturonic acid solutions (0 to 100 mg/L). The degree of methylesterification of uronic acids (DE) was measured by saponification of the AIS pectins in the presence of KOH, thus allowing the release of methanol. Methanol was converted to formaldehyde that was determined using the colorimetric method of Klavons and Bennet [33].

2.5 Amino acid composition of AISs

Cell wall material (5 mg) was hydrolysed in 1 mL of 6N HCl for 24 h at 120°C. Norleucine was added as an internal standard. After evaporation of the acidic aqueous solution under air stream, samples were washed twice in water and then in 95% ethanol. Finally, samples were dissolved in a 0.2 M pH 2.2 lithium citrate loading buffer and filtered through a 0.22 µm filter (Millipore Millex-GV). Amino acids were quantified by ion exchange chromatography with a Biochrom 30 amino acid analyzer (Biochrom, Cambridge, England), as described in Vicens *et al*. [21].

2.6 Polyphenol extraction from skins and precipitates.

Frozen initial skins and frozen skins after extraction in wine-like conditions were finely ground to a fine powder in liquid nitrogen and using a mortar grinder (Pulverisette 2, Fritsch). 150 mg of powder were treated first with methanol (750 µL) then extracted with 5.25 mL of 60/40/1 (v/v/v) acetone/water/formic acid at room temperature on an orbital shaker (Precellys 24, Bertin technologies, program 5000-3*40-20). The methanolic and acetone/water/ formic acid extracts were pooled and after centrifugation (3000 rpm, 5 min, 4°C), 1mL aliquots were dried in a rotary evaporator under vacuum at 35°C for 2h (EZ-2 plus, Genevac SP service). Dried extracts were re-dissolved in a wine-like solution (ethanol 12%, 3 g/L tartaric acid, 50 mM NaCl, 40 mg/L SO_2, pH 3.5) for UV–visible spectrophotometry and High Performance Liquid Chromatography (HPLC) analysis, or in dimethyl formamide for High Performance Size Exclusion Chromatography (HPSEC). The same procedure except grinding was applied for the extraction and analysis of polyphenols in the precipitates recovered at the end of the wine-like maceration experiments.

2.7 Polyphenol analysis.

Total polyphenols Index (TPI) and total red pigments (TRP) were determined by UV–visible spectrophotometry (spectrophotometer UV-1800, Shimadzu) at 280 and 520 nm (1 cm path length) after adequate dilution in HCl 1 M. Free anthocyanins were analysed by HPLC using a Waters chromatography system equipped with DAD detection and a C18 reversed-phase column (Atlantis T3, Waters) as described by Fournand *et al.* [27]. Anthocyanins were quantified at 520 nm, in equivalent of malvidin-3-*O*-glucoside.

The size distribution of polyphenols in the samples and the concentrations of polymeric tannins (in eq. epicatechin) were determined by HPSEC, according to the procedure described before[34]. Commercial epicatechin, B2 dimer, epigallocatechin gallate, malvidin and home prepared and characterized tannin fractions were used to evaluate retention times corresponding to monomers, oligomers and polymers (supplementary data **S2**).

2.8 Extraction of skin phenolic compounds by diffusion in model wine-like solution

Thirty berries of each modality were manually peeled and fresh skins were weighed and immediately immersed in 42 mL of a model solution containing 3 g/L tartaric acid, 50 mM NaCl and 40 mg/L SO_2, at pH 3.5 (adjusted with NaOH 1M). This value (42 mL) was chosen from the data obtained by grape sampling (average weight of berries, skins, seeds, pulp) and microvinifications of 900 g of berries in triplicate of each modality (average volumes of wine obtained for 900 g of berries). These averages slightly differed depending on the berry size, and the volume varied between 28-31 mL (vol-) and 38-44 mL (vol+). The value of 42 ml corresponded then to the vol+ modalities and the highest liquid to solid ratios, to avoid differences in extraction between modalities related to different solid-liquid equilibria. Simulated maceration experiments were carried out by increasing stepwise the ethanol content from 0 to 15% (**Fig. 1B**). All experiments were performed in triplicate. Flasks were placed under argon and gently stirred in dark at 22 °C. Polyphenol diffusion was followed during 11 days by measuring the TPI and TRP daily, as well as the HPLC and HPSEC profiles of samples taken

and centrifuged (15000 g, 15 min, 15°C) at the end of each ethanol increase step. The dilution induced by the sampling and the addition of ethanol was considered. To account for differences in initial fresh skin mass (dependent on the modality), results were divided by this initial mass and reduced to 1 g fresh skin for all modalities. At the end of the diffusion, skins were recovered, and the diffusion solutions clarified by centrifugation. Skins were then washed first with 0% (3 times for Grenache and 7 times for Carignan) and then with 15% (5 times for Grenache and 7 times for Carignan) fresh model wine-like solutions until no further extraction of skin polyphenols could be observed (**Fig. 1B**). Centrifugation pellets recovered from the diffusion solutions and "washed" skins were stored at -80 °C for further polyphenol analysis.

2.9 Statistical analysis

Statistical analysis was performed using Statistica software. The results obtained were assessed by factorial and one-way ANOVA analysis followed by a Tukey Test. Principal Component Analysis was performed on the AIS compounds (monosaccharides and amino acids) to assess the differences between varieties.

3. RESULTS AND DISCUSSION

3.1.Grape skin cell walls Alcohol Insoluble Solids (AIS)

AIS amounts in skins and their composition in monosaccharides and amino acids are reported for both variety and the four modalities (**Fig. 1**) in **Table 1.** No significant differences were observed between samples with respect to their content of AIS (mg AIS/g fresh skin) and the total content of neutral and acidic monosaccharides. For a given variety, these results are in accordance with those obtained previously on Syrah grapes[21]: total amounts of skin AISs remained constant during ripening and only minor changes in their monosaccharide composition were observed. However, factorial ANOVA followed by Tukey Test indicated (Supplementary Data **S3**) a significant difference between

varieties (Grenache vs Carignan) regarding their galactose, mannose, and glucose composition. A centered reduced principal component analysis (PCA) was done on the obtained data set (**Fig. 2A**). Variables and individuals were represented on the first 2 principal components (PC 1-2), describing 70% of the variability in the data. Mannose, galactose and glucose variables were negatively correlated on axis 1 to rhamnose and fucose. The axis 2 represented arabinose and xylose. The clear separation between the two varieties confirmed the results of the factorial ANOVA. The AISs of Grenache skins were richer in mannose, galactose and glucose compared to Carignan.

The primary cell wall of grape skins is formed by cellulose microfibrils tethered to a hemicellulosic matrix and of a more soluble domain consisting of pectic polysaccharides. Hemicellulosic polysaccharides are mainly xyloglucans, which account for about 10 % of the wall polysaccharides. Pectic polysaccharides are homogalacturonans (HG, smooth regions of pectins), rhamnogalacturonans I (RG-I) carrying side chains of arabinose and arabinogalactans (hairy regions of pectins) and rhamnogalacturonans II (RG-II). They are embedded within the primary cell wall cellulose-xyloglucan framework and in the middle lamella [15]. As expected, galacturonic acid (from pectins) and glucose (from cellulose and hemicellulose) were the major sugars in skin AISs. Other neutral sugars were arabinose, galactose and rhamnose (from pectins), along with minor contents of xylose and fucose (from hemicellulosic polysaccharides) [1]. The higher glucose content found in the Grenache may then indicate higher cellulose/hemicellulose content in this variety. The calculation of different specific ratios between neutral sugars has been proposed to characterize cell wall polysaccharides [35]. Only the arabinose/galactose ratio differed between the two varieties (**Table 1**). This ratio is characteristic of the PRAGs-like structures (polysaccharides rich in arabinose and galactose) [36]. It was higher in Carignan than in Grenache AISs, indicating different compositions in the insoluble PRAGs of the hairy regions of pectins. The degree of esterification (DE) of skin cell wall pectins ranged from 55 to 85% (**Table 1**), which is consistent with that found for other grape

varieties harvested at maturity [37]. The high deviations found for a given sample did not reveal any significant impact of variety or berry size and maturity.

Amino acids of cell wall insoluble proteins represented from 8 to 12% of skin AISs **(Table 1).** These amino acids belong to network of structural proteins. In general, our results indicated higher amino acid/monosaccharide ratios for Carignan than for Grenache. A factorial ANOVA followed by Tukey Test (Supplementary data **S3**) indicated an impact of both the variety and the maturity (deg^+ vs deg^- modalities). The AIS of the Carignan skins were significantly richer in amino acids than those of the Grenache and deg^+ modalities were richer than deg^- ones. Centered reduced PCA is shown on **Fig. 2B**. Variables and individuals were represented on the first 2 axes (1-2) that described 97% of the variability. All the variables were positively correlated on the axe 1 (90% of the variability).

3.2 Fresh skin polyphenol composition.

The polyphenol compositions in the fresh skins (Initial Skin Polyphenols, **ISP)** of the different Carignan and Grenache modalities were determined using UV-visible spectrophotometry (TPI, TRP), HPLC-DAD (total free anthocyanins) and HPSEC-DAD chromatography. UV-visible spectrophotometry and HPLC-DAD data showed differences between varieties, with significantly higher total polyphenol and anthocyanin contents in Carignan skins than in Grenache ones (**Table 1**). They also evidenced a different composition, related to the respective proportions of non-acylated and *p*-coumaroylated anthocyanins, further discussed later. HPSEC analysis (**Fig. 3**) was used to determine the concentrations and the size distributions of polymeric tannins (aDP > 3). HPSEC profiles evidenced three main populations, eluted between 13 and 21 min. The first one corresponds to polymeric tannins with aDP > 3, the second one (maximum A_{280} at 19 min.) to oligomeric tannins (dimers and trimer) co-eluted with anthocyanins and the third one (maximum A_{280} at 20 min) to non-acylated anthocyanins. Polyphenols eluted after are lower molecular weight compounds. The concentrations and size distributions of polymeric tannin were similar in all cases (**Table 1**). Thus,

the main differences between the two varieties and between the deg^+ and deg^- modalities for a given variety were their anthocyanin content and anthocyanin/polymeric tannin ratios. The size of the berries (vol^+, vol^-) had no significant impact. Berry size may affect the skin to juice ratio and then their concentration in wines but also the concentration of secondary metabolites in skins[6]. Such an impact on skin composition was not observed in our experimental conditions.

3.3 Polyphenol diffusion kinetic in wine-like maceration conditions.

Polyphenol diffusion in wine-like conditions was followed first by UV-visible spectrophotometry. The ethanol content in the solvent was progressively increased from 0 to 15% in 11 days to mimic solvent change during fermentation and maceration (**Fig. 1B**). However, unlike the conditions in red winemaking, samples were kept under constant stirring, i.e. under forced convective conditions in the liquid. Thus, the mass transfer is expected to be governed by the internal diffusion of polyphenols from within the skins to the solid-liquid boundary layer and to be faster than in winemaking [38]. In the present work, results have been reduced to the same mass of fresh skin (1 g) to compare the different modalities. TPI and TRP analysis indicated similar diffusion kinetic for both varieties and all modalities (**Fig. 4**). A large and rapid increase in total polyphenols and red pigments was observed during the first few hours of the diffusion, up to a maximum reached after 30 hours, which corresponds to 5% ethanol **(Fig.4B and 4D)**. After that, a gradual decrease in TRP continued until the end of the experiment. This decrease was concomitant with a decrease in anthocyanins (measured by HPLC-DAD, **Table 2**). It may have several origins: i) degradation into low molecular weight compounds such as vanillic and syringic acids; ii) involvement in chemical reactions leading to the formation of both colorless or pigmented derived compounds with different molar extinction coefficients [13,39]; iii) involvement in physico-chemical interactions with other skin soluble components in the medium (polysaccharide, proteins)[12,40] or solubility losses, leading to precipitation; iv) re-adsorption by solid parts. In parallel, total polyphenols also decreased for all Grenache modalities, but to a much lower extent, whereas a pseudo-plateau value was observed for Carignan

ones. As anthocyanins present a higher extinction coefficient than flavanols at 280 nm, their decrease may hide the extraction of other polyphenols as quantified by UV spectrophotometry [41]. Thus, after 30 h extraction, TPI profiles reflected equilibrium between polyphenol extraction from skins and anthocyanin decrease.

HPSEC analyses were also performed at the end of three different steps of the diffusion experiment (0, 5 and 15% ethanol) to follow the extraction of polymeric tannins. These steps were chosen based on the analyses performed at the end of all steps (t_0 to t_5) with the vol^+deg^+ modalities of the two varieties. Since the volume had no impact, results detailed in **Fig. 5** are those obtained with vol^+deg^+ and vol^+deg^- only. Polymeric tannin concentrations in the diffusion solutions increased for all Carignan modalities until the end of the 15% ethanol step (t_5), but only very slightly after the 12% one. By contrast, after a maximum reached at 5% ethanol, a slight decrease of polymeric tannins was observed for all Grenache modalities, more pronounced for the deg^- than the deg^+ ones.

3.4 Anthocyanin and tannin extraction: impact of variety, berry maturity and size

In wine-like maceration conditions, the concentrations of anthocyanins and tannins will be dependent on their extraction from skins but also from losses, which may be induced by chemical or physicochemical mechanisms. Maximum and final TRPs, anthocyanins and polymeric tannins concentrations in the diffusion solutions are reported for all modalities in **Table 2** and compared to their concentration in the fresh skin 60% acetone extracts.

Anthocyanin extraction strongly differed between the two cultivars. At their maximum in the diffusion medium, the % TRP extracted were twice higher for the Grenache modalities (61-75%) than for the Carignan ones (28-32%). So, similar concentrations of red pigments were obtained by diffusion for the Carignan vol^+deg^- and the Grenache vol^+deg^+ modalities whereas anthocyanin contents in the Carignan vol^+deg^- skins were twice higher. The % of extracted anthocyanins was calculated for non-acylated and *p*-coumaroylated anthocyanins (**Table 2**). An ANOVA analysis

showed that they were significantly different between the two varieties, but not between different berry size and maturity for a given variety. Besides, soluble anthocyanins were mainly the non-acylated ones, from the beginning to the end of the experiment. The coumaroylated/non acylated anthocyanin ratios varied between 0.3 (deg^+) and 0.5 (deg^-) in the Carignan skins, and between 0.15 (deg^+) and 0.25 (deg^-) in the Grenache ones. In solution, these ratios fell to values between 0.05 and 0.1. These results are consistent with previous ones[27,42] that showed a poor extraction of *p*-coumaroylated anthocyanins in both simulated extraction experiments and winemaking for other grape varieties. However, these structural features alone did not account for the differences observed here between Carignan and Grenache, as lower extraction were also found for non-acylated anthocyanins in Carignan. According to Ortega-Regules *et al.*[1,37], higher anthocyanin extractability could be associated with low concentrations in galactose, glucose and mannose in the skin AISs, along with a low DE of pectins. These results, obtained from the comparison of four varieties (Cabernet-Sauvignon, Syrah, Merlot and Monastrell), are not in total agreement with those obtained here: Carignan had in fact lower galactose content than Grenache (and higher arabinose/galactose ratios), but also lower glucose and mannose contents (cellulose, hemicelluloses). This suggest that differences in the insoluble PRAGs of pectins could be of importance in anthocyanin extractability.

Polymeric tannins extraction at maximum only represented a small proportion of skin polymeric tannins, as expected [1,10,27]. It was higher for Grenache (from 13 to 28%, depending on the modalities), than for Carignan (10 to 20%), especially for the deg^+ modalities (**Table 2**). It has been previously observed with Syrah that higher anthocyanin/tannin ratios in skins led to a higher extraction of skin tannins [26]. This ratio was higher and statistically different (Tukey test) for Carignan, and among the Grenache modalities, differences were significant between deg^+ and deg^- modalities (**Table 1**). It could be concluded from present results and as for Syrah that higher anthocyanin/tannin ratios favour the extraction of tannins for Grenache (deg^+ vs deg^- modalities, **Table 2**). However, this was not the case for Carignan and was not verified when comparing the two varieties. Differences in

the composition of AISs may have offset a positive impact of anthocyanins. Differences in tannin extractability are largely attributed to differences in the polysaccharide composition of skin AISs [3,4] and/or to their protein content, higher in Carignan than in Grenache. In addition to higher maximums in solution, tannin diffusion was faster for Grenache than for Carignan, and favored by increasing ethanol contents for the latter. This may be due to stronger interactions of tannins with cell walls, hydrophobic interactions and H-bonds being weakened by the ethanol concentration. In accordance with literature, the comparison of polymeric tannin size distribution in skins and in solutions (**Fig. 3** and **5, supplementary data S2**) indicated that the highest molecular weight tannins are not extracted in wine-like solvents [24,27]. Indeed, skin polymeric tannins were eluted between 13 and 18.2 min., with a peak maximum around 15-16 min whereas extracted tannins were eluted between 15 and 18.2 min, with a maximum around 17-17.3 min.

After a maximum, a decrease in polymeric tannins measured by SEC (peak 1, **Fig.5**) was observed for all Grenache modalities that reduced differences between varieties at the end of the maceration (**Table 2**). A much more pronounced decrease was also observed in all cases for red pigments. At the end of the diffusion, skins were recovered and the whole diffusion media clarified by centrifugation. Red-coloured pellets were present in all cases. Their visual observation showed the existence of two types of materials: one, located at the bottom of the centrifuge tube and having a fibrous-like appearance, and another, above, having a gel-like appearance. The exact nature of these deposits was not further investigated here and will be the subject of future work. Their presence indicated that either precipitation of soluble material from skin cells and degradation of some insoluble material leading to the presence of cell fragments/fibers occurred during our maceration experiments. This material was found in higher amounts in the Carignan modalities than in the Grenache ones (**Table 2**).

Polyphenols in these pellets (**PSP**, **Fig.1**), were extracted and quantified. Results confirmed the presence of anthocyanins and tannins, related to precipitation or adsorption phenomena. They

represented a small but non negligible part of skin polyphenols, and were in higher amount for Carignan. Red pigments in **PSP**s accounted for 7-9% of the initial skin ones for Carignan, to be compared to 3-5% for Grenache, with no clear incidence of berry maturity or size. Polymeric tannins accounted for 6-9% of the initial skin tannins for Carignan and for 3-6% for Grenache. HPLC-DAD analyses evidenced a preferential involvement of coumaroylated anthocyanins in the precipitates (**Table 2**), with coumaroylated/non-acylated anthocyanin ratios between 3.8 (deg^+) and 6.3 (deg^-) in Carignan **PSP**s and around 1 in Grenache ones. This can be compared to the same ratio in **ISPs:** within the range [0.30- 0.50] for Carignan and [0.14-0.27] for Grenache. This indicated a lower solubility of coumaroylated anthocyanins and/or favored interactions with other constituents extracted from skins.

Whatever the variety or the modality, anthocyanins in **PSPs** did not explain the anthocyanin losses observed in solution during maceration (SSP at max *versus* SSP at the end). Considering first Carignan, losses in *p*-coumaroylated anthocyanins during maceration represented between 0.1 and 0.7 $mg.L^{-1}$ and were then much lower than their content in PSPs (7.3 to 10.1 $mg.L^{-1}$), whereas losses in non-acylated anthocyanins represented between 10 and 12.4 $mg.L^{-1}$ and where much higher than their contents in PSPs (1.1 to 2.3 $mg.L^{-1}$). For Grenache, *p*-coumaroyled anthocyanins in PSPs could be related to their precipitation from solution in all cases but the vol^-deg^+ modality. In all cases and as with Carignan, losses in non-acylated anthocyanins (between 2.4 and 10.3 $mg.L^{-1}$, depending on the maturity) were much higher than their content in PSPs (0.07 to 0.2 $mg.L^{-1}$). This indicated that there were also losses of anthocyanins related to chemical reactions. TRP at the end of the maceration experiment represented between 40 (deg^+) and 50% (deg^-) of the maximum TRP value for the Grenache modalities (to be compared to 42 and 54 % for anthocyanins) respectively, and between 30 (deg^+) and 43% (deg^-) for the Carignan ones (42 and 53 % for anthocyanins). Thus, anthocyanin degradation and/or the formation of non-pigmented derived compounds, such as those from anthocyanin-tannin adducts, were likely the predominant phenomenon in the observed decreases. In

the Carignan, part of anthocyanin losses could be attributed to the formation of derived red pigments such as tannin-anthocyanin adducts.

As for coumaroylated anthocyanins, it is unclear whether tannins in **PSP**s were precipitated or adsorbed very quickly after their solubilisation or whether their presence was related to their interaction inside the cells with debris/fibers released during maceration. A slight decrease in tannin concentrations was observed during the experiment with the Grenache, not with the Carignan (**Fig.5**, **Table 2**). As extraction was more gradual for this variety and analyses only carried out at the end of the various ethanol additions, extraction may have counterbalanced losses related to physico-chemical mechanisms.

3.5 Residual polyphenols in skins after maceration

Skins after diffusion experiments were successively washed with new 0 and 15% ethanol wine-like solvents until total polyphenol extraction (**Fig. 1B**). These washings led to the recovery of so-called residual extractible polyphenols in wine-like solvents (**RESP**, **Table 2**). The latter represented from 7 to 12% of the initial skin anthocyanins for both variety and from 6 to 9% of the polymeric tannins. As solid/liquid diffusion is driven by a partition coefficient between the solid and the liquid phases, their extraction in new solvents indicated that an equilibrium between phases had been reached in our conditions at the end of the maceration. Finally, residual polyphenols in skins (non-extractable skin polyphenols **NESP**s) were extracted in 60% acetone, as for **ISP**s and **PSP**s. Only very small amounts of red pigments were recovered (**Table 2**). **NESP**s were mainly polymeric tannins (**Table 2**, **Fig. 6**), along with small amounts of oligomers. Polymeric tannins recoveries in **NSEP** were higher for the Carignan (between 24 and 40%) than for the Grenache (between 12 and 30%) and, within a given variety, about twice higher for the deg^+ (39 and 27%) than for the deg^- (24 and 13%) modalities.

Similar HPSEC profiles were found for **SSP** and **RESP (Fig. 6)**, whatever the variety and the modality. HPSEC also evidenced a wider size distribution of polymeric tannins in **PSPs** by

comparison to diffusion media, related to the presence of higher molecular polymers, and strong differences between Carignan and Grenache, related to *p*-coumaroylated anthocyanins (supplementary data **S4**). As expected, **NESPs** were preferentially the highest molecular weight tannins in skins. A mass balance was performed with the whole results obtained from our diffusion/extraction experiments (**Table 2, Fig.6**). Taking the sum of TRP in **SSP** (end of the maceration), **PSP**, **ESP** and **NESP** resulted in the recovery of only 45 to 64% of the initial skin red pigments for Grenache (to be compared to 19-36% of the anthocyanins) and of 31 to 51% for Carignan (25-28% of the anthocyanins), with no clear impact of the maturity (deg^+ vs deg^- modalities). Although no fermentation was carried out under our experimental conditions, our results are consistent with those of Morel-Salmi *et al.* [43], who observed a drastic loss (around 70 %) of anthocyanins during fermentation when they compared anthocyanins present in the initial skins, the wines and the pomaces. Contrary to that observed with anthocyanins, higher tannin recoveries were found for the Carignan (72-74% for the deg^+ modalities and 53-56% for the deg^-) than for the Grenache (60-70% for deg^+ and 33-38% for deg^-), but as for anthocyanins, they were not complete. Besides, HPSEC indicated an excess of A_{280nm} in the range of oligomers and smaller polymers (17 - 19 minutes) for all modalities except Grenache deg^-. These elution times correspond to trimers-heptamers according to **Figure S2**. This excess, along with the differences observed between TRPs and anthocyanins, agrees with the occurrence of chemical changes during maceration. The latter may affect anthocyanins as well as tannins alone. Indeed, even in the absence of fermentation and under conditions of protection against oxidation, cleavage and rearrangements reactions can also occur within tannins that does not imply anthocyanins [13,43,44]. These chemical reactions may also occur within skins when the integrity of the berries is broken. The quantification of polymeric tannins by HPSEC was based here on A_{280nm} measurements and was done in equivalent epicatechin. The molar absorptivity of tannins differs as a function of their degree of polymerization, even if their chemical nature is not changed [45] and can also be modified by chemical reactions. As SEC separation is based

on the size, polymers with the same size but different chemical features (including different molar extinction coefficients) are coeluted, which may induce a misquantification.

Besides chemical changes, it must be considered that the same extraction procedure was performed on fresh skins, precipitates and skins after maceration and washings, whereas these samples have different characteristics. Changes in cell-wall structures during the maceration process (11 days), leading to different permeability and solvent accessibility, as well as to stronger physico-chemical interactions than those observed in fresh skins or possibly the formation of covalent bonds, may have resulted in a lower extraction at the end (**NESP** vs **ISP**). The fact that both anthocyanins and tannins total recovery differed between the Carignan and Grenache varieties and for tannins, between the deg^+ and the deg^- modalities, is of interest and likely indicated structural differences in cells/cell walls in the initial skins and/or different changes during maceration that deserve to be further explored.

4. Conclusion

The diffusion results obtained under model conditions, as well as the analyses carried out on the precipitates observed at the end of maceration and on the residual skins, made it possible to highlight differences between the two varieties studied and between berries with different levels of maturity. The impact of berry size was much less evident. In agreement with the literature, these differences concerned first the extraction of anthocyanins and tannins. The former was proportionally much more important in Grenache than in Carignan. This could be partly explained by the higher proportion of *p*-coumaroylated anthocyanins in Carignan skins but the extraction of non-acylated anthocyanins also was lower for this variety. The extraction of tannins was also slightly higher but above all much faster in Grenache than in Carignan, and higher for the deg^+ modalities. No decisive impact of the anthocyanin-to tannin ratio on the tannin final contents in solution was observed.

Carbohydrate and amino-acid analysis of skin AISs evidenced also differences in composition between the two varieties, but not between the different modalities (especially ripeness). These differences were related to glucose (cellulose/hemicelluloses), in higher content in Grenache than in Carignan, and to different arabinose/galactose ratios reflecting different structures of PRAGs. Higher protein contents were found in Carignan skin AISs. However, compositional analyses on AISs do not provide any structural information on the initial polymeric network in skin cell walls. This information, combined with a better knowledge of its changes during maceration and a better understanding of the part played by the chemical reactivity of tannins and anthocyanins in solution or in skins, is needed to understand the impact of variety and/or berry maturity on tannin and anthocyanin extractability.

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Table 1. Alcohol Insoluble Solids contents (AIS) and their monosaccharide and amino acid compositions, and polyphenol contents of the four modalities of Carignan and Grenache initial skin berries. Results are expressed in mg/g fresh skin or AIS. Different letters indicate significant differences between samples for a given parameter (Tukey's test for $p < 0.05$). DE (%) is the percentage of methylesterification.

		Carignan				Grenache			
		vol$^+$deg$^+$	vol$^-$deg$^+$	vol$^+$deg$^-$	vol$^-$deg$^-$	vol$^+$deg$^+$	vol$^-$deg$^+$	vol$^+$deg$^-$	vol$^-$deg$^-$
AIS (g.kg^{-1} fresh skin)		30.9 ± 7.1 ^{a}	35.7 ± 6.2 ^{a}	40.3 ± 6.7 ^{a}	42.7 ± 5.5 ^{a}	41.8 ± 7.2 ^{a}	39.0 ± 7.0 ^{a}	43.6 ± 3.0 ^{a}	39.0 ± 3.4 ^{a}
Amino acid/monosaccharide ratio		0.34	0.32	0.30	0.32	0.25	0.26	0.26	0.21
Monosaccharides (mg.kg^{-1} AIS)	Rhamnose	7.4 ± 0.6	7.6 ± 0.5	7.6 ± 0.2	6.5 ± 0.9	6.2 ± 0.5	8.0 ± 1.5	7.2 ± 2.0	6.9 ± 0.9
	Fucose	1.8 ± 0.2	2.0 ±0.4	1.8 ± 0.0	1.8 ± 0.0	1.7 ± 0.1	1.7 ± 0.3	1.7 ± 0.2	1.7 ± 0.1
	Arabinose	22.4 ± 2.5	21.9 ± 1.7	20.8 ± 1.4	19.6 ± 1.9	18.7 ± 1.1	21.2 ±2.8	19.3 ± 2.6	19.5 ± 0.6
	Xylose	8.5 ± 1.2	7.5 ± 0.8	8.4 ±0.5	7.9 ± 1.9	8.2 ± 0.6	9.0 ±1.2	8.2 ± 0.3	9.0 ± 0.1
	Mannose	14.4 ± 1.6	14.5 ± 1.7	14.6 ± 0.7	14.8 ± 1.9	18.4 ± 1.2	16.0 ± 0.8	14.8 ± 5.9	18.0 ± 0.9
	Galactose	19.7 ± 1.0	22.2 ± 1.8	22.0 ±1.3	21.3 ± 3.5	27.3 ± 3.0	26.0 ± 1.0	24.6 ± 1.8	25.5 ± 2.5
	Glucose	138.6 ± 6.1	142.9 ±15.1	135.0 ± 6.5	134.0 ± 16.5	154.9 ± 11.8	157.7 ± 4.8	132.4 ± 26.1	159.9 ± 15.7
	Uronic acids	173.1 ± 8.4	178.1 ± 22.0	171.2 ± 13.9	163.4 ± 9.1	148.6 ± 26.0	168.2 ±33.1	162.7 ± 22.8	158.8 ± 22.2
	Total	**385.9 ± 20.3^a**	**397.0 ± 28.7^a**	**381.6 ± 23.9^a**	**369.4 ± 30.8^a**	**384.0 ± 18.5^a**	**407.9 ± 41.7^a**	**370.9 ± 38.3^a**	**399.3 ± 11.5^a**
	Ara/Gal ratio	**1.1 ± 0.1**	**1.0 ± 0.0**	**1.0 ± 0.0**	**1.0 ± 0.1**	**0.7 ± 0.0**	**0.8 ± 0.1**	**0.8 ± 0.1**	**0.8 ± 0.1**
	DE (%)	**55.2 ± 4.1^c**	**61.4 ± 0.8^{bc}**	**54.0 ±0.3^c**	**85.2 ± 3.4^a**	**71.9 ± 4.2^{abc}**	**71.9 + 11.8^{abc}**	**60.7 ± 7.6^{bc}**	**78.6 ± 10.9^{ab}**
Amino acids (g.kg^{-1} AIS)	Hydroxyproline	3.8 ± 0.3	4.0 ± 0.4	2.6 ± 0.2	3.0 ± 0.0	2.0 ± 0.2	2.4 ± 0.1	1.8 ± 0.2	1.8 ± 0.6
	Proline	7.2 ± 0.4	7.5 ± 0.6	6.2 ± 0.3	6.5 ± 0.1	5.2 ± 0.2	6.1 ± 0.2	5.1 ± 0.5	4.8 ± 1.6
	Alanine	7.4 ± 0.3	7.5 ± 0.7	6.8 ± 0.3	7.2 ± 0.2	5.4 ± 0.2	6.0 ± 0.3	5.3 ± 0.3	4.7 ± 1.5
	Arginine	5.5 ± 0.7	4.9 ± 0.2	4.6 ±0.3	4.5 ± 0.4	4.5 ± 0.3	5.1 ± 0.2	4.6 ± 0.8	4.2 ± 1.3
	Aspartic acid	12.9 ± 0.5	13.3 ± 1.0	11.5 ± 0.5	12.2 ± 0.3	8.9 ± 0.4	10.2 ± 0.5	8.8 ± 0.7	7.9 ± 2.4
	Glutamic acid	14.6 ± 0.6	14.5 ± 1.1	12.8 ± 0.5	13.4 ± 0.7	10.9 ± 0.6	12.2 ± 0.7	10.7 ± 1.0	9.6 ± 3.3
	Glycine	8.3 ± 0.4	8.4 ± 0.6	7.7 ± 0.3	8.2 ± 0.3	6.4 ± 0.3	7.0 ± 0.4	6.2 ± 0.4	5.6 ± 1.7
	Histidine	4.8 ± 0.2	4.7 ± 0.5	4.2 ± 0.1	4.6 ± 0.2	3.7 ± 0.1	4.1 ± 0.3	3.6 ± 0.2	3.5 ± 1.1
	Isoleucine	6.0 ± 0.4	5.8 ± 0.4	5.2 ± 0.1	5.3 ± 0.3	4.7 ± 0.2	5.4 ± 0.3	4.7 ±0.5	4.2 ± 1.3
	Leucine	10.0 ± 0.6	9.7 ± 0.8	8.8 ± 0.3	9.0 ± 0.3	7.8 ± 0.3	8.8 ± 0.4	7.7 ± 0.7	6.9 ± 2.2
	Lysine	10.6 ± 0.6	10.5 ± 0.9	9.2 ± 0.4	9.8 ± 0.6	8.2 ± 0.2	9.0 ± 0.5	7.9 ± 0.4	7.1 ± 2.3
	Phenylalanine	7.2 ± 0.4	7.0 ± 0.6	6.3 ± 0.3	6.5 ± 0.2	5.1 ± 0.2	5.8 ± 0.3	5.0 ± 0.6	4.6 ± 1.4
	Serine	8.0 ± 0.4	8.1 ± 0.8	7.2 ± 0.3	7.6 ± 0.2	5.4 ± 0.2	6.1 ± 0.3	5.3 ± 0.4	4.7 ± 1.5
	Threonine	7.0 ± 0.2	7.2 ± 0.5	6.1 ± 0.3	6.4 ± 0.3	4.8 ± 0.2	5.6 ± 0.3	4.7 ± 0.4	4.3 ± 1.3
	Tyrosine	4.0 ± 0.9	3.0 ± 0.1	3.0 ± 0.8	2.5 ± 0.2	2.5 ± 0.5	3.2 ± 0.4	2.7 ±1.0	2.7 ± 0.9
	Valine	7.6 ± 0.5	7.5 ± 0.4	6.6 ± 0.1	6.9 ± 0.4	5.9 ± 0.3	6.7 ± 0.3	5.8 ± 0.5	5.2 ± 1.6
	Total	**127.4 ± 8.3^a**	**126.2 ± 10.0^a**	**111.4± 5.5^{ab}**	**116.3 ± 5.4^a**	**95.7 ±8.2^{ab}**	**106.5 ± 5.7^{ab}**	**94.9 ± 10.7^{ab}**	**83.3 ±26.6^b**

Polyphenols								
TPI	316.4 ± 34.3 [a]	325.9 ± 38.7 [a]	245.0 ±19.4[bc]	274.0±21.7[ab]	209.0 ± 11.4 [cd]	165.3 ±14.9 [de]	149.5±13.1 [de]	138.4 ± 10.2 [e]
TRP	289.8± 32.5 [a]	302.4 ± 20.8 [a]	264.6±10.2[b]	252.0±21.5 [b]	105.0 ± 9.7 [c]	96.6 ± 2.2 [c]	42.0 ± 3.5 [d]	37.8 ± 2.9 [d]
Anthocyanins (HPLC) ($mg.g^{-1}$ fresh skin)	5.4 ± 0.8 [a]	5.6 ± 0.6 [a]	4.9 ± 0.2 [b]	4.75 ± 0.4 [b]	2.0 ± 0.1 [c]	2.0 ± 0.2 [c]	0.9 ± 0.1 [d]	0.7 ± 0.1 [d]
Polymeric tannins ($mg.g^{-1}$ fresh skin)	8.6 ±1.7 [a]	9.2 ± 1.4 [a]	10.5 ± 1.8 [a]	10.5 ± 1.4 [a]	9.9 ± 0.8 [a]	9.6 ± 1.0 [a]	9.8 ± 0.5 [a]	9.5 ± 0.9 [a]
Anthocyanin / tannin ratio	**0.63** [a]	**0.61** [a]	**0.47** [a]	**0.45** [a]	**0.20** [b]	**0.21** [b]	**0.09** [c]	**0.08** [c]

Table 2. Polyphenol extraction. Values represent concentrations reduced considering 1 g fresh skin in 42 mL solvent (in mg/L eq. malvinin-3-*O*-glucoside for anthocyanins and mg/L eq. epicatechin for tannins). ISP: initial skin polyphenols (60% acetone); SSP: soluble extracted skin polyphenols (at maximum and at the end of the maceration experiment); PSP: Precipitated skin polyphenols; RESP: residual extractable polyphenols in wine-like solvents; NESP: non extracted polyphenols (60% acetone). Non-acyl. (non-acylated) and *p*-coum. (*p*-coumaroylated) anthocyanins. Different letters indicate significant differences between samples for a given parameter (Tukey's test for $p<0.05$).

		Carignan				Grenache			
		vol$^+$deg$^+$	vol$^-$deg$^+$	vol$^+$deg$^-$	vol$^-$deg$^-$	vol$^+$deg$^+$	vol$^-$deg$^+$	vol$^+$deg$^-$	vol$^-$deg$^-$
ISP	TRP	6.9 ± 0.1^{a}	7.2 ± 0.1^{a}	6.3 ± 0.1^{b}	6.00 ± 0.03^{b}	2.5 ± 0.2 ^{c}	2.3 ± 0.1 ^{c}	1.0 ± 0.1^{d}	0.9 ± 0.1 ^{d}
	Non-acyl. anthocyanins	**82.0 ± 20.3** ^{ab}	**96.7 ± 8.6** ^{a}	**56.0 ± 12.8**^{bc}	**60.5 ± 8.7** ^{bc}	**38.5 ± 2.7** ^{cde}	**44.6 ± 12.0** ^{cd}	**15.4 ± 1.3** ^{de}	**13.1 ± 1.2** ^{e}
	***p*-coum. anthocyanins**	**26.3 ± 5.6** ^{a}	**29.3 ± 3.6** ^{a}	**28.5 ± 7.4a**	**28.8 ± 2.6** ^{a}	**6.0 ± 0.3** ^{b}	**6.1 ± 0.9** ^{b}	**4.1 ±0.5** ^{b}	**3.4 ± 0.2** ^{b}
	Polymeric tannins	204.5 ± 41.0 ^{a}	220 ± 33.6^{a}	250.5 ±42.0^{a}	250.2 ±33.6 ^{a}	236.4 ±20.1^{a}	229.0 ±24.1 ^{a}	233.4 ±11.3^{a}	225.4 ±21.1^{a}
SSP at max	TRP	1.9 ± 0.1 ^{ab}	2.2 ± 0.1 ^{a}	1.8 ±0.3 ^{ab}	1.9 ±0.3 ^{ab}	1.6 ± 0.3 ^{b}	1.6 ± 0.2 ^{b}	0.6 ± 0.1 ^{c}	0.6 ±0.2 ^{c}
	% skin TRP	*27.5 ± 1.9* ^{b}	*31.0 ± 1.2* ^{b}	*28.0 ±4.9* ^{b}	*32.0 ±4.2* ^{b}	*62.0 ±10.9* ^{a}	*67.0 ± 8.7* ^{a}	*64.0 ± 9.0* ^{a}	*75.0 ± 20.4* ^{a}
	non-acyl. anthocyanins	**23.3 ± 0.4** ^{ab}	**26.7 ± 1.4** ^{a}	**21.7 ±3.2** ^{ab}	**23.1 ± 3.6** ^{ab}	**20.0 ± 3.7** ^{ab}	**18.8 ± 2.3** ^{b}	**6.9 ± 0.7** ^{c}	**5.6 ± 1.2** ^{c}
	% skin non acyl. anthocyanins	*29.7 ±7.8* ^{b}	*27.8 ± 3.1* ^{b}	*39.6 ±3.9* ^{b}	38.2 ± 3.0 ^{b}	*52.1 ± 9.2* ^{a}	*44.5 ± 5.2* ^{a}	*45.2 ± 7.8* ^{a}	*43.3 ± 5.0* ^{a}
	***p*-coum. anthocyanins**	**2.4 ± 0.9** ^{a}	**2.0 ± 0.2** ^{ab}	**1.5 ± 0.4** ^{abc}	**1.2 ± 0.2** ^{bcd}	**1.2 ± 0.2** ^{bcd}	**0.9 ± 0.2** ^{cd}	**0.7 ± 0.04** ^{cd}	**0.5 ± 0.1** ^{d}
	% skin p-coum. anthocyanins	*9.9 ± 5.1* ^{b}	*6.7 ± 1.2* ^{b}	*5.5 ± 2.1* ^{b}	*4.2 ± 0.5* ^{b}	*20.4 ± 2.8* ^{a}	*14.4 ± 3.5* ^{a}	*16.9 ± 1.5* ^{a}	*14.0 ± 2.7* ^{a}
	Polymeric tannins	39.0 ± 4.5 ^{bc}	40 ± 0.6 ^{bc}	39.0±10.5 ^{bc}	34.0 ±8.8 ^{c}	64.4 ± 7.2 ^{a}	54.0 ± 6.1 ^{ab}	45.0 ± 3.6 ^{bc}	40.0 ± 7.1 ^{bc}
	% skin polymeric tannins	*19.0 ± 2.2* ^{bc}	*18.0 ± 0.3* ^{bc}	*16.0 ± 4.2* ^{c}	*13.6 ± 3.5* ^{c}	*27.0 ± 3.0* ^{a}	*23.6 ± 2.6* ^{ab}	*19.0 ± 1.5* ^{bc}	*18.0 ± 3.1* ^{bc}
SSP at end	TRP	1.3 ± 0.1 ^{ab}	1.5 ±0.0 ^{a}	1.0 ± 0.2 ^{b}	1.1 ± 0.3 ^{b}	0.9 ± 0.2 ^{b}	1.0 ± 0.1 b	0.3 ± 0.0 ^{c}	0.3 ± 0.1 ^{c}
	% skin TRP	*18.1 ±1.3* ^{d}	*21.0±0.4* ^{cd}	*16.0 ± 2.9* ^{d}	*18.5 ± 4.2* ^{d}	*34.0 ± 6.0* ^{a}	*45.0 ± 6.2* ^{ab}	*27.0 ± 3.0* ^{bcd}	*33.0 ± 7.0* ^{abc}
	non-acyl. anthocyanins	**13.3 ± 1.3** ^{ab}	**15.6 ± 0.2** ^{a}	**9.6 ± 1.6** ^{b}	**11.3 ± 3.0** ^{ab}	**9.7 ± 1.5** ^{b}	**12.4 ± 1.7** ^{ab}	**2.9 ± 0.2** ^{c}	**3.2 ± 0.8** ^{c}
	***p*-coum. A**	**0.7 ± 0.1** ^{abc}	**1.0 ± 0.0** ^{a}	**0.8 ± 0.2** ^{ab}	**1.1 ± 0.3** ^{a}	**0.4 ± 0.07** ^{cd}	**0.5 ± 0.1** ^{bc}	**0.03 ± 0.01** ^{d}	**0.06 ± 0.02** ^{d}
	Polymeric tannins	39.0 ± 4.5 ^{bc}	40.0 ± 0.6 ^{bc}	39.0 ± 10.5 ^{bc}	34.0 ± 8.8 ^{a}	58.0 ± 7.9 ^{a}	54.0 ± 6.1 ^{ab}	32.0 ± 2.7 ^{c}	33.0 ± 5.0 ^{c}
	% skin polymeric tannins	*19.0 ± 2.2* ^{ab}	*18.0 ± 0.3* ^{ab}	*16.0 ± 4.2* ^{b}	*13.6 ±3.5* ^{b}	*25.0 ± 3.4* ^{a}	*23.6 ± 2.6* ^{a}	*14.0 ± 1.1* ^{b}	*15.0 ± 2.2* ^{b}
PSP	mg fresh weight/g fresh skin	63 ± 2	78 ± 6	59 ± 19	101 ± 7	38 ± 11	65 ± 14	34 ± 2	67 ± 18
	TRP	0.55 ± 0.02 ^{a}	0.50 ± 0.06 ^{a}	0.45 ± 0.15 ^{a}	0.54 ± 0.06 ^{a}	0.10 ± 0.02 ^{b}	0.08 ± 0.01 ^{b}	0.05 ± 0.00 ^{b}	0.04 ± 0.01 ^{b}
	% skin TRP	*7.9 ± 0.2* ^{ab}	*7.0 ± 0.8* ^{abc}	*7.2 ± 2.4* ^{abc}	*9.0 ± 1.0* ^{a}	*4.1 ± 0.6* ^{cd}	*3.3 ± 0.3* ^{d}	*4.8 ± 0.2* ^{bcd}	*4.5 ± 1.5* ^{cd}
	non-acyl. anthoyanins	**2.1 ± 0.1** ^{ab}	**2.3 ± 0.2** ^{a}	**1.6 ± 0.6** ^{ab}	**1.5 ± 0.4** ^{b}	**0.24 ± 0.2** ^{c}	**0.70 ± 0.07** ^{c}	**0.07 ± 0.02** ^{c}	**0.17 ± 0.09** ^{c}
	***p*-coum. anthocyanins**	**8.6 ± 0.4** ^{a}	**7.3 ± 0.8** ^{a}	**10.0 ± 3.7** ^{a}	**10.1 ± 2.6** ^{a}	**0.21 ± 0.2** ^{b}	**0.71 ± 0.04** ^{b}	**0.07 ± 0.02** ^{b}	**0.19 ± 0.08** ^{b}
	Polymeric tannins	19.0 ± 1.7 ^{abc}	20.0 ± 2.0 ^{ab}	14.0 ± 4.4 ^{bcd}	23.5 ± 3.8 ^{a}	8.6 ± 1.1 ^{de}	13.0 ± 0.8 ^{cde}	5.8 ± 0.2 ^{e}	7.4 ± 2.6 ^{de}
	% skin polymeric tannins	*9.4 ± 0.8* ^{a}	*9.1 ± 0.9* ^{a}	*5.7 ±1.8* ^{b}	*9.4 ± 1.5* ^{a}	*3.6 ± 0.5* ^{bc}	*5.6 ±0.4* ^{b}	*2.5 ±0.1* ^{c}	*3.3 ± 1.2* ^{bc}
RESP	TRP	0.64 ± 0.1 ^{a}	0.62 ± 0.0 ^{a}	0.45 ± 0.1 ^{b}	0.42 ± 0.1 ^{bc}	0.24 ± 0.0 ^{d}	0.3 ± 0.0 ^{cd}	0.07 ± 0.0 ^{c}	0.10 ± 0.0 ^{c}
	% skin TRP	*9.3 ± 0.3* ^{a}	*8.7 ± 0.3* ^{ab}	*7.1 ± 0.8* ^{d}	*7.0 ± 1.8* ^{d}	*9.6 ±1.2* ^{bc}	*12.6 ± 0.9* ^{a}	*7.0 ± 0.0* ^{bc}	*13.0 ± 1.2* ^{cd}
	Polymeric tannins	11.0 ± 0.4 ^{bc}	15.0 ± 0.3 ^{bc}	16.0 ± 2.2 ^{b}	21.4 ± 2.8 ^{a}	16.0 ± 3.0 ^{bc}	23.2 ±1.3 ^{a}	11.0 ± 0.8 ^{c}	16.0 ± 2.2 ^{bc}
	% skin polymeric tannins	*5.4 ± 0.2* ^{cd}	*6.9 ±0.2* ^{bc}	*6.4 ± 0.9* ^{bcd}	*8.6 ± 1.1* ^{ab}	*6.7 ± 1.3* ^{bcd}	*10.1 ± 0.6* ^{a}	*4.5 ± 0.3* ^{d}	*6.9 ± 1.0* ^{bc}

	TRP	0.09 ±0.02 [a]	0.06 ± 0.0 [ab]	0.03 ±0.01 [b]	0.03 ± 0.01 [b]	0.08 ± 0.0 [a]	0.09 ± 0.0 [a]	0.03 ±0.0 [b]	0.03 ± 0.0 [b]
	% skin TRP	*1.3 ± 0.3 [b]*	*0.8 ± 0.5 [b]*	*0.5 ± 0.2 [b]*	*0.5 ± 0.2 [b]*	*3.1 ± 0.3 [a]*	*3.9 ± 0.5 [a]*	*3.1 ± 0.3 [a]*	*3.6 ± 0.4 [a]*
	Polymeric tannins	81.9 ± 4.9 [ab]	83.8 ± 0.3 [a]	61.1 ± 6.1 [c]	61.0 ± 6.4 [c]	58.4 ± 2.0 [c]	70.4 ± 6.1 [bc]	28.9 ±2.1 [d]	29.2 ± 1.5 [d]
	% skin polymeric tannins	*40.0 ± 2.4 [a]*	*38.1 ± 0.1 [a]*	*24.4 ± 2.4 [c]*	*24.4 ± 2.6 [c]*	*24.7 ± 0.9 [c]*	*30.8 ± 2.7 [b]*	*12.4 ± 0.9 [d]*	*13.0 ± 0.7 [d]*

Figure captions

Figure 1. A) Preparation of the different modalities. D = berry diameter; §: sugar content expressed in $g.L^{-1}$, Car = Carignan, Gre = Grenache.
B) Experiments performed on each modality. First step: diffusion in wine-like solvent with a gradual increase of the ethanol content. t_0, 0% ethanol – 24h; t_1 5 % ethanol – 48 h; t_2 10 % ethanol – 48 h; t_3 12% ethanol – 48 h; t_4 14 % ethanol – 48 h; t_5 15 % ethanol – 48 h. Recovery of the model wine-like solution and of the skins at the end of the experiment and centrifugation of the solution to separate soluble compounds (Soluble Extracted Skin Polyphenols, *SSP*) and precipitates (Precipitated Skins Polyphenols, *PSP*). Second step: successive washings of skins with new wine-like solutions (0 and 15% ethanol) until no further extraction is observed (residual extractable polyphenols in wine-like solvents, *RESP*). Extraction in 60 % acetone (non-extracted skin polyphenols, *NESP*,).

Figure 2. PCA analysis of the variables and individual distribution regarding monosaccharides constitutive of polysaccharides (A) and amino acids constitutive of proteins (B) in the skin AISs of Carignan and Grenache different modalities.

Figure 3. Initial polyphenols contents in the skins of the different modalities ($vol^+ deg^+$, $vol^- deg^+$, vol^+deg^- and vol^-deg^-) by HPSEC. Carignan: car; Grenache: gre. Peak 1: polymeric tannins with aDP > 3; peak 2: oligomeric tannins (dimers and trimer) co-eluted with acylated anthocyanins; peak 3: non-acylated anthocyanins.

Figure 4. Polyphenols diffusion during skin maceration experiments in wine-like solvents, followed by UV-visible spectrophotometry. TPI: Total Polyphenol Index ($A_{280\ nm}$). TRP: Total Red Pigments ($A_{520\ nm}$). Arrows indicate the different ethanol additions. **A**) Carignan, TPI; **B**) Carignan TRP; **C**) Grenache TPI, **D**) Grenache TRP.

Figure 5. HPSEC analysis of polyphenols at the end of different steps of the diffusion experiments. Peak 1: polymeric tannins; Peak 2: di-trimers and anthocyanins, Peak 3: anthocyanins. Integration of the 3 main peaks of the HPSEC spectra at the end of the first (t_0, 0% ethanol), second (t_1, 5% ethanol) and last (t_5, 15% ethanol) steps of the maceration experiment for the vol^+deg^+ and vol^+deg^- modalities of the Carignan (**A**) and the Grenache (**B**) varieties. Comparison of the HPSEC profiles of the vol^+deg^+ (**C**) and vol^+deg^- (**D**) modalities of Grenache and Carignan at the end of t_1 and t_5.

Figure 6. Comparison of the HPSEC profiles of the different phenolic extracts for the different Grenache and Carignan vol^+deg^+ and vol^+deg^- modalities. **A**) Carignan vol^+deg^+ **B**) Carignan vol^+deg^- **C**) Grenache vol^+deg^+ **D**) Grenache vol^+deg^-. ISP: initial skin polyphenols; *SSP*: soluble skin polyphenols at the end of the maceration experiment*; PSP*: Precipitated skin polyphenols at the end of the maceration experiment; *RESP*: residual extractable skin polyphenols in wine-like solvents (extraction in 60 % acetone solvent); *NESP*: non-extracted skin polyphenols in wine-like solvents; SUM = *SSP* + *PSP* + *RESP* + *NESP*.

Figure 1

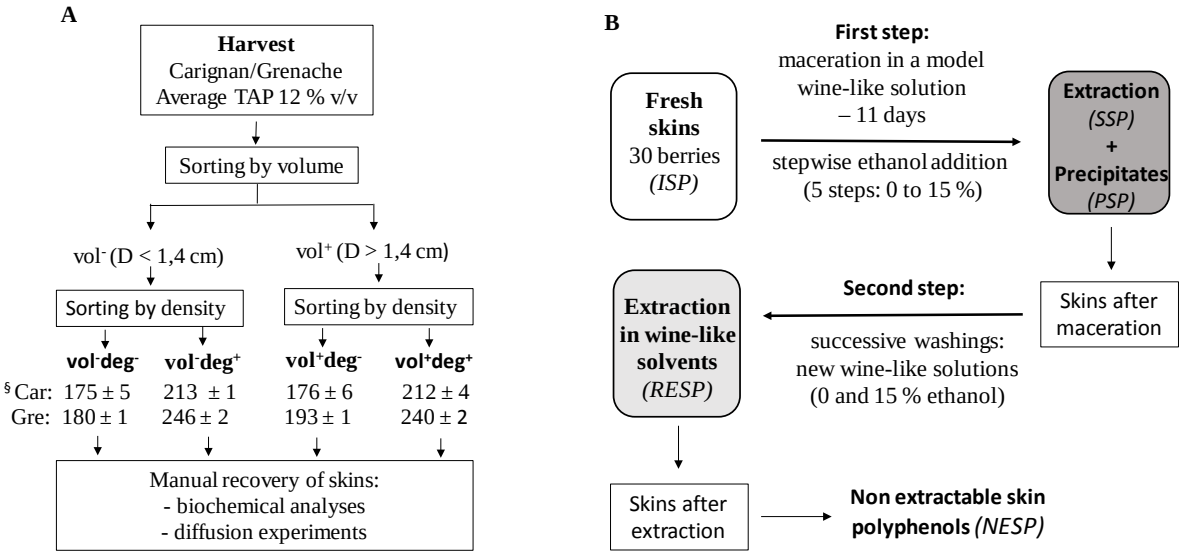


Figure 2

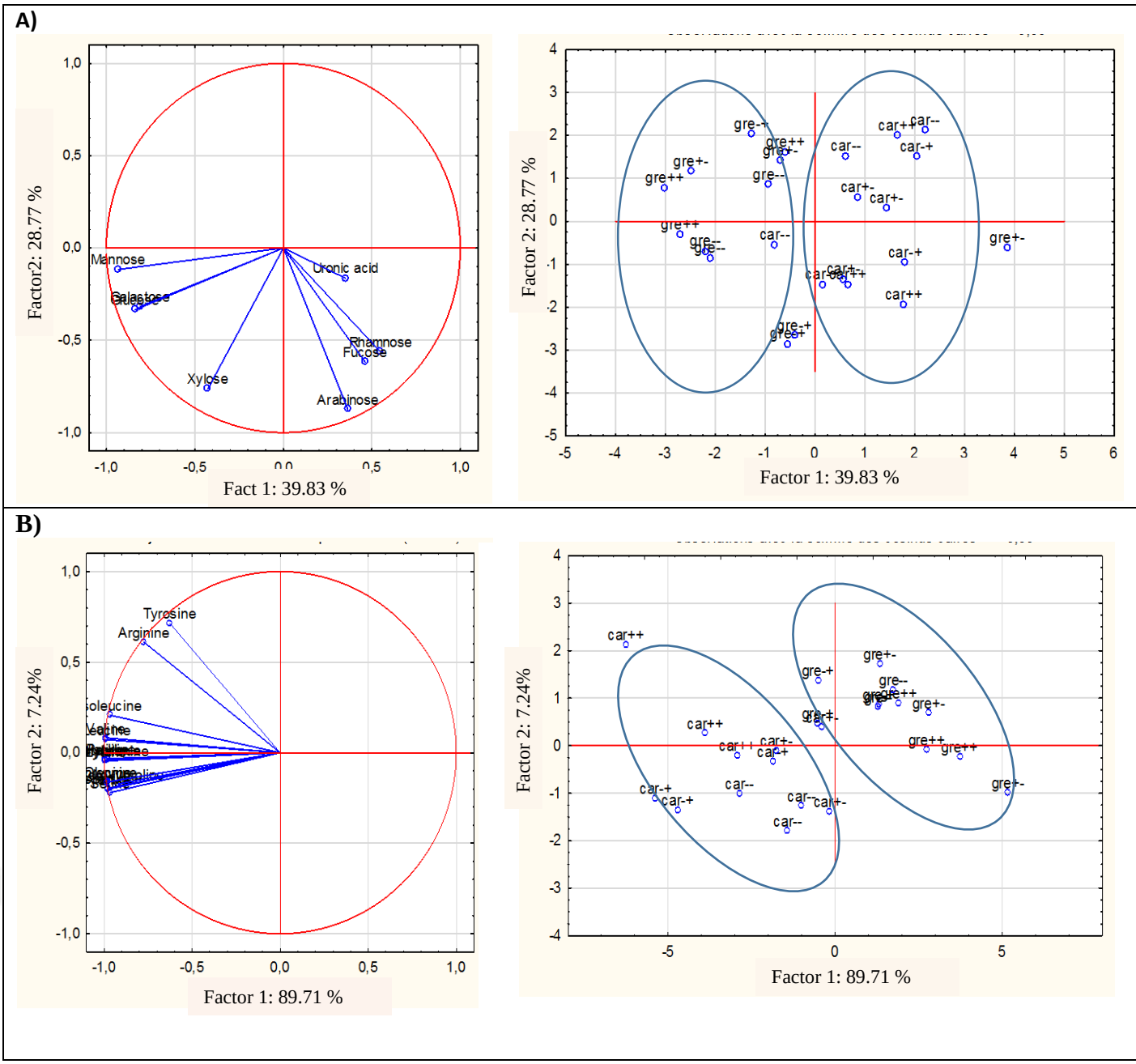


Figure 3

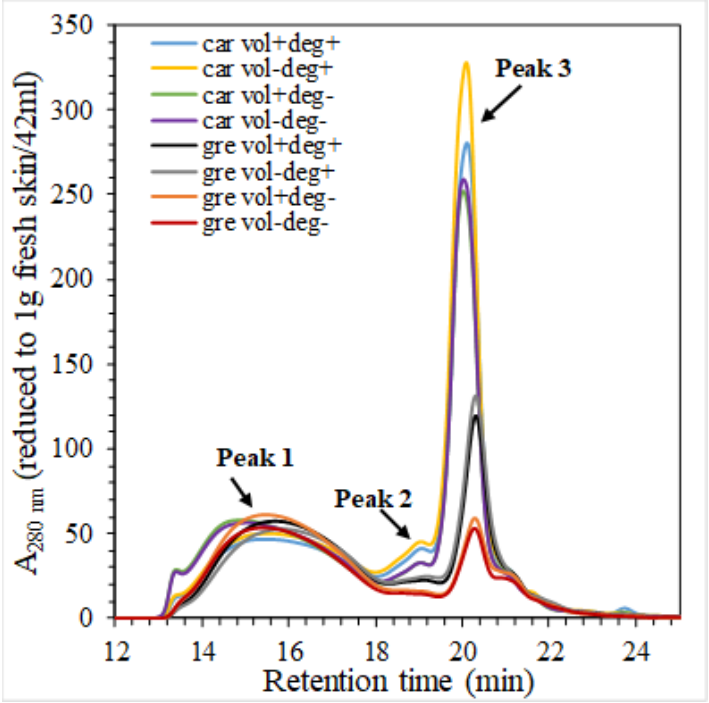


Figure 4

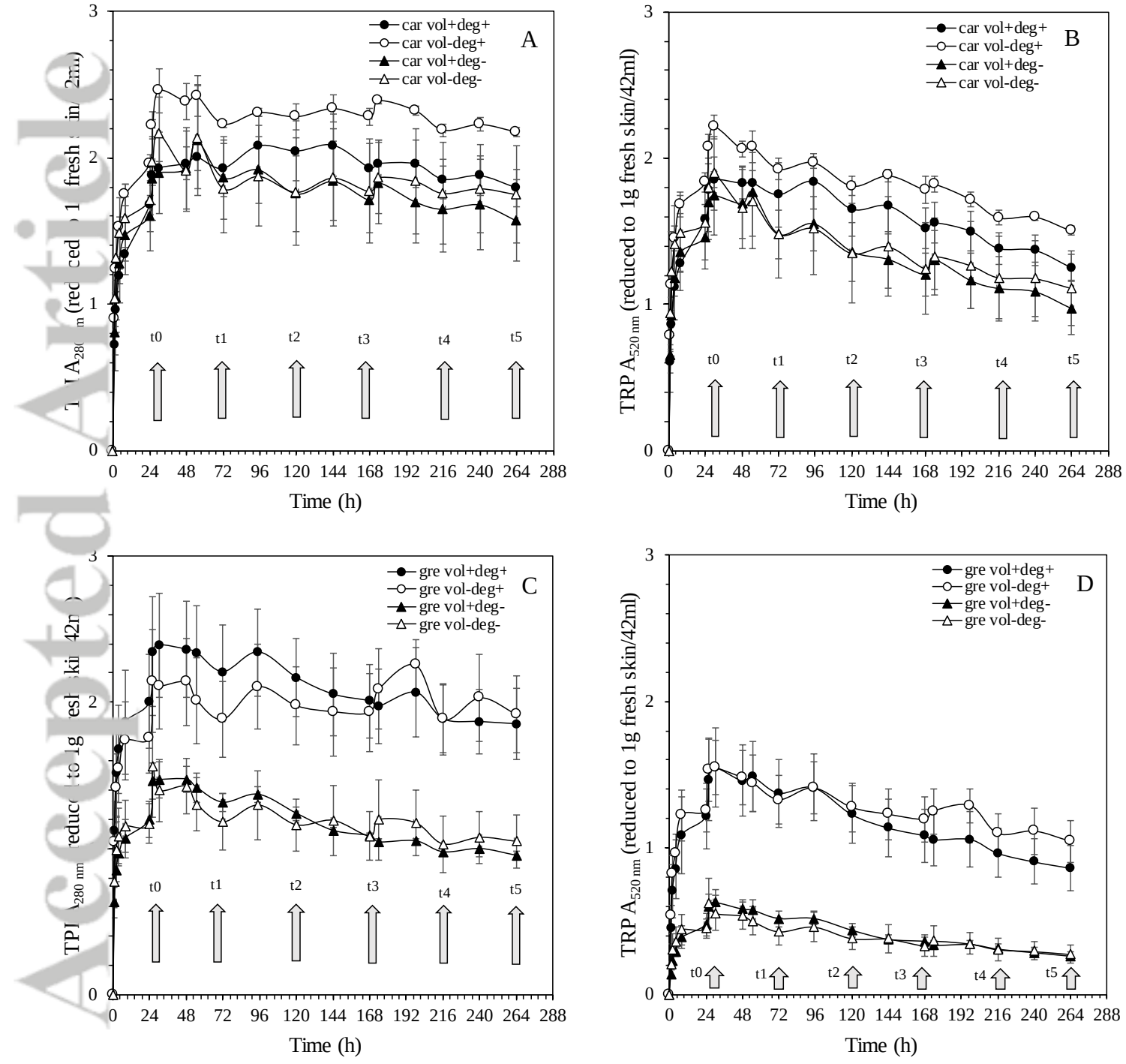
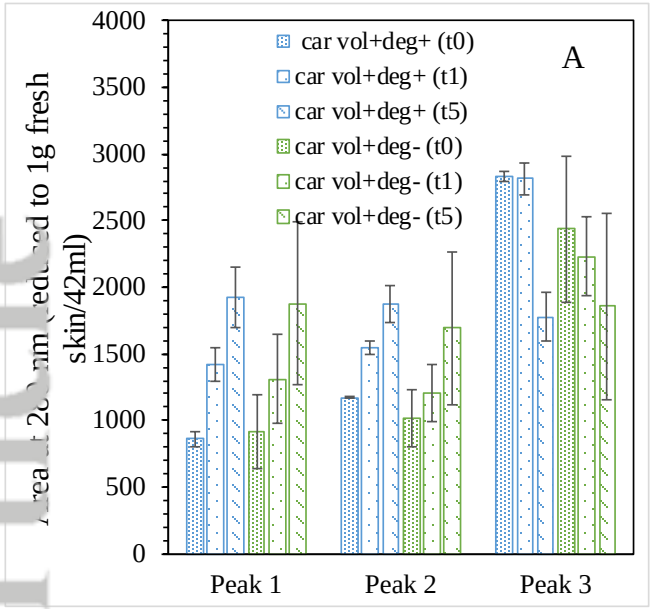
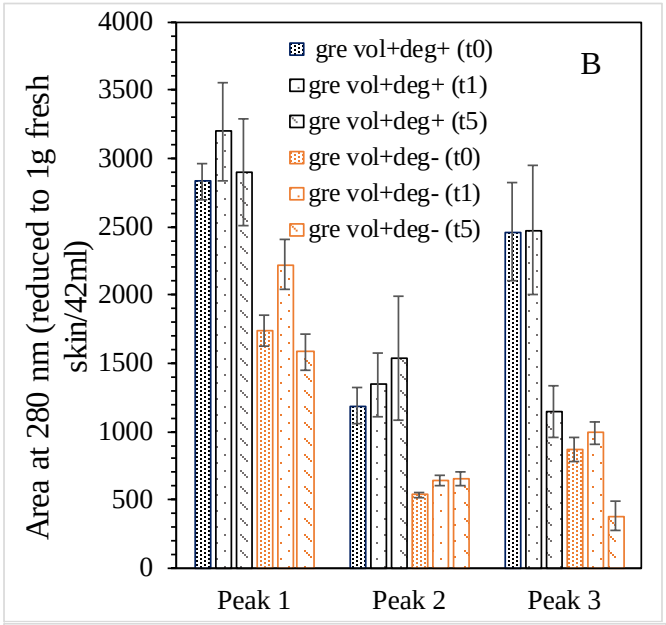
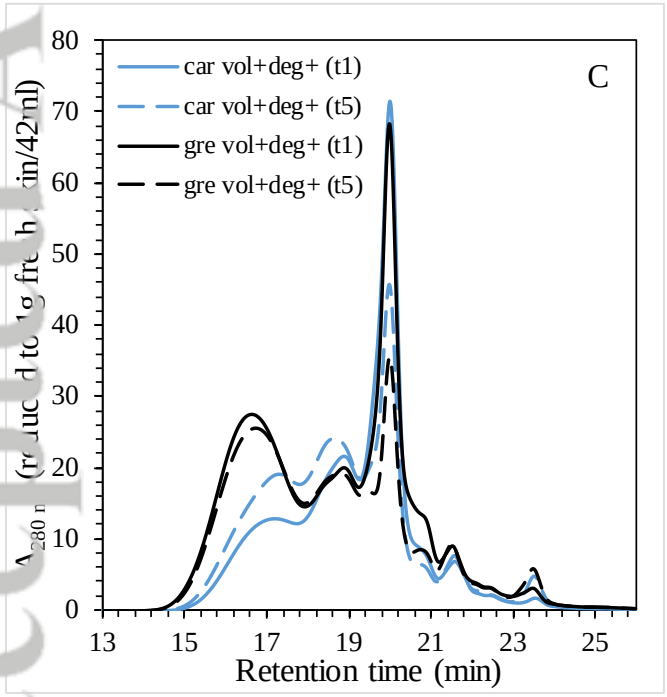


Figure 5







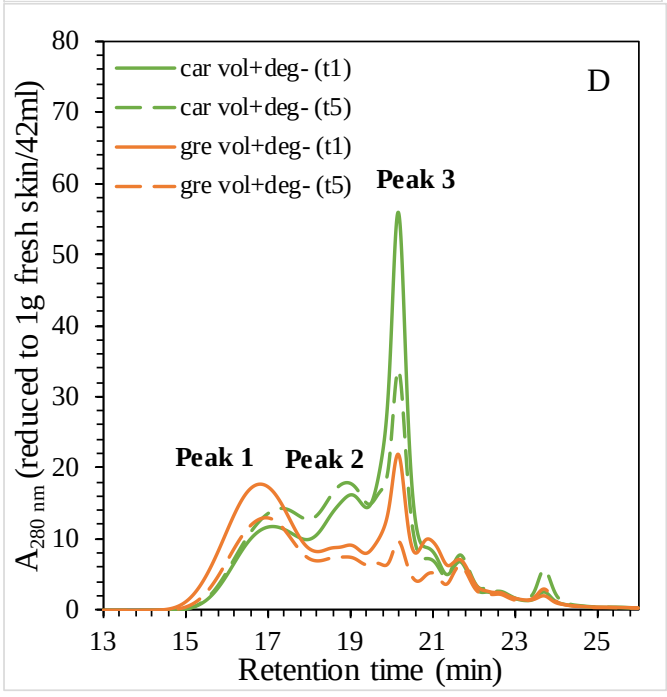


Figure 6