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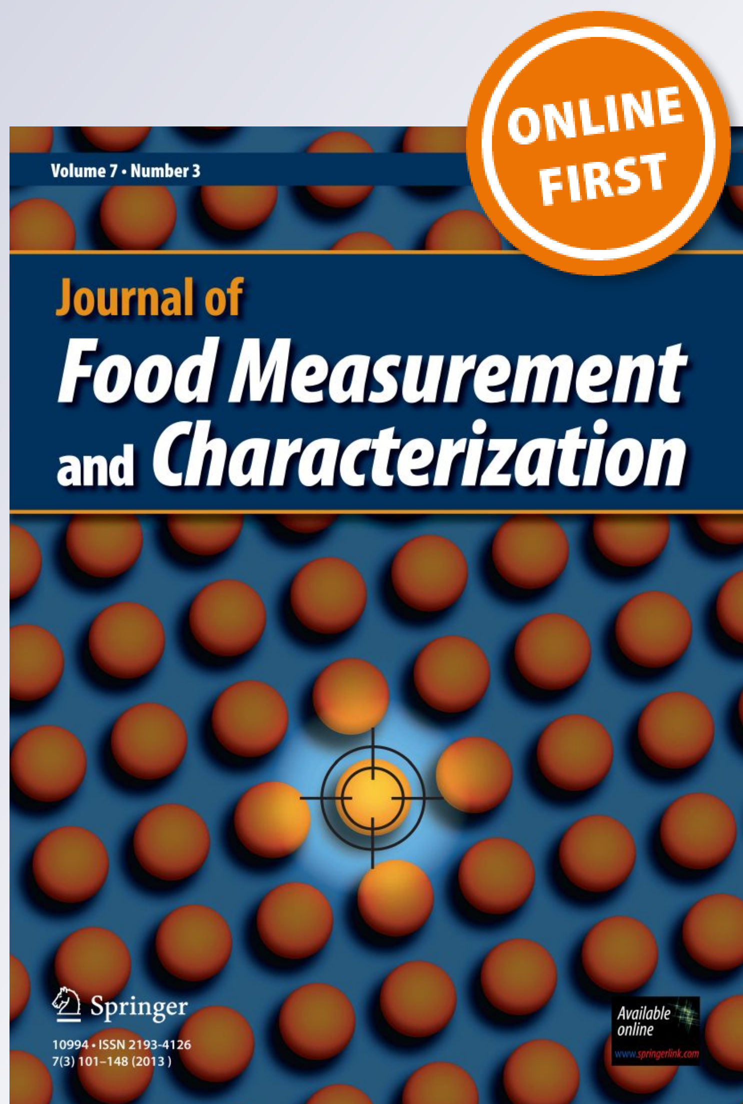
Journal of Food Measurement and Characterization

ISSN 2193-4126

Food Measure
DOI 10.1007/s11694-019-00126-3



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Journal of Food Measurement and Characterization
https://doi.org/10.1007/s11694-019-00126-3

ORIGINAL PAPER

Proanthocyanidins and anthocyanins contents, chromatic and antioxidant properties of red grape pomaces from morocco

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Received: 5 January 2019 / Accepted: 8 April 2019
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Abstract

Proanthocyanidins (PAs) and anthocyanins contents of four different red grape pomaces (GPs) from Morocco were analysed using chromatographic methods. The GP skin pigments characteristics were determined using spectroscopic methods while their antioxidant activities were determined using both spectroscopic methods as ABTS and DPPH radical cations, ferric reducing/antioxidant power (FRAP) and electrochemical technique: cyclic Voltammetry (CV). The PA of seeds extracts varied significantly among the types of GP and ranged from 56.1 ± 0.3 mg g^{-1} dry weight (DW) in *Cot* to 88.4 ± 2.3 mg g^{-1} DW in non-fermented *Cabernet Sauvignon* (CSNF). The skins of CSNF contained the highest levels of PAs (22.1 ± 0.3 mg g^{-1} DW). The PAs average degree of polymerization distribution ranged from 2 to 45 subunits for the skins and from 2 to 12 subunits for the seeds. The maximum anthocyanins amounts and antioxidant activities were measured in *Cot* skin extracts (32.8 mg g^{-1} DW, DPPH: 0.23 mmol Trolox mg g^{-1} DW; ABTS: 0.38 mmol Trolox mg g^{-1} DW and CV: 1.73×10^{-3}mA V) followed by *Arinarnoa* skin extracts which had the maximum corrected pigments (14.8 ± 0.1) and color power (152.4 ± 4.6 Unit color). The principal components analysis (PCA) of GP skins showed three different groups relevant to anthocyanins, PAs content, color and antioxidant properties.

Keywords Grape pomace · Proanthocyanidins · Thioglycolysis · Anthocyanins · Antioxidant assays · Cyclic voltammetry

Abbreviations

GP	Grape pomace
PAs	Proanthocyanidins
CS	Fermented *Cabernet Sauvignon*
Ari	Fermented *Arinarnoa*
Cot	Fermented *Cot*
CSNF	Non-fermented *Cabernet Sauvignon*
aDP	Average degree of polymerization
% gall	Percentage of galloyalation
% prod	Percentage of prodelphenidin
% oxi	Percentage of oxidation

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Introduction

According to FAO (Food Agriculture Organisation) statistics, Morocco is classified as the 28th country regarding the total grapes annual production [1]. Winemaking industry is one of the most important economic activities in Morocco generating large amounts of solid residues named grape pomace (GP). The GP consists of the remaining skins, seeds and stems after pressing the grape. It represents about on fifth of the quantity of grapes which are transformed to wine [2]. The method of valorization of this waste adopted at the scale of Morocco is its destination to elaborate compost. This solution is not good enough to benefit from the wealth of waste. The GP contains high level of natural bio-based compounds such as polyphenols, tartaric acid, cellulose, hemicellulose, lignin, …, which makes it very attractive bio-sourced material for bioenergy, food, pharmaceutical and cosmetic applications [3, 4]. In relation, the interest of polyphenols (stilbenes, phenolic acids, proanthocyanidins and anthocyanins) is currently increasing due to their natural antioxidant power and their health benefits (anticarcinogenic and anti-inflammatory activities among others) [5]. GP is a very rich source of anthocyanins mostly found

Published online: 13 April 2019

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in the vacuoles and cell walls of grape skins and it gives color to grapes [6, 7]. They are glucosides formed from the combination of an aglycone called anthocyanidin and a sugar substituted in the 3-position. These sugars may be acylated by aliphatic acids (malonic acid, succinic, etc.) or aromatic (coumaric acid, ferulic acid, etc.) [8]. The color of anthocyanins is affected by the physicochemical conditions in which it is viewed and the structure of anthocyanins. Furthermore, GP is also a very rich source of proanthocyanidins which contribute to the sensorial qualities of food such as color, bitterness and astringency [9]. They are biopolymers consisting of flavan–3–ol subunits of very similar structure which are catechin, epicatechin, epigallocatechin and epicatechin gallate [10]. The proanthocyanidins biopolymers are found in different parts of GP and their characteristics vary from one part to another [11, 12]. The skin proanthocyanidins are characterised by a high level of epigallocatechin (prodelphinidin), an average degree of polymerisation and a lower amount of epicatechin gallate (galloylated derivative) compared to seed proanthocyanidins [11]. They are used in food industry as solubiliser, colorant and antioxidant stabilizers [13]. Concerning analytical tools, liquid chromatography is the most used technique used to separate and identify the anthocyanin and proanthocyanidin compounds of wine, grape and plant extracts. In order to determine the antioxidant properties, the DPPH (1, 1'-diphenyl-2-picrylhydrazyl radical), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)), oxygen radical absorbance capacity (ORAC), ferric reducing antioxidant potential (FRAP), DMPD (*N,N,N′,N′*-tetramethyl-*p*-phenylenediamine) and TRAP (total peroxyl radical-trapping potential) assays are chemical assays classically used [14]. Nevertheless, electrochemical techniques like cyclic voltammetry (CV) can also be applied. It has emerged as an alternative method for economic, a quick and precise measurement [15]. The antioxidant potential of the wine and white GPs was evaluated by CV as previously described by [16]. But, to the best of our knowledge, red GPs is evaluated for the first time in this study. Moreover, in Morocco, phenolic composition of GPs generated by wine cellars has never been studied. Therefore, the purpose of this work is to characterize the proanthocyanidin and anthocyanin extracts and evaluate the coloring and antioxidant activities of different red grape pomaces skins [*Arinarnoa* (*Ari*), *Cot* (*Cot*), and *Cabernet Sauvignon* (*CS*) before and after fermentation].

Materials and methods

Grapes pomaces

In this study, the GP samples provided from Château Roslane (Meknès, Morroco). The three fermented GPs of *CS*, *Ari* and *Cot* were generated from red winemaking process. In red winemaking process, grapes were passed through a mechanical destemmer-crusher to remove the stems and slowly press the berries. The juice and solid parts (stems, seeds and skins) were transferred to a fermentation tank (300 hL), then commercial yeasts (*Saccharomyces Cerevisiae*) were added at 10 g hL^{-1} in order to convert the grape sugar into alcohol. During the alcoholic fermentation, the temperature value was comprised between 24 and 30 °C and the fermentation duration was comprised between 5 to 7 days to be finished (≈ 13% ABV). The wine was then separated from the solid parts (called grape pomaces) by passing it through a press. After the pressing step, grape pomaces were isolated and three samples of grape pomaces were collected. CSNF: *Cabernet Sauvignon* non-fermented was generated from rosé winemaking process. The grapes were passed through a destemmer-crusher, and the must (juice) was immediately passed through a press that applied pressure to separate the juice from the skins, seed and stems (juice was separated from the skins and stems before fermentation). After the pressing step, grape pomaces were isolated and samples (n = 3) of grape pomaces were collected. The seeds and skins of the different GPs were separated and lyophilized.

Preparation of polyphenols extracts from grape pomace

Grape pomace seeds and skins were reduced to powder using liquid nitrogen, pestle and mortar. 5 g of powders were weighed into erlenmeyer flasks (seed powders were extracted three times with chloroform (250 mL) to remove lipophilic materials). Then they were extracted with 50 mL of ethanol/water (30/70, v/v) containing 1 g L^{-1} of SO_2 for 30 min under stirring after saturation with argon in order to prevent polyphenols oxidation. The extracts were centrifuged (15 min, 3000 rpm) and the residue was re-extracted twice with the organic solvent. The supernatants were merged, concentrated, frozen at – 80 °C and lyophilized.

Fractionation, chemical depolymerization (thioglycolysis) and UPLC analysis of proanthocyanidins

Around 250 mg mL^{-1} of extracts were injected on a Flash Chromatography system (PuriFlash 430, Interchim) and fractionated on a Toyo pearl TSK gel HW-50 (F) column (3.8,13 cm) (methyl acrylate copolymer in 2% of aqueous ethanol solution) [17, 18]. The solvents used for elution were the followings: solvent A (CH_3CH_2OH/TFA, 99.95:0.05, v/v); solvent B (H_2O/TFA, 99.95:0.05, v/v) and solvent C (CH_3COCH_3/TFA, 99.95:0.05, v/v). The fractions studied in this work were separated according to the UV profile on line with the flash chromatography system. The

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recovered fractions were lyophilized. A 100 µL of fraction solution (4 g L^{-1}, prepared in methanol) was combined to 100 µL of solution of thioglycolic acid (4 mL of thioglycolic acid added to 496 mL of methanol/concentrated HCl, 95:5, v/v) in a sealed glass. The thioglycolysis reaction of proanthocyanidins was on 90 °C heat in 6 min [19]. The reversed-phase UPLC–ESI/MS analyses were conducted as described above [17, 19]. The undepolymerized samples (2 µL) were directly injected into the UPLC system yielding the flavan-3-ol monomers composition. The depolymerized samples give the proanthocyanidin contents. The liquid chromatography system was an Acquity UPLC (Waters, Milford, MA) equipped with a Diode Array Detector. The flow rate was 0.55 mL min^{-1} and the gradient conditions were solvent A (H_2O/CHOOH, 99/1, v/v); solvent B (CH_3CN/H_2O/CHOOH, 80/19/1, v/v/v); initial 0.1% B; 0–2 min, 25% B linear; 2–4 min, 35% B linear; 4–5 min, 35% B isocratic; 5–6 min, 40% B linear; 6–8 min, 99.9% B linear and 8–10 min, 99.9% B isocratic. UPLC analysis of depolymerized proanthocyanidins allow the determination of its structural properties such as the galloylation percentage (% gall), the prodelphinidins percentage (% prod) and the average degree of polymerization (aDP) [17, 20]. The percentage of tannins oxidation (% oxi) is calculated according to the method described by [17]. An example of UPLC chromatogram of depolymerization products from *Cabernet Sauvignon* seeds tannins is presented in Fig. 1a.

HPLC analysis of anthocyanins

The anthocyanins were analyzed by direct injection of around 5 mg mL^{-1} of extracts into the reversed-phase HPLC–DAD system. The chromatography liquid analyses were conducted using a Waters 2690 system equipped with

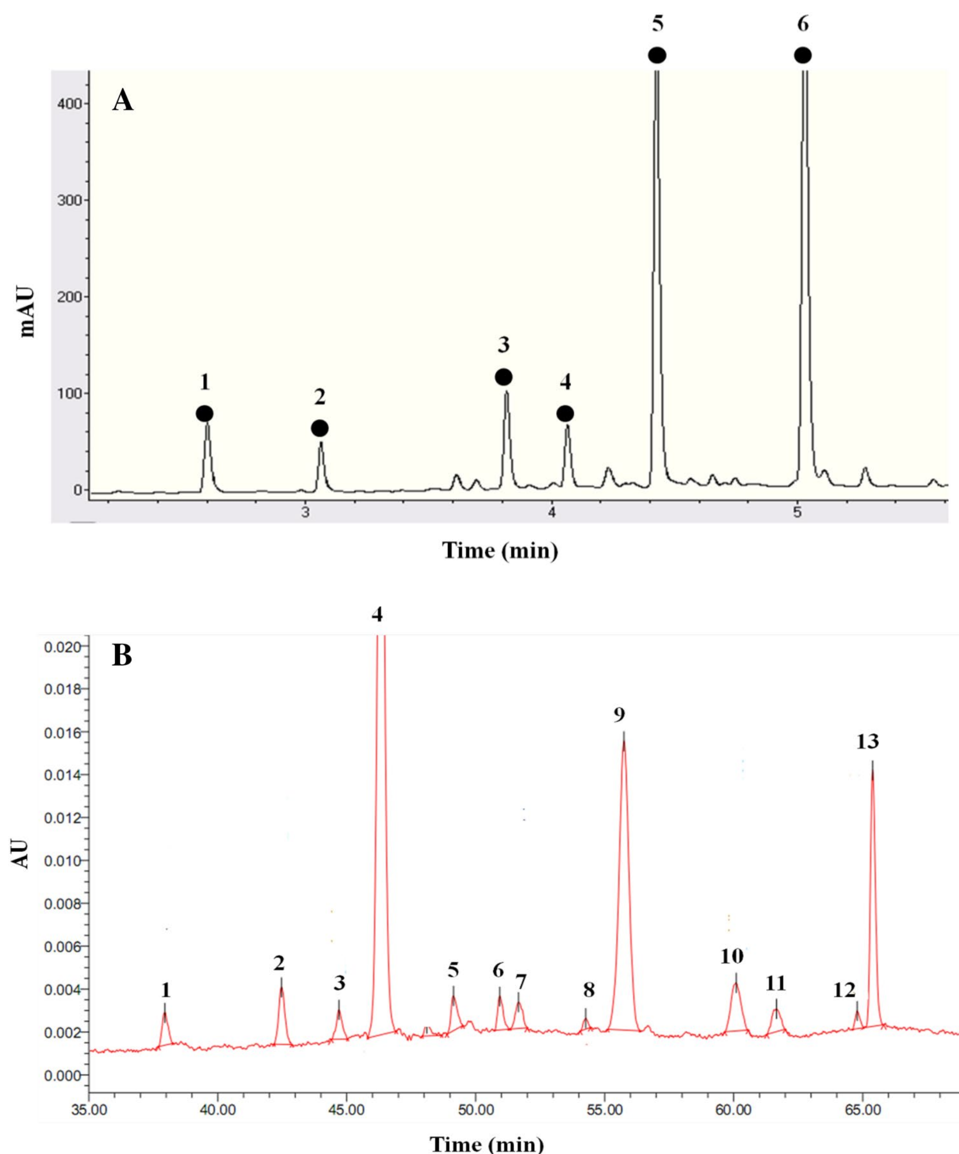

Fig. 1 **a** UPLC chromatogram of depolymerization products from *Cabernet Sauvignon* pomace seed tannins: peaks numbered and marked with a black dot; 1, catechin; 2, epicatechin; 3, epicatechin 3-*O*-gallate; 4, catechin-thiol; 5, epicatechin-thiol; 6, epicatechin 3-*O*-gallate-thiol. **b** HPLC chromatogram of anthocyanins compounds of *Cabernet Sauvignon* skin recorded at 520 nm. 1, delphinidin-3-*O*-glucoside; 2, petunidin-3-*O*-glucoside; 3, peonidin-3-*O*-glucoside; 4, malvidin-3-*O*-glucoside; 5, delphinidin-3-*O*-acetylglucoside; 6, cyanidin-3-*O*-acetylglucoside; 7, petunidins-3-*O*-acetylglucoside; 8, peonidin-3-*O*-acetylglucoside; 9, malvidin-3-*O*-acetylglucoside; 10, cyanidin-3-*O*-p-coumarylglucoside; 11, petunidin-3-*O*-p-coumarylglucoside; 12, peonidin-3-*O*-p-coumarylglucoside; 13, malvidin-3-*O*-p-coumarylglucoside

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a reversed-phase Atlantis dC18 column (250 × 2.1 mm i.d., 5 µm packing) for separation, a Waters 996 photodiode array detector, and Millennium 32 chromatography manager software (Waters, Milford, MA). The elution conditions were as follows: oven temperature 30 °C; 0.250 mL min^{-1} flow rate; solvent A: water/formic acid (95/5 v/v); solvent B: acetonitrile/water/formic acid (80/15/5 v/v/v); elution began isocratically with 0% B during 2 min, then continued with linear gradients from 0 to 8% B in 20 min, from 8 to 20% B in 15 min, from 20 to 25% B in 15 min, from 25 to 65% B in 15 min, from 60 to 0% B in 5 min, followed by washing and re-equilibration of the column. A calibration curve was established with the anthocyanin malvidin 3-*O*-glucoside to quantify the anthocyanins amount at 520 nm as equivalent malvidin 3-*O*-glucoside. The anthocyanin profile of *Cabernet Sauvignon* GP skin extracts which were analysed by the HPLC system and chemical structure of grape pomaces anthocyanins are shown in Figs. 1b and 2 rspectively.

Chromatic measurements

Absorbance measurements were effected with a SAFASUV mc2 spectrophotometer (Monaco) and colour indices were deduced from these absorbance measurements as previously described [21–23]. All the absorbances were measured at 520 nm and converted to a 10-mm light path cell. Corrected pigments (CP) were measured 30 min after addition of acetaldehyde. The sulfite bleaching resistant pigments ($PRSO_2$) were determined after 30 min of reacting of a sulfite solution with extracts. Total pigments at acidic pH ($pH < 1$) were determined 4 h after a 100-fold dilution in HCl 1 M. The coloring power ($E^{1\%}$) was determined according to the method of [24]. A mass (g) of extract was introduced into a 100 mL vial of pH 3 buffer and the absorbance was measured. Then, the coloring power was expressed in UC kg^{-1} (Units Color per kilo).

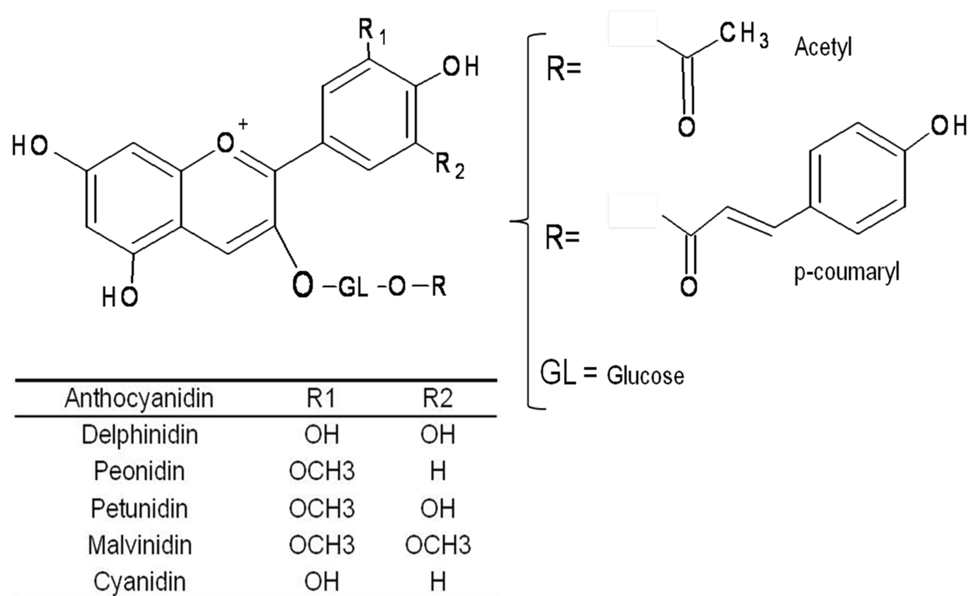
Total phenolic measurements

The total phenolics content of skin extracts was determined directly after dilution with water at 280 nm [TPI (Total Polyphenols Index) 280 nm] and by the Folin–Ciocalteu assay [25]. For this later, the results were expressed as milligram of gallic acid equivalents per gram of dry weight (mg GAE g^{-1} DW).

Antioxidant assays

Cyclic voltammetry

CV experiments were carried outusing an Autolab Potentiostat/Galvanostat (PGSTAT302 N) coupled with GPES software (Metrohm-Autolab, Switzerland). The voltammetry system was connected to a standard three-electrode electrochemical cell with a glassy carbon electrode (GCE) of 3 mm diameter (Metrohm, Switzerland) as working electrode, asilver/silver chloride (Ag/AgCl, KCl 3 M) as reference electrode and a platinum wire as auxiliary electrode. Prior to each measurement, alumina powder (0.3 µm) was used to polish the GCE surface, then thoroughly rinsed with highly purified water and passed to the ultrasonic bathfor 5 min. Following this mechanical treatment, the electrochemical study was carried out at 22 °C with skin extract dissolved in acidic hydroalcoholic solution (12% vol. ethanol, 33 mM L-tartaric acid and pH 3.6 adjusted with 1 M NaOH). The potential range was from 100 mV up to 1000 mV and the



Anthocyanidin	R1	R2
Delphinidin	OH	OH
Peonidin	OCH3	H
Petunidin	OCH3	OH
Malvinidin	OCH3	OCH3
Cyanidin	OH	H

Fig. 2 Chemical structure of grape pomaces anthocyanins

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scan rate was 100 mVs^{-1}. The solutions containing the skin extracts were diluted before measurement 100fold in the hydroalcoholic solution in order to get a peak intensity proportional to the polyphenols concentration and also to overcome any influence of the presence of sulfites on the cyclic voltammograms like for red wines [26].

Ferric reducing antioxidant potential assay

FRAP assay was developed by [27]. The working FRAP reagent was prepared by mixing 10 volumes of 300 mmol L^{-1} acetate buffer, pH 3.6, with 1 volume of 10 mmol L^{-1} TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mmol L^{-1} hydrochloric acid and with 1 volume of 20 mmol L^{-1} ferric chloride. 150 µL of deionized water and 50 µL of skin extracts (dilution of the skin extract in model wine) were added to 1.5 mL of the FRAP reagent, incubated at 37 °C for 30 min in a water bath and the absorbance was measured at 593 nm using spectrophotometer. The change in absorbance between the sample absorbance and the blank sample were selected for calculation of FRAP values. Standard curve was prepared using different concentrations of $FeSO_4 \cdot 7H_2O$ (50–1000 µmol L^{-1}).

ABTS assay

The antioxidant power of the skin extracts was also characterized using the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) assay [28]. ABTS radical cation (ABTS + .) was produced by reaction of two solutions: 7 mM ABTS and 2.45 mM potassium persulfate, followed by 12–16 h of incubation in the dark at room temperature. The ABTS + solution was diluted, in H_2O/ethanol to an absorbance of 0.700 ± 0.020 at 734 nm. 0.1 mL of sample (dilution of the skin extract in model wine) was added to 1 mL of ABTS + solution and absorbance was measured at 734 nm after 10 min of reaction. The results were expressed as µmol Trolox equivalents g^{-1} dry weight.

DPPH assay

The ability of the skin extracts to scavenge the DPPH was evaluated as described previously by [29]. In test tubes, 0.1 mL of sample (dilution of the skin extract in model wine) was added to 3.9 mL of DPPH solution (6×10^{-5} mol L^{-1} in methanol), and the mixture was well mixed. The absorbance at 515 nm was measured after 30 min. A blank was prepared for each sample using methanol instead of the DPPH solution. A linear regression for the Trolox standards was constructed. Results were expressed as µM Trolox equivalent g^{-1} dry weight.

Statistical analysis

The data are presented as mean ± standard deviation of triplicate samples. Analysis of variance (ANOVA), ACP and the least significant difference (LSD) test according to Student–Newman–Keuls were done using the XLSTAT 2013 statistics software.

Results and discussion

Quantification of grape pomace proanthocyanidins (seeds and skins)

The flavan-3-ols content of the GP seed and skin, determined by the UPLC–ESI/MS after thioglycolysis reaction, is shown in Fig. 3. The phenolic compounds differed according to the part of the grape which was subjected to chemical extraction, the proanthocyanidins in the skin amount being lower than in the seed [11, 30, 31]. Significant differences ($p < 0.05$) of proanthocyanidins seed and skin contents among the four GPs are illustrated on Fig. 3. The proanthocyanidins of seed extracts varied significantly among the type of GP and ranged from 56.1 ± 0.3 mg g^{-1} DW in *Cot* to 88.4 ± 2.3 mg g^{-1} DW in CSNF (Fig. 3a). The results were lower than those obtained by [4, 12, 32]. CSNF skins contained higher levels of proanthocyanidins (22.1 ± 0.3 mg g^{-1} DW) than the other three GP skins (*Cot*, *Ari* and *CS*) studied (Fig. 3b). GP skins of *Cot*, *Ari* and *CS* had similar amounts of proanthocyanidins (around 13.8 mg g^{-1} DW) which were also lower than those obtained by [12]. The comparison of proanthocyanidins amounts of the four GPs (Fig. 3) showed that various factors, such as grapes varieties and extraction treatments, influence the proanthocyanidins amounts in the GPs [33]. The *Cabernet Sauvignon* GP, obtained from white winemaking, is unfermented pomace and consequently is richer in phenolic compounds than fermented pomaces [34].

Structural characterisation of seeds and skins proanthocyanidin fractions

Proanthocyanidins extracted from seeds and skins GPs were also fractionated on a Toyo pearl TSK gel HW-50 (F) column, eluted with a gradient of ethanol/water/acetone. The distribution of proanthocyanidins amount related to the average degree of polymerisation is investigated in Fig. 3. The proanthocyanidins degree of polymerization distribution ranged from 2 to 45 subunits for the skins and from 2 to 12 subunits for the seeds. For all grape pomaces, the seed proanthocyanidins at aDP of 8–10 represent around 72–89% of the total proanthocyanidins (Fig. 3c). This amount was higher than the one reported in *Syrah* and in *Cabernet Sauvignon* at aDP of 7 and 6 respectively, and

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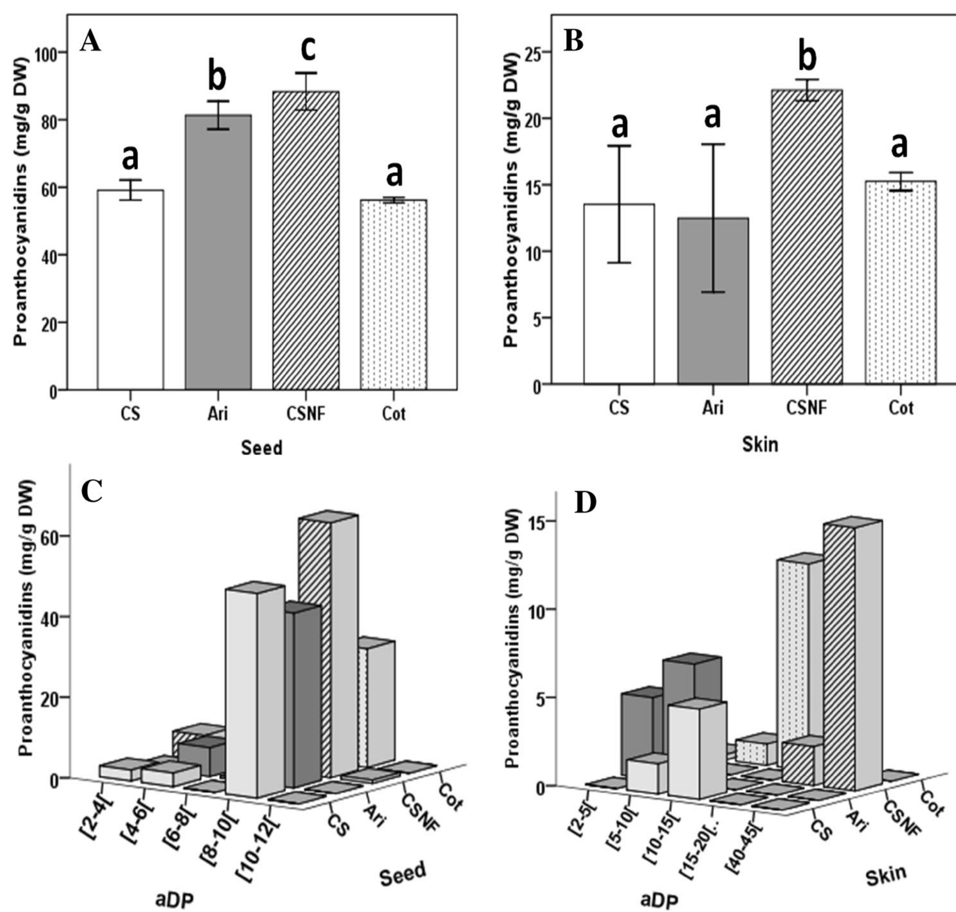

Fig. 3 Proanthocyanidins contents [seeds (**a**) and skins (**b**)] and average degree of polymerization (aDP) distribution [seeds (**c**) and skins (**d**)] (fermented *Cot* (*Cot*), *Arinarnoa* (*Ari*), *Cabernet Sauvignon* (*CS*) and non-fermented *Cabernet Sauvignon* (CSNF)). Diffrent letters above bars indicate statistically significant differences between grape pomaces (Student-Newman-Keulstest, P < 0.05)

which represented 30% of the total proanthocyanidins [35, 36] have reported that the aDP of the GPs seeds was higher than these of grapes. Indeed, the grapes lose low molecular weight proanthocyanidins into wines during maceration/fermentation and as a result, the remaining seed pomace has a higher aDP. As already noted, the non-fermented *Cabernet Sauvignon* had the largest quantities of proanthocyanidins and showed distribution of tannin fraction as follows: 12% of 15–20 aDP and 87% of 40–45 aDP (Fig. 3). [11] have reported that the aDP of *Cabernet Sauvignon* varies from 81 to 83 subunits. Moreover, the aDP, % gall, % prod and % oxi of grape pomace seed and skin proanthocyanidins are summarised in Table 1. The composition and the structural characteristics of proanthocyanidins are dependent on the grape localisation (seed or skin) and on the grape variety [11]. Seed proanthocyanidins had a lower average degree of polymerisation, % oxi and a higher percentage of galloylated subunits (% gall) than those from skins. Grape skin proanthocyanidins are composed of prodelphinidins which are absent in all seed proanthocyanidins extracts (Table 1) [11]. For structural parameters of seeds proanthocyanidins, no difference was noticed in aDP (Table 1) and in the % gall and % oxi among the different types of GP. The proanthocyanidins of seeds showed an aDP, % gall and % oxi ranging from 5.2 to 7.4, 38.4 to 51.1% and 15 to 21% respectively. The proanthocyanidins of skins showed an aDP ranging from 10.0 ± 1.3 to 13.2 ± 1.2 (Fig. 3d).The % gall, % prod and % oxi in the skin proanthocyanidins ranged from 4.8 to 9.4%, 12 to 38.4% and 62.3 to 68% respectively (Table 1). A tendency for proanthocyanidins with higher aDP also presenting a higher % prod was put in evidence. These results have been noted in previous works [11, 30, 37].

Grape pomace anthocyanins

Anthocyanins are the focus of numerous studies because of their exhaustiveness, their instability and their use as a natural food coloring [8]. Table 1 represents the anthocyanins concentrations in the four red GP skin extracts determined by HPLC. The total anthocyanins contents ranged from 5.90 ± 0.02 to 32.8 ± 0.3 mg g^{-1} DW in *CS* and *Cot* respectively. CSNF samples possessed the highest levels of 3-*O*-glucoside and 3-*O*-acetylglucoside anthocyanins (17.80 ± 0.14 and 11.1 ± 0.1 mg g^{-1} DW respectively) while *Cot* possessed more glycosylated and coumaroylated anthocyanins (9.8 ± 0.0 mg g^{-1} DW and 20.10 ± 0.08 mg g^{-1} DW respectively). Previous works showed that anthocyanins compounds are extracted during the maceration–alcoholic fermentation

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Table 1 Structural characterization of GP proanthocyanidins: Percentage of galloylation (% gall), percentage of prodelphinidins (% prod), percentage of oxidation (% oxi) and anthocyanins content (mg g^{-1} dry weight) are reported for seeds and skins extracts of GPs. Values followed by the same letter are not significantly different (LSD, 5%) (n = 3)

	CS	*Ari*	CSNF	*Cot*	Sig
Seed					
aDP	6.6 ± 0.2^{a}	6.0 ± 0.0^{a}	7.4 ± 0.4^{a}	5.6 ± 1.4^{a}	n.s
% gall	17.1 ± 2.2^{a}	21.8 ± 3.1^{a}	17.4 ± 2.1^{a}	18.1 ± 8.7^{a}	n.s
%pro	n.d	n.d	n.d	n.d	–
% oxi	51.1 ± 2.5^{a}	49.7 ± 3.1^{a}	38.4 ± 0.8^{a}	49 ± 0.5^{a}	n.s
Skin					
aDP	10.0 ± 1.3^{a}	11.1 ± 0.8^{a}	13.2 ± 1.2^{a}	11.9 ± 0.4^{a}	n.s
% gall	8.9 ± 1.8^{a}	$8,4 \pm 3.9^{a}$	4.8 ± 1.9^{a}	9.4 ± 1.1^{a}	n.s
%pro	25.2 ± 4.5^{ab}	19.7 ± 0.5^{ab}	38.4 ± 0.6^{b}	18.8 ± 0.4^{a}	*
% oxi	66.6 ± 0.8^{b}	$62,3 \pm 0.4^{a}$	66.2 ± 0.1^{b}	68 ± 0.14^{b}	**
De-3-gl	0.1 ± 0.0^{a}	1.1 ± 0.0^{d}	0.3 ± 0.0^{b}	0.5 ± 0.0^{c}	***
Cy-3-gl	n.d	n.d	n.d	n.d	–
Pet-3-gl	0.2 ± 0.0^{a}	1.6 ± 0.0^{d}	0.8 ± 0.0^{b}	1.0 ± 0.0^{c}	***
Peo-3-gl	0.1 ± 0.0^{a}	0.2 ± 0.02^{a}	0.5 ± 0.0^{a}	0.3 ± 0.0^{a}	***
Mv-3-gl	2.8 ± 0.0^{a}	7.4 ± 0.4^{b}	16.2 ± 0.2^{d}	8.0 ± 0.0^{c}	***
De-3-ac	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.2 ± 0.0^{a}	0.1 ± 0.0^{a}	n.s
Cya-3-ac	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	$0,2 \pm 0.1^{a}$	0.03 ± 0.0^{a}	n.s
Pet-3-ac	0.1 ± 0.0^{a}	0.9 ± 0.0^{b}	$0,1 \pm 0.0^{a}$	0.2 ± 0.1^{a}	***
Peo-3-ac	n.d	0.2 ± 0.0^{a}	0.3 ± 0.0^{a}	0.4 ± 0.4^{a}	n.s
Mv-3-ac	1.5 ± 0.0^{a}	4.4 ± 0.0^{c}	10.3 ± 0.0^{d}	2.4 ± 0.0^{b}	***
De-3-coum	n.d	n.d	n.d	n.d	–
Cy-3-coum	0.2 ± 0.0^{a}	0.8 ± 0.0^{a}	0.5 ± 0.0^{a}	0.7 ± 0.0^{a}	n.s
Pet-3-coum	0.1 ± 0.0^{a}	1.7 ± 0.0^{b}	0.1 ± 0.0^{a}	2.6 ± 0.0^{c}	***
Peo-3-coum	0.05 ± 0.0^{a}	0.4 ± 0.0^{b}	0.1 ± 0.0^{a}	0.6 ± 0.0^{c}	**
Mv-3-coum	0.7 ± 0.0^{a}	8.1 ± 0.05^{c}	1.0 ± 0.0^{b}	16.2 ± 0.0^{d}	***
Anth-gl	3.1 ± 0.0^{a}	10.3 ± 0.1^{c}	17.8 ± 0.1^{d}	9.8 ± 0.0^{b}	***
Anth-ac	1.8 ± 0.0^{a}	5.6 ± 0.04^{c}	11.1 ± 0.1^{d}	3.0 ± 0.2^{b}	***
Anth-coum	1.0 ± 0.0^{a}	11.0 ± 0.5^{b}	1.7 ± 0.6^{a}	20.1 ± 0.0^{c}	***
Total	5.9 ± 0.0^{a}	26.8 ± 0.2^{b}	30.6 ± 0.6^{c}	32.8 ± 0.3^{d}	***

ns no significant ($p > 0.05$); significant levels: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; *nd* means not detected; *De-3-gl* delphinidin-3-*O*-glucoside, *Cy-3-gl* cyanidin-3-*O*-glucoside, *Pet-3-gl* petunidin-3-*O*-glucoside, *Peo-3-gl* peonidin-3-*O*-glucoside, *Mv-3-gl* malvidin-3-*O*-glucoside, *De-ac* delphinidin-3-*O*-acetylglucoside, *Cy-ac* cyanidin-3-*O*-acetylglucoside, *Pet-ac* petunidins-3-*O*-acetylglucoside, *Peo-ac* peonidin-3-*O*-acetylglucoside, *Mv-ac* malvidin-3-*O*-acetylgluco-side, *De-coum* delphinidin-3-*O*-p-coumarylglucoside, *Cy-coum* cyanidin-3-*O*-p-coumarylglucoside, *Pet-coum* petunidin-3-*O*-p-coumarylglucoside, *Peo-coum* peonidin-3-*O*-p-coumarylglucoside, *Mv-coum* malvidin-3-*O*-p-coumarylglucoside, *Anth-gl* Anthocyanins-3-*O*-glucoside, *Anth-ac* Anthocyanins-3-*O*-acetylglucoside, *Anth-coum* Anthocyanins-3-*O*-p-coumarylglucoside *CS Fermented Cabernet Sauvignon, Ari, Fermented Arinarnoa,* CSNF *Non-fermented Cabernet Sauvignon, Cot Fermented Cot*

stage [12]. Nevertheless, a large amount of anthocyanins still remained in grape pomace skins after the winemaking process, *Cot* presenting weaker quantities (Table 1). The profiles of anthocyanins were not similar for the different skin extracts. Malvidin-3-*O*-monoglucoside was the most abundant anthocyanin for skin extracts of fermented and non-fermented *Cabernet Sauvignon* representing 52–47% of the total anthocyanins respectively (Table 1). However, malvidine-3-*O*-coumaroylglucoside was the most present anthocyanin for *Ari* and *Cot* with 30 and 40% respectively. These results are different from those obtained by [12] but similar to those obtained for Malbec of Argentina [38]. This anthocyanins profile variation could be related probably to grape variety, vintage and grapes ripening or to the contents modification in the solid/liquid ratio of the anthocyanins and their solubility in the wine. This factors can change the anthocyanins diffusion in wine. As a consequence, the GPs are obtained with varied concentrations of phenolic compounds [38, 39].

Chromatic characteristics of grape pomace skin extracts

As already mentioned, the interest to use the anthocyanins as natural food coloring is constantly increasing. Grape skins

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represent about 50% of the GPs mass, contain the highest amount of anthocyanins compared to other grape parts and offer shades of colours ranging from bright red to purple [40, 41]. Table 2 represents colour indices of the GP skin extracts which were deduced from the absorbance measurements. Noticeable differences ($p < 0.05$) were observed in the absorbance values of extracts. *Ari* samples possessed the highest level of corrected pigments (CP) which a contribution of 17% of sulfite bleaching resistant pigments ($PRSO_2$), while fermented and non-fermented *Cabernet Sauvignon* samples possessed lowest CP which contributed by 24% and 13% of $PRSO_2$ respectively (Table 2). The literature data have reported that during maceration-fermentation step, there are conversions of GP anthocyanins onto derivative pigments (polymerized colored anthocyanin-tannin complexes) which are resistant to bleaching by sulfite [42]. These latter are confirmed by a higher level of sulfite bleaching resistant pigments ($PRSO_2$) in the GP skins of *Ari*, *Cot* and fermented *Cabernet Sauvignon* than in the non-fermented *Cabernet Sauvignon* GP. The coloring power is particularly intense in the *Ari* and CSNF skin extracts. This indicates that color intensity is not only correlated with the concentration of anthocyanins (Table 3), but depends also on the molecular structure of anthocyanins formed during the maceration step and on the copigmentation phenomenon. Several authors have reported that the increase in colour intensity of wines and model wine solutions is probably due to a greater amount of pigments derived or a higher incidence of co-pigmentation due to better extraction of proanthocyanidins [42–44].

Antioxidant activities of grape pomace skin extracts measured by CV

The GP skin extracts antioxidant properties were assessed using an electrochemical technique, the CV. Figure 4 shows cyclic voltammogram of GP skin extracts and cyclic voltammogram for grape pomace *Cot* F skin extract after background (acidic hydro-alcoholic solution) subtraction. The cyclic voltammograms present three characteristics oxidation peaks (Fig. 4a). The first observed peak with a low potential of around 400 mV corresponds to the most easily oxidizable molecules since the potential of the peak is the weakest. This peak concerns the oxidation of the phenolic compounds that possess a catechol or a gallyol group on the B-ring. Among these molecules, catechin-like flavonoids [flavanols, but also oligomeric and polymeric (tannins) structures] are mainly concerned in addition to flavonols, caffeic acid and derivatives, gallic acid and anthocyanins [4, 22]. The second peak at around 620 mV is attributed to the oxidation of the malvidin anthocyanins which are present to a large extent. Other polyphenols can also contribute to a smaller extent like *trans*-resveratrol (or ferulic acid). At last,

Table 2 Total phenolics (TPI and FC assay), chromatic parameters (corrected pigments, sulfite bleaching resistant pigments, total pigments at acidic pH and the coloring power) and antioxidant capacity (cyclic voltammetry, DPPH, ABTS and FRAP assays) of grape pomace skins extracts. Values followed by the same letter are not significantly different (LSD, 5%) (n = 3)

Sample	TPI (AU)	FC (mg g^{-1} DW)	CP	$PRSO_2$	pH < 1	$E^{1\%}$	DPPH (mmol g^{-1}DW)	ABTS (mmol g^{-1}DW)	FRAP (µmol g^{-1}DW)	CV (mA.V)
CS	6.3 ± 0.2^a	70.5 ± 1.4^a	7.8 ± 0.8^a	1.9 ± 0.0^b	12.2 ± 0.1^a	128.3 ± 4.0^a	0.12 ± 0.0^a	0.31 ± 0.0^a	6231 ± 767^a	$1.60 \times 10^{-3} \pm 0.0^a$
Ari	9.2 ± 0.5^b	115.6 ± 1.7^c	14.8 ± 0.1^b	2.6 ± 0.0^d	17.2 ± 6.7^a	152.4 ± 4.6^b	0.21 ± 0.0^b	0.41 ± 0.0^b	6904 ± 508^a	1.68×10^{-3} 0.0^a
CSNF	8.6 ± 0.6^b	105.3 ± 5.5^b	8.2 ± 0.2^a	1.1 ± 0.0^a	16.2 ± 4.6^a	152 ± 4.1^b	0.20 ± 0.1^b	0.40 ± 0.0^b	7328 ± 271^a	$1.66 \times 10^{-3} 0.0^a$
Cot	12.5 ± 0.2^c	129.5 ± 1.7^d	13.6 ± 0.4^b	2.1 ± 0.0^c	19.2 ± 4.6^a	132.4 ± 8.8^a	0.23 ± 0.0^c	0.38 ± 0.0^b	6323 ± 841^a	$1.73 \times 10^{-3} 0.0^a$

TPI total polyphenols index at 280 nm, *FC* folin ciocalteu, *GA* gallic acid, *Cat* catechin, *CP* corrected pigments, $PRSO_2$ sulphite bleaching resistant pigments, *pH* <total pigments at acidic pH, $E^{1\%}$ coloring power, *CV* cyclic voltammetry, *CS* Fermented *Cabernet Sauvignon*, *Ari* Fermented *Arinarnoa*, CSNF non-fermented *Cabernet Sauvignon*, *Cot* fermented *Cot*, *mA.V* milliAmpere. Volt

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Table 3 Correlation coefficient (R^2) between: (1), color parameters (corrected pigments, coloring power) and total Anthocyanins; (2), polyphenols (TPI and FC assay) and antioxidant capacity (cyclic voltammetry, DPPH, ABTS and FRAP assays) of grape pomace skins extracts

	TPI	FC	Total Ant	DPPH	ABTS	FRAP	CV
DPPH	0.81	0.98	0.94	1	0.66	0.12	0.91
ABTS	0.24	0.58	0.79	0.66	1	0.60	0.38
FRAP	0.00	0.05	0.26	0.12	0.60	1	0.00
CV	0.97	0.95	0.78	0.91	0.38	0.00	1
CP	0.50	0.61	0.29	0.51	0.33	0.00	0.58
$E^{1\%}$	0.00	0.13	0.30	0.22	0.71	0.87	0.03

TPI total polyphenols index at 280 nm, *FC* folin ciocalteu, *Total Ant* total antocyanins, *CP* corrected pigments, $E^{1\%}$ coloring power, *DPPH* 1, 1'-diphenyl-2-picryl-hydrazyl radical, *ABTS* 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), *FRAP* ferric reducing antioxidant potential, *CV* cyclic voltammetry

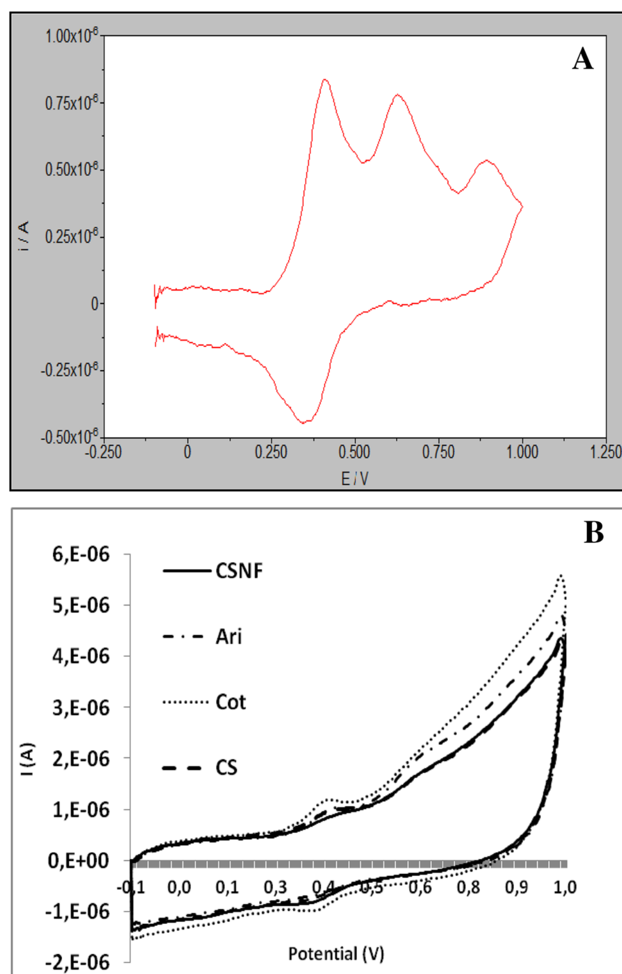

Fig. 4 **a** Cyclic voltammogram of fermented *Cot* skin extract after background subtraction. **b** Cyclic voltammograms of GP skin extracts (fermented *Cot* (*Cot*), *Arinarnoa* (*Ari*), *Cabernet Sauvignon* (*CS*) and non-fermented *Cabernet Sauvignon* (CSNF)

the third observed peak with the highest potential (around 890 mV) corresponds mostly to a second oxidation of the flavonoids which are present in consequent quantities [22]. Some phenolic acids with higher oxidation potentials can also contribute to a smaller extent to the intensity of the peak (mainly *para*-coumaric acid).

Antioxidant capacity of grape pomace skin extracts measured by spectrophotometric methods

The phenolic amounts and the antioxidant power of the different grape pomaces skin extracts are illustrated in Table 2. The DPPH and ABTS tests showed high significantly differences in antioxidant power of skin extracts. The highest antioxidant activities were found in *Cot* skin extract (DPPH: 0.23 mmol Trolox g^{-1} DW; ABTS: 0.38 mmol Trolox g^{-1} DW) followed by *Ari* skin extract (DPPH: 0.21 mmol Trolox g^{-1} DW; ABTS: 0.41 mmol Trolox g^{-1} DW). These results are in agreement with the phenolic contents obtained by the TPI (12.5 AU), FC: (129 mg AGE g^{-1} DW) and anthocyanins (32.8 g g^{-1} DW) (Tables 1 and 2). These differences related to the amount of polyphenols and the antioxidant capacity between the GP skin extracts depend not only on the variety but also on the extraction methods of pomace cap constituents during vatting (more or less extensive polyphenols extraction of pomace cap by pumping over, unballasting, intensity and duration of maceration,…). The four antioxidant tests did not give the same skin extracts classification. The FRAP test classified CSNF skin extract as the first extract showing the highest antioxidant capacity. CS skin extract presented a weak antioxidant activity in all antioxidant tests as a result of its low phenolic contents (Table 2). Table 3 shows the correlation of the polyphenols amounts with the antioxidant assays using regression analyses. The strongest correlations between phenolic contents (TPI, FC assay and total anthocyanins) and antioxidant assays were obtained for CV ($R^2=0.97$, $R^2=0.95$ and $R^2=0.78$ respectively), and DPPH ($R^2=0.81$, $R^2=0.98$ and $R^2=0.94$ respectively). Weaker correlations were obtained with FRAP ($R^2=0.03$, $R^2=0.05$ and $R^2=0.26$ respectively). These results do not agree with those of [36] which reported that strongest correlations were established with

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FRAP followed by ABTS and DPPH assays. The strongest correlations between pigments (total anthocyanins and coloring power) and antioxidant assays indicate that antioxidant capacity of anthocyanins extracts relies on their chemical structure and concentration (Table 3). According to [43], scavenging activities are increased by acylation of anthocyanins with hydroxycinnamic acid derivatives and o-diphenols. For the correlation between antioxidant assays (Table 3), on one hand a strong correlation ($R^2=0.91$) was obtained between the CV and the DPPH assays and on the other hand the better correlation between chemical tests was established between the ABTS and DPPH assays ($R^2=0.66$). The differences in correlation levels may be due to the variation in the reactivity of each assay [36]. Finally, PCA was used to determine the relationship between the phenolic compounds and chromatic and antioxidant characteristics of grape pomace skin extracts (Fig. 5). The first axis (F1) of PCA accounted for the largest part of variance (48.41%). As shown in Fig. 5, the grape pomaces are separated along this axis (F1) in two groups (*CS*) and (*Ari*, *Cot* and CSNF) according to their amounts of polyphenols, monomeric anthocyanins and their chromatic and antioxidant attributes (*Ari*, *Cot* and CSNF are richer in polyphenols, monomeric anthocyanins and have high levels of pigments and antioxidant activity). The second component (F2, 29.97%, Fig. 5) distinguished two groups: *Ari*, *Cot* and *CS* and CSNF according to the quantitative and qualitative proanthocyanidins characteristics. Indeed, CSNF is associated with large amounts of polymerised proanthocyanidins, prodelphinidins and lesser amounts of resistant pigments to sulfite. The other group (*Ari*, *Cot* and *CS*) is associated with higher content of pigments and monomeric anthocyanins and a lesser level of prodelphinidins.

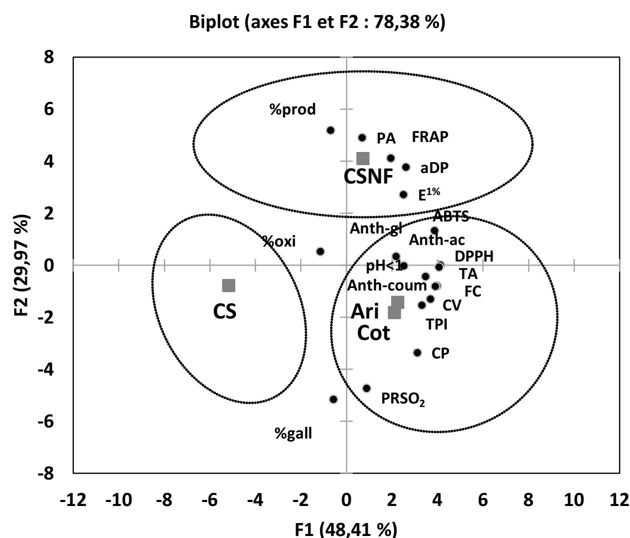

Fig. 5 Principal Component Analysis (PCA) of GPskins (PC1-48.41%, PC2-29.97%). Relationship between phenolic compounds and, chromatic and antioxidant characteristics GP skins (score and loading biplot). *TPI* total polyphenols index at 280 nm; *FC* folin ciocalteu; *PA* proanthocyanidins; *TA* total anthocyanins; *Anth-gl* anthocyanins-3-*O*-glucoside; *Anth-ac* Anthocyanins-3-*O*-acetylglucoside; *Anth-coum* Anthocyanins-3-*O*-p-coumarylglucoside; *aDP* Average degree of polymerization, *% gall* percentage of galloylation, *% prodel* percentage of prodelphinidin, *% oxi* percentage of oxidation, *CP* Corrected pigments; $PRSO_2$ Sulphite bleaching Resistant Pigments; $pH<1$ Total pigments at acidic pH; $E^{1\%}$ coloring power; *CV* cyclic voltammetry; DPPH, ABTS and FRAP assays; Fermented *Cot* (*Cot*), *Arinarnoa* (*Ari*), *Cabernet Sauvignon* (*CS*) and non-fermented *Cabernet Sauvignon* (CSNF))

Acknowledgements Thanks are addressed to Frederic Veran for supervising analysis and helpful discussion and to The Agence Universitaire de la Francophonie (AUF) and Vlir-IUC program for their assistance.

Conclusions

The phenolic compounds of four GP samples (white and red winemaking) from Moroccan vineyard were studied. Grape pomace seed and skin extracts maintained noticeable quantities of proanthocyanidins and anthocyanins. Their quantities and structural profile were highly variable, depending on grape variety, type of extract (seed or skin) and grape pomace origin (red or white winemaking). The seed and skin extracts from no fermented GP (*Cabernet Sauvignon*) and *Cot* were the richest in proanthocyanidins and anthocyanins respectively. *Ari*, CSNF and *Cot* skin extract express high levels of coloring and antioxidant capacity. The evaluation of the polyphenolic potential of GPs showed heterogeneous results due to several factors. In fact, their valorization necessitates a selection of polyphenol-rich GPs in order to extract pigments and proanthocyanidins for food or pharmaceutical applications.

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