POTENTIAL OF LANDRACE WINERY BY-PRODUCTS (*VITIS VINIFERA* L.) AS A SOURCE OF PHENOLIC COMPOUNDS WITH ANTIOXIDANT PROPERTIES

Maria Carme GARAU[1], María Reyes GONZÁLEZ-CENTENO[2*], Joana Maria LUNA[1], Antoni NEGRE[1], Carmen ROSSELLÓ[2] and Antoni FEMENIA[2]

1 : Institute of Agricultural and Fishing Research and Training (IRFAP) of the Government of the Balearic Islands, C/ Eusebi Estada, 145, 07009, Palma, Spain
2 : Department of Chemistry, University of the Balearic Islands, Ctra. Valldemossa, km 7.5, 07122, Palma, Spain

Abstract

Aim: To evaluate the potential of the main winery by-products – pressed pomaces, fermented pomaces and stems –, derived from minor grape varieties (Escursac, Gorgollassa and Sabater as red varieties, Giró ros and Quigat as white varieties) native to the Balearic Islands (Spain), as raw material for the production of phenolic concentrates with antioxidant properties.

Methods and results: Total phenolics, tannins and anthocyanins, as well as antioxidant capacity of winery by-products were spectrophotometrically evaluated and compared to those of Cabernet-Sauvignon and Chardonnay varieties. In general, stems presented higher average total phenolic (5.57 ± 1.25 g/100 g dm) and total tannin (10.26 ± 2.10 g/100 g dm) contents than the corresponding pomaces, with the landrace variety Escursac being that which exhibited the highest values ($p < 0.05$). **Conclusion**: The present research demonstrated that landrace minor varieties denoted similar, or even higher, phenolic and antioxidant potential than the reference grape varieties. The characterization performed might be the basis for their integrated use and revalorization as promising sources of phenolic concentrates, despite not having still undergone the selection process that the traditional grape varieties have been subjected to as a result of decades of intensive production.

Significance and impact of the study: To the best of our knowledge, this is the first time that the phenolic composition and antioxidant properties of winery by-products from grape varieties native to the Balearic Islands are examined. Due to the increasing use of these minor grape varieties in winemaking, the phenolic characterization of their by-products is of great interest for the wine sector, which could exploit these underutilized resources more efficiently and extensively so as to support sustainable agricultural production.

Key words: landrace minor grape varieties, winery by-products, total phenolic content, antioxidant capacity, total anthocyanin content, total tannin content

Résumé

Objectif: Évaluer le potentiel des principaux sous-produits de vinification – marcs pressés, marcs fermentés et rafles –, provenant de cépages minoritaires (Escursac, Gorgollassa et Sabater pour les cépages rouges, Giró ros et Quigat pour les cépages blancs) originaires des Îles Baléares (Espagne), comme matière première pour la production de concentrés phénoliques aux propriétés antioxydantes.

Méthodes et résultats: Les teneurs en phénols totaux, tanins totaux et anthocyanes totales, ainsi que l'activité antioxydante des sous-produits de vinification ont été évaluées par spectrophotométrie et comparées à celles des cépages Cabernet-Sauvignon et Chardonnay. En général, les rafles ont montré des teneurs en phénols totaux (5.57 ± 1.25 g/100 g dm) et tanins totaux (10.26 ± 2.10 g/100 g dm) plus élevées que les marcs du même cépage. Le cépage autochtone Escursac a présenté les valeurs les plus élevées pour ces paramètres ($p < 0.05$).

Conclusion: Cette étude a montré que les cépages autochtones présentent un potentiel phénolique et antioxydant similaire, et même plus important, à celui des cépages de référence. La caractérisation des sous-produits réalisée pourrait être à la base de leur exploitation intégrée et de leur valorisation en tant que sources prometteuses de concentrés phénoliques, malgré le fait de ne pas avoir subi le processus de sélection appliqué aux cépages traditionnels dans le cadre d'une production intensive depuis plusieurs décennies.

Signification et impact de l'étude: À notre connaissance, il s'agit de la première évaluation de la composition phénolique et des propriétés antioxydantes de sous-produits de vinification provenant de cépages originaires des Îles Baléares. En raison de l'utilisation croissante de ces cépages minoritaires dans la production de vin, la caractérisation phénolique de leurs sous-produits est d'un grand intérêt dans le secteur du vin, ceci afin d'exploiter ces ressources sous-utilisées de manière plus efficace et plus intensive, pour soutenir une production agricole durable.

Mots clés: cépages autochtones, sous-produits de vinification, phénols totaux, capacité antioxydante, anthocyanes totales, tanins totaux

*Corresponding author : reyes.gonzalez.centeno@gmail.com

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INTRODUCTION

The intensification of the *Vitis vinifera* crop over recent decades has led to a continuous vineyard renewal, which has caused the disappearance of many indigenous minor grape varieties. In most cases, this choice has not been properly assessed with regard to their oenological potential and the critical loss of biodiversity (Gómez Gallego *et al.*, 2012b). Nevertheless, consumers today are looking for particular wines with enhanced varietal aroma. Thus, the wine sector around the world is promoting landrace minor grape vines, searching for a touch of authenticity and originality linked to their geographical origin and enhanced agroclimatic adaptation (Bertuccioli, 2010 ; García-Muñoz, 2011). In fact, different landrace minor varieties have been recently authorized for winemaking in various Spanish Appellations of Origin (AO) (ie., *Giró ros* and Gorgollassa for the AO Binissalem-Mallorca [BOIB n. 23, 2013] ; Maturana and Turruntés for the AO Rioja [BOE n. 64, 2008 ; BOE n. 130, 2009]).

However, few studies have focused on the oenological potential of these minor varieties (Escalona *et al.*, 2009 ; Gómez Gallego *et al.*, 2012a) and even fewer have investigated the potential of their corresponding by-products as inexpensive and easily available sources of bioactive compounds for the pharmaceutical, cosmetic and food industries.

Winery by-products are known to be rich in phenolic compounds with i) antioxidant capacity to preserve food (Ping *et al.*, 2011) and ii) protective effects against certain diseases such as cancer, atherosclerosis and cardiovascular pathologies, among others (Olas *et al.*, 2012 ; Gollucke *et al.*, 2013). There is a great diversity of grape varieties whose phenolic content, antioxidant capacity and health-promoting properties can significantly differ from one another. The question remains as to whether the better agroclimatic adaptation of landrace grape varieties results in a greater potential for winemaking by-products as a source of bioactive constituents.

Although there are numerous studies regarding the phenolic composition of seeds and/or skins (Negro *et al.*, 2003 ; Lafka *et al.*, 2007 ; Cosme *et al.*, 2009 ; Deng *et al.*, 2011 ; Lorrain *et al.*, 2011), research evaluating the phenolic and antioxidant potential of all by-products derived from winemaking is still scarce, especially in the case of stems (Anastasiadi *et al.*, 2012 ; González-Centeno *et al.*, 2012). Furthermore, with regard to grape pomace by-products, it is difficult to compare reported results since most studies do not clearly specify if they proceeded from rosé (pressed pomaces) or red winemaking (fermented pomaces).

In this context, the potential of the three main winery by-products (pressed pomaces, fermented pomaces and stems) from both red and white landrace grape varieties has been evaluated, as a basis for their future integrated exploitation. The main aims were (i) to evaluate their potential to be used as raw material for the production of phenolic concentrates with antioxidant properties and (ii) to compare them with those of well-known varieties cultivated worldwide. For these purposes, the total phenolic, anthocyanin and tannin contents were investigated, together with the *in vitro* antioxidant capacity.

MATERIALS AND METHODS

1. Samples

Winery by-products from Majorcan landrace grape varieties (*Vitis vinifera* L.) from the 2010 vintage were considered. The selected varieties were : the red Escursac, Gorgollassa and Sabater, and the white Giró ros and Quigat. Cabernet-Sauvignon (red) and Chardonnay (white) were considered as reference varieties.

All grape varieties, both autochthonous and reference, shared the same vineyard location, cultivation system, climate, soil type, cultivation practices, harvesting time and oenological treatment, at the experimental station of the Institute of Agricultural and Fishing Research and Training of the Government of the Balearic Islands (Mallorca, Spain).

The winemaking process was carried out separately for each grape variety to obtain the corresponding winery by-products. Specifically, stems were directly collected at the beginning of the winemaking process after the grape destemming step, and pressed pomaces (PP) were separated just after the pressing process for both red and white grape varieties. In the case of the red grape varieties, fermented pomaces (FP) were also collected after two weeks of alcoholic fermentation at 25 ºC. Both types of pomace mainly consisted of peels, seeds and some pulp residues. All by-products were vacuum-packed and stored at -80 ºC until further processing and analysis.

2. Extraction of phenolic compounds

Samples were lyophilized and mechanically ground to a homogeneous powder. The phenolic extraction was carried out according to the experimental conditions previously described by González-Centeno *et al.* (2012) by using an ASE 350

Accelerated Solvent Extraction System equipped with a solvent controller (Dionex Corporation, Sunnyvale, CA). Briefly, the ground samples (~ 10 g) were submitted to eight solid/liquid extractions by acetone/water (80 : 20, v/v) and three more by using the solvent system MeOH/water (60 : 40, v/v). All extracts were combined and evaporated under reduced pressure. The obtained solid residue was redissolved in 30 mL of water prior to lyophilization and stored under dark conditions until analysis. The extraction yields were recalculated from the moisture content of the samples and the weights considered. All samples were extracted in duplicate.

3. Determination of total phenolic content

Total phenolic content (TPC) of the winery by-products was estimated by the Folin-Ciocalteu assay, using a Beckman Coulter DU series UV/Vis spectrophotometer (Spain). TPC was determined by mixing 0.5 mL of an aqueous extract solution (~ 10 mg/mL), 0.5 mL of the Folin-Ciocalteu reagent and 10 mL of sodium carbonate (20 % v/v) to a final volume of 25 mL in a calibrated flask. The solution was homogenized and allowed to react for 30 min at room temperature in the dark. The absorbance was then measured at 750 nm. TPC was calculated from a calibration curve using gallic acid as standard (50-500 mg/L), the data being expressed as mg of gallic acid equivalents (GAE), averaged from three measurements.

4. Determination of total anthocyanin content

Total anthocyanin content (ANT) was estimated spectrophotometrically according to the sodium bisulfite discoloration method described by Ribéreau-Gayon *et al.* (2006). Briefly, 1 mL of an aqueous extract solution (~ 10 mg/mL), 1 mL of EtOH 96 % and 20 mL of HCl 0.7 % were combined. A volume of 5 mL of this solution was mixed with 2 mL of a 7 % sodium bisulfite solution. After 10 min under dark conditions, absorbance was measured at 520 nm using Beckman Coulter DU series UV/Vis spectrophotometer (Spain). The value was corrected by subtraction of the blank sample, which was prepared following the same methodology but replacing the bisulfite solution by distilled water. Each determination was performed in triplicate.

5. Determination of total tannin content

For the estimation of the total tannin content (TC), 2 mL of an aqueous extract solution (~ 10 mg/mL) were mixed with 1 mL of distilled water and 6 mL of HCl 37 % in glass tubes, according to the experimental conditions reported by Ribéreau-Gayon *et al.* (2006). The tubes were hermetically sealed, shaken and heated at 100 ºC for 30 min. After ice cooling, the absorbance was measured at 550 nm. The value was corrected by subtraction of the blank sample, prepared in the same way but standing for 30 min under dark conditions at room temperature rather than being heated. Final values were the mean of three determinations.

6. Antioxidant capacity evaluation

There are multiple mechanisms through which phenolic compounds may act as antioxidants in grapes and/or wine. Thus, no single analytical assay is able to assess the antioxidant potential entirely. Therefore, different *in vitro* antioxidant capacity assays should be applied to yield a more accurate characterization of the antioxidant properties of the samples (Pellegrini *et al.*, 2003). In the present study, the spectrophotometric ABTS, DPPH, FRAP and CUPRAC assays were performed.

Extract solutions (~ 4 mg/mL) were prepared in EtOH/water (25 : 75, v/v). All the spectrophotometric determinations were carried out in a microplate spectrophotometer (Thermo Scientific Multiskan Spectrum, Vantaa, Finland). All data, reported as trolox equivalents (TE), were calculated from a calibration curve ranging from 25 to 800 µM trolox. Final values were the mean of six determinations.

ABTS assay. The ABTS assay was conducted by discoloration of the radical cation 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$), based on a slightly modified version of the experimental procedure described by González-Centeno *et al.* (2012). Specifically, dilution of the $ABTS^{\bullet+}$ solution was carried out with EtOH 80 %.

DPPH assay. Scavenging activity was determined by following the method of Brand-Williams *et al.* (1995), with some modifications to fit in 96-well microplates. Firstly, a 30 µM 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) solution was prepared in EtOH 80 %. Secondly, 190 µL of $DPPH^{\bullet}$ solution were included in each well and incubated at 25 ºC for 25 min. Then, 10 µL of extract solution were added and absorbance was recorded at 515 nm after 30 min of reaction. A control sample, prepared by replacing the extract solution by EtOH/water (25 : 75, v/v), was used to measure the maximum $DPPH^{\bullet}$ absorbance.

FRAP assay. The ferric reducing antioxidant power method is based on the absorbance increase at 593 nm due to the formation of 2,4,6-Tri-(2-pyridyl)-s-triazine complexes with iron (II) in the presence of a reductive agent (Özgen *et al.*, 2006). The protocol

and experimental conditions applied were as previously reported by González-Centeno *et al.* (2012).

CUPRAC assay. The cupric reducing antioxidant capacity was determined according to the method of Apak *et al.* (2004), with some modifications to fit in 96-well microplates. The CUPRAC reagent was prepared by combining a 10 mM Cu(II) aqueous solution, a 7.5 mM neocuproine solution and a 1 M (pH 7.0) ammonium acetate buffer (1:1:1, v/v). For the analysis, 150 µL of this solution were placed in each well and incubated for 25 min at 25 ºC. Then, 30 µL of water and 25 µL of the extract solution were added. The absorbance was measured at 450 nm after 30 min of reaction.

7. Statistical analyses

All experimental values were reported as mean values ± standard deviations. For all the parameters evaluated, the statistical analysis of the variability among grape varieties and by-products was performed using the statistical package R version 2.14.2 (R Foundation for Statistical Computing, Vienna, Austria). Differences at $p < 0.05$ were considered statistically significant. Correlations were assessed at 95 %, 99 % and 99.9 % significance levels.

Table 1. Phenolic extraction yield of winery by-products (g of extract/100 g dm).

Grape variety	By-product	Extraction yield
Red varieties		
Escursac	PP	32.4 ± 0.4 d
	FP	17.9 ± 0.2 b
	Stems	42.8 ± 1.2 bc
Gorgollassa	PP	35.8 ± 0.5 c
	FP	16.4 ± 0.2 c
	Stems	40.2 ± 1.1 c
Sabater	PP	28.7 ± 0.4 e
	FP	19.8 ± 0.3 a
	Stems	42.2 ± 1.1 bc
C. Sauvignon*	PP	41.3 ± 0.5 a
	FP	16.9 ± 0.2 c
	Stems	37.4 ± 1.0 d
White varieties		
Giró ros	PP	39.0 ± 0.3 b
	Stems	43.0 ± 0.6 b
Quigat	PP	39.8 ± 0.3 b
	Stems	48.4 ± 0.7 a
Chardonnay*	PP	32.0 ± 0.2 d
	Stems	46.2 ± 0.7 a

* Reference varieties. PP, pressed pomace; FP, fermented pomace. a, b, c, d, e Within the same by-product, means followed by different letters show significant differences among varieties ($p < 0.05$).

RESULTS AND DISCUSSION

1. Phenolic extraction yield

The phenolic extraction yields of pressed pomaces (PP), fermented pomaces (FP) and stems from the landrace and reference grape varieties are shown in Table 1. Results are expressed as g of extract per 100 g of lyophilized samples (dm).

As expected, FP from red grape varieties presented significantly lower extraction yields (average 17.8 ± 1.5 g/100 g dm) than the corresponding PP (average 35.6 ± 2.1 g/100 g dm) and stem (average 42.9 ± 7.1 g/100 g dm) by-products ($p < 0.05$).

In the case of pomaces, FP extraction yields for red landrace varieties ranged from 16.4 to 19.8 g/100 g dm for Gorgollassa and Sabater, respectively, with the reference variety, Cabernet-Sauvignon, being within the same interval. In addition, PP extraction yields varied from 28.7 g/100 g dm for Sabater to 39.8 g/100 g dm for Quigat, this interval being slightly lower than the yield obtained for Cabernet-Sauvignon (41.3 ± 0.5 g/100 g dm).

In the case of stem by-products, extraction yields ranged from 40.2 to 48.4 g/100 g dm for Gorgollassa and Quigat, respectively, being higher than that exhibited by Cabernet-Sauvignon (37.4 ± 1.0 g/100 g dm).

Therefore, there were significant differences ($p < 0.05$) in the phenolic extraction yields, depending not only on the grape variety but also on the type of by-product analysed.

2. Total phenolic content (TPC)

TPC results of winery by-products from the seven grape varieties under study, together with the corresponding statistical analyses, are shown in Table 2. Taking into account that all by-products were collected at the same geographical location and vintage (same edafoclimatic characteristics, cultivation practices and winemaking techniques), differences detected in the TPC may be mainly attributed to the intrinsic properties of each grape variety.

For landrace varieties, TPC varied from 0.91 to 2.30 g GAE/100 g dm for PP; from 3.88 to 5.27 g GAE/100 g dm for FP; and from 4.39 to 7.95 g

Table 2. Total phenolic content (TPC), total anthocyanin content (ANT) and total tannin content (TC) of winery by-products from different grape varieties.

Grape variety	By-product	TPC (g GAE/100 g dm)	ANT (g/100 g dm)	TC (g/100 g dm)
Red varieties				
Escursac	PP	z 1.96 ± 0.01 cd	y 0.40 ± 0.03 b	z 3.94 ± 0.42 b
	FP	y 5.27 ± 0.16 a	x 0.72 ± 0.03 a	y 5.25 ± 0.23 b
	Stems	x 7.95 ± 0.22 a	z 0.18 ± 0.01 a	x 13.05 ± 2.90 a
Gorgollassa	PP	z 0.91 ± 0.02 e	x 0.30 ± 0.01 c	z 2.19 ± 0.29 d
	FP	y 3.88 ± 0.17 c	y 0.18 ± 0.01c	y 4.65 ± 0.72 b
	Stems	x 4.92 ± 0.26 d	z 0.02 ± 0.00 c	x 12.16 ± 1.31 ab
Sabater	PP	z 1.77 ± 0.05 d	y 0.15 ± 0.01 d	z 2.84 ± 0.46 bcd
	FP	x 5.14 ± 0.14 a	x 0.19 ± 0.02 c	y 6.56 ± 0.71 a
	Stems	y 4.39 ± 0.11 e	z 0.05 ± 0.01 b	x 9.89 ± 1.68 bcd
C. Sauvignon*	PP	z 2.47 ± 0.12 b	x 0.61 ± 0.05 a	y 5.20 ± 0.16 a
	FP	y 4.13 ± 0.14 b	y 0.37 ± 0.03 b	y 4.98 ± 0.11 b
	Stems	x 6.56 ± 0.26 b	z 0.06 ± 0.01 b	x 10.16 ± 2.15 bc
White varieties				
Giróros	PP	y 2.21 ± 0.03 bc	x 0.02 ± 0.01 e	y 3.28 ± 0.28 bc
	Stems	x 5.31 ± 0.05 c	x 0.04 ± 0.03 b	x 7.08 ± 0.98 d
Quigat	PP	y 2.30 ± 0.04 bc	x 0.02 ± 0.01 e	y 2.87 ± 0.64 cd
	Stems	x 5.06 ± 0.17 cd	x 0.02 ± 0.01 c	x 11.21 ± 0.32 abc
Chardonnay*	PP	y 2.97 ± 0.55 a	x 0.05 ± 0.03 e	y 3.45 ± 0.58 bc
	Stems	x 4.80 ± 0.04 d	x 0.01 ± 0.00 c	x 8.25 ± 0.07 cd

* Reference varieties. PP, pressed pomace; FP, fermented pomace. x, y, z Within the same variety, means preceded by different letters show significant differences among by-products ($p < 0.05$). a, b, c, d, e Within the same by-product, means followed by different letters show significant differences among varieties ($p < 0.05$).

GAE/100 g dm for stem by-products. TPC of the reference varieties (Cabernet-Sauvignon and Chardonnay) were included within the same ranges, except for PP by-products which exhibited slightly higher TPC values.

In general, among the three types of winery by-products derived from the same grape variety, PP presented the lowest and stem by-products the highest TPC values, except for Sabater ($p < 0.05$). This greater TPC of stems compared to their corresponding grape pomaces has been previously reported in the literature (Alonso *et al.*, 2002; Llobera and Cañellas, 2007, 2008; Besharati and Taghizadeh, 2009).

In contrast to expected behaviour, the TPC of FP was higher than that observed for the corresponding PP of the same red grape variety. This fact might be explained by the differing dry matter basis of both pomaces, since those collected after the fermentation process have transferred most of their components, in particular soluble sugars, to the wine, thus concentrating the remaining fraction of phenolic compounds.

Among red pomaces, FP residues from Escursac and *Sabater* landrace varieties displayed significantly higher TPC values than Cabernet-Sauvignon, whereas for red PP by-products, the reference variety exhibited the highest value ($p < 0.05$). In contrast, the Gorgollassa variety presented the lowest results, on both PP and FP by-products ($p < 0.05$).

Comparison with other studies is rather difficult, since a literature review of the TPC of red grape pomaces reveals that most studies do not specify whether the by-product comes from rosé vinification (PP) or from the fermentation process of red wines (FP). In any case, the results obtained in the present study were in broad agreement with the wide range of TPC values reported in the literature (Alonso *et al.*, 2002; Negro *et al.*, 2003; Amico *et al.*, 2004; Llobera and Cañellas, 2007; Vatai *et al.*, 2009; Rockenbach *et al.*, 2011), pointing out that the landrace minor varieties considered in this research exhibit a phenolic potential comparable to that of different varieties cultivated elsewhere. For example, Alonso *et al.* (2002) observed TPC values ranging from 2.00 to 4.5 g GAE/100 g dm for pomaces from three different red grape varieties (Cabernet-

Sauvignon, Tempranillo and Syrah). Deng et al. (2011) reported TPC values of 2.14 and 2.67 g GAE/100 g dm, respectively, for Pinot noir and Cabernet sauvignon. And Negro *et al*. (2003) determined TPC values for Negro amaro pomaces (4.19 g GAE/100 g dm) similar to those obtained for the FP by-products in this study.

Among white pomaces, it is noteworthy that Chardonnay presented the highest TPC, not only compared with the white varieties, but also with the red ones. In the present study, white pomaces exhibited TPC values slightly lower than those found in Roditis (4.83 g GAE/100 g dm) (Makris *et al.*, 2007), Prensal blanc (3.49 g GAE/100 g dm) (Llobera and Cañellas, 2007) and Chardonnay, Macabeu and Parellada white pomaces (3.09-4.65 g GAE/100 g dm) (González-Centeno *et al.*, 2013). In contrast, higher TPC values for landrace white varieties were observed when comparing the results to those reported by Deng *et al.* (2011) in pomaces from Muller thurgau and Morio muscat (1.16-1.58 g GAE/100 g dm), and by Alonso *et al*. (2002) in pomaces from Palomino fino (1.50 g GAE/100 g dm).

With regard to stem by-products, the landrace variety *Escursac* stood out clearly from the others, exhibiting the highest TPC ($p < 0.05$), even higher than the corresponding value for the Cabernet-Sauvignon variety. The results included in Table 2 were similar to those previously established by Makris *et al.* (2007) in stems from Roditis white variety (5.79 g GAE/100 g dm), but higher than those published by Spigno and De Faveri (2007) and Bustamante *et al.* (2008). In contrast with these studies, Llobera and Cañellas (2007, 2008) and González-Centeno *et al.* (2012) reported considerably higher TPC values for stems of both red and white varieties.

When comparing wines, it is well known that there is a higher phenolic content in red wines than in white wines. Nevertheless, according to the results of the present study, this behaviour was not reflected in landrace winery by-products, as no significant differences were observed between red and white varieties ($p > 0.05$) when considered separately, neither in pomaces nor in stems. There are contradictory results in the literature regarding this, since some authors did not detect differences in TPC between red and white by-products (Bravo and Saura-Calixto, 1998; Llobera and Cañellas, 2008; Deng *et al.*, 2011) (stems, González-Centeno *et al.*, 2012), whereas others have reported higher values for by-products from red varieties (Alonso *et al.*, 2002; Püssa *et al.*, 2006; Makris *et al.*, 2007).

3. Total anthocyanin content (ANT)

ANT values of winery by-products are also shown in Table 2. Experimental data of red landrace varieties ranged from 0.15 to 0.40 g/100 g dm for PP and from 0.18 to 0.72 g/100 g dm for FP by-products. In the case of PP by-products, landrace ANT values were lower than those exhibited by Cabernet-Sauvignon, while for FP by-products, the reference variety presented an ANT value within the same interval as landrace red varieties. It is important to point out that the highest ANT values, for both PP and FP by-products, were exhibited by Escursac landrace variety (0.40 and 0.72 g/100 g dm, respectively). In particular, its FP by-products presented a significantly higher content than Cabernet-Sauvignon (0.37 g/100 g dm) ($p < 0.05$).

ANT values for red grape pomaces were higher than those detected in Merlot and Cabernet-Sauvignon (0.03-0.14 g/100 g dm) by Deng *et al.* (2011) but lower than those found in Negro amaro (0.98 g/100 g dm) by Negro *et al.* (2003).

As expected, anthocyanins were almost negligible in both stems and white PP (< 0.06 g/100 g dm, in all cases), since these compounds are mainly concentrated within the vacuoles of red grape skins (Moutounet *et al.*, 1996). In fact, it is noteworthy to mention that the sodium bisulfite discoloration method used to measure the anthocyanin content of grape by-products has strong limitation at very low concentration and that the results may be impacted by the other phenolic compounds (tannins, flavanols, flavonols) present in the extract. Then, both stems and white PP probably did not exhibit the anthocyanin levels reported in Table 2, being a result of the interaction between sodium bisulfite and the other phenolic compounds.

4. Total tannin content (TC)

TC values of winery by-products are also presented in Table 2. Experimental TC results for landrace varieties ranged from 2.19 to 3.94 g/100 g dm, from 4.65 to 6.56 g/100 g dm, and from 7.08 to 13.05 g/100 g dm for PP, FP and stems, respectively. Regardless of the by-product considered, no significant differences were observed between red and white varieties when studied separately ($p > 0.05$).

For the same grape variety, stem by-products presented significantly larger amounts of tannins (average 10.26 ± 2.10 g/100 g dm) than the corresponding pomaces ($p < 0.05$). With regard to the red varieties, FP (average 5.36 ± 0.30 g/100 g

dm) showed higher tannin contents than the corresponding PP (average 3.39 ± 0.97 g/100 g dm) ($p < 0.05$), except for the Cabernet-Sauvignon variety.

In the case of red PP by-products, the reference red variety achieved the highest TC ($p < 0.05$), whereas for FP, the landrace minor variety Sabater contained significantly higher amounts of tannins than Cabernet sauvignon ($p < 0.05$). In the case of white PP by-products, no significant differences were observed between landrace and reference varieties with regard to the TC ($p > 0.05$).

Due to the different methods and standards used to evaluate TC, data in the literature vary enormously. In comparison with the experimental results of the present research, Negro *et al.* (2003) reported lower TC for Negro amaro pomaces (2.23 g/100 g dm), and Llobera and Cañellas (2007, 2008) published higher TC values for Manto negro and Prensal blanc pomaces (2.30 and 16.80 g/100 g dm, respectively). Meanwhile, González-Centeno *et al.* (2013) described TC values for white grape pomaces similar to those observed in the present study (5.08-9.21 g/100 g dm).

Among stems, the landrace red varieties Escursac and Gorgollassa, and the landrace white variety Quigat showed higher TC ($p < 0.05$) than the corresponding reference varieties. According to the literature, stem TC values were consistent with those reported by González-Neves *et al.* (2004) for Mulo negro stems (10.30 g/100 g dm), and by González-Centeno *et al.* (2012) for Cabernet-Sauvignon, Merlot, Chardonnay and Macabeu stems (7.91-12.49 g/100 g dm). On the contrary, TC results from this study were slightly lower than those described by Prozil *et al.* (2012) for stems from different red grape varieties (average 15.9 g/100 g dm).

5. Total antioxidant capacity

Studies on the *in vitro* antioxidant potential of winery by-products are still very scarce. Furthermore, the different determination methods, antioxidant activity units, and standards used for calibration curves make it difficult to establish quantitative comparisons.

Results of the antioxidant capacity of winery by-products evaluated by four different methods (ABTS, DPPH, FRAP and CUPRAC) are depicted in Table 3. In general, regardless of the methodology used, stems exhibited the highest antioxidant capacity for both landrace and reference varieties ($p < 0.05$). This behaviour has been previously reported in the literature (Llobera and Cañellas, 2007 ; Spigno and De Faveri, 2007 ; Llobera and Cañellas, 2008 ; González-Centeno *et al.*, 2013).

For pomaces obtained from the same red variety, FP generally exhibited a higher antioxidant capacity than the corresponding PP ($p < 0.05$). This general trend might be explained by the same reasons as for the TPC.

With regard to the PP by-products of landrace varieties, the antioxidant activities varied from 0.30 to 0.46 mmol TE/g dm, from 0.11 to 0.16 mmol TE/g dm, from 0.23 to 0.32 mmol TE/g dm and from 0.50 to 0.65 mmol TE/g dm for ABTS, DPPH, FRAP and CUPRAC measurements, respectively. This variation can be explained by the different reagents and/or chemical reactions involved in each analytical method. It is important to point out that for the white varieties, the total antioxidant capacity of PP from both Giró ros and Quigat landrace varieties did not differ significantly from that from Chardonnay ($p > 0.05$), regardless of the analytical method used.

The ranges of the antioxidant potential for FP by-products were 0.36-0.47 mmol TE/g dm, 0.18-0.27 mmol TE/g dm, 0.56-0.83 mmol TE/g dm and 1.47-1.79 mmol TE/g dm for ABTS, DPPH, FRAP and CUPRAC, respectively. Apart from the CUPRAC assay, all methods agreed with the largest antioxidant power exhibited by FP from the minor red variety *Sabater* (even higher than that of the reference red variety), and the lowest values for Gorgollassa ($p < 0.05$). In the case of the CUPRAC assay, no statistical differences were reflected among the three red landrace varieties and the reference Cabernet-Sauvignon variety with respect to the antioxidant capacity of their FP by-products ($p > 0.05$).

Stem by-products from landrace varieties showed antioxidant capacity values ranging from 0.65 to 0.79 mmol TE/g dm, from 0.48 to 0.61 mmol TE/g dm, from 0.78 to 0.94 mmol TE/g dm and from 1.81 to 2.50 mmol TE/g dm for ABTS, DPPH, FRAP and CUPRAC, respectively. As observed in Table 3, stems from the landrace red varieties Escursac, Gorgollassa and Sabater showed a similar, or even higher, antioxidant potential than stems from the Cabernet-Sauvignon reference variety. The same behaviour was observed in the case of white varieties with regard to the DPPH, FRAP and CUPRAC results : a similar antioxidant potential for both reference and landrace varieties.

According to the results depicted in Table 3, when comparing red and white varieties, antioxidant capacity values were comprised within the same

Table 3 - Total antioxidant capacity of winery by-products evaluated by ABTS, DPPH, FRAP, and CUPRAC methods

Grape variety	By-product	ABTS (mmol TE/g dm)	DPPH (mmol TE/g dm)	FRAP (mmol TE/g dm)	CUPRAC (mmol TE/g dm)
Red varieties					
Escursac	PP	z 0.46 ± 0.07 ab	y 0.16 ± 0.02 a	y 0.25 ± 0.04 c	z 0.51 ± 0.04 d
	FP	y 0.39 ± 0.04 bc	y 0.18 ± 0.01 c	x 0.73 ± 0.06 b	y 1.76 ± 0.39 a
	Stems	x 0.79 ± 0.06 b	x 0.61 ± 0.05 a	x 0.78 ± 0.21 cd	x 2.50 ± 0.11 a
Gorgollassa	PP	y 0.40 ± 0.12 b	y 0.15 ± 0.02 a	z 0.32 ± 0.03 a	z 0.65 ± 0.02 bc
	FP	y 0.36 ± 0.02 c	y 0.18 ± 0.02 c	y 0.56 ± 0.07 c	y 1.47 ± 0.29 a
	Stems	x 0.72 ± 0.06 cd	x 0.53 ± 0.04 b	x 0.94 ± 0.09 ab	x 2.33 ± 0.25 abc
Sabater	PP	z 0.40 ± 0.04 b	z 0.11 ± 0.06 ab	y 0.24 ± 0.02 c	y 0.50 ± 0.04 d
	FP	y 0.47 ± 0.06 a	y 0.27 ± 0.03 a	x 0.83 ± 0.01 a	x 1.79 ± 0.21 a
	Stems	x 0.75 ± 0.04 bc	x 0.51 ± 0.06 b	x 0.80 ± 0.11 bcd	x 2.11 ± 0.56 bcd
C. Sauvignon*	PP	y 0.53 ± 0.05 a	z 0.14 ± 0.04 ab	z 0.35 ± 0.02 a	z 0.74 ± 0.06 a
	FP	z 0.42 ± 0.04 b	y 0.22 ± 0.05 b	y 0.70 ± 0.03 b	y 1.54 ± 0.40 a
	Stems	x 0.59 ± 0.01 f	x 0.49 ± 0.05 b	x 1.05 ± 0.06 a	x 2.41 ± 0.58 ab
White varieties					
Giró ros	PP	y 0.31 ± 0.03 c	y 0.14 ± 0.01 ab	y 0.26 ± 0.03 bc	y 0.65 ± 0.03 bc
	Stems	x 0.67 ± 0.04 de	x 0.48 ± 0.07 b	x 0.78 ± 0.04 d	x 1.81 ± 0.27 d
Quigat	PP	y 0.30 ± 0.02 c	y 0.12 ± 0.03 b	y 0.23 ± 0.03 c	y 0.61 ± 0.02 c
	Stems	x 0.65 ± 0.05 ef	x 0.53 ± 0.04 b	x 0.87 ± 0.07 bcd	x 1.99 ± 0.08 cd
Chardonnay*	PP	y 0.27 ± 0.01 c	y 0.12 ± 0.02 b	y 0.29 ± 0.02 b	y 0.68 ± 0.06 b
	Stems	x 0.94 ± 0.08 a	x 0.55 ± 0.06 ab	x 0.93 ± 0.05 abc	x 1.85 ± 0.15 d

* Reference varieties. PP, pressed pomace; FP, fermented pomace. x, y, z Within the same variety, means preceded by different letters show significant differences among by-products ($p < 0.05$). a, b, c, d, e Within the same by-product, means followed by different letters show significant differences among varieties ($p < 0.05$).

interval ($p > 0.05$), for both PP and stem by-products. This general trend has been previously observed between Roditis white pomace and Agiorgitiko red pomace (Makris *et al.*, 2007), and also among stems from 10 different red and white varieties (González-Centeno *et al.*, 2012). No significant differences ($p > 0.05$) were found between red (Manto negro) and white (Prensal blanc) varieties by Llobera and Cañellas (2007, 2008) for both pomaces and stems.

6. Correlations

Pearson correlation coefficients between the antioxidant activity assessed by the four different techniques and the polyphenol compounds (TPC, ANT and TC) were determined (Table 4).

For all combinations, the relationship between the antioxidant potential and TPC and TC was high, positive and significant ($r \geq 0.78$; $p < 0.001$), apart from ABTS with TPC, which presented a slightly lower correlation ($r \geq 0.67$; $p < 0.001$). For TC, DPPH showed the highest *Pearson* correlation coefficient ($r \geq 0.88$; $p < 0.001$). With regard to ANT, the correlation with y was positive and significant, but very weak in all cases ($r \leq 0.32$; $p < 0.05$).

Several authors have reported good correlations ($r \geq 0.83$; $p \leq 0.05$) between the antioxidant capacity and the TPC of grape derivatives (Deng *et al.*, 2011; Anastasiadi *et al.*, 2012). In the case of anthocyanins, Gómez-Plaza *et al.* (2006) also obtained a poor correlation with the antioxidant potential for grape pomace by-products. In fact, as previously reported by Fukumoto and Mazza (2000) and Spigno and De Faveri (2007), the degree of correlation depends on the type of compound, although it is generally higher for total phenolics than for anthocyanins.

In order to evaluate the uniformity of the antioxidant capacity results based on the four assays applied, *Pearson* correlation coefficients were also calculated (Table 5). The results revealed a high, significant and positive correlation among the different antioxidant methods ($0.66 \leq r \leq 0.90$), suggesting that all four assays give comparable values for the winery by-products considered. The best relationships were found between FRAP–CUPRAC and ABTS–DPPH methods. This could be explained by their similar

Table 4. Pearson correlation coefficients between the analysed parameters and the antioxidant potential evaluated by ABTS, DPPH, FRAP and CUPRAC methods.

	ABTS (mmol TE/g dm)	DPPH (mmol TE/g dm)	FRAP (mmol TE/g dm)	CUPRAC (mmol TE/g dm)
TPC (g/100 g dm)	0.67***	0.83***	0.78***	0.87***
ANT (g/100 g dm)[a]	0.32*	0.12*	0.10*	0.12*
TC (g/100 g dm)	0.82***	0.88***	0.78***	0.78***

Significance at *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ by ANOVA test; ns, not significant. TPC, total phenolic content; ANT, total anthocyanin content; TC, total tannin content. aPearson correlation values calculated by considering just pressed and fermented pomaces of red grape varieties.

Table 5. Pearson correlation coefficients among the different methods for quantifying the antioxidant potential.

	ABTS (mmol TE/g dm)	DPPH (mmol TE/g dm)	FRAP (mmol TE/g dm)	CUPRAC (mmol TE/g dm)
ABTS (mmol TE/g dm)	1.00	0.88***	0.69***	0.66***
DPPH (mmol TE/g dm)		1.00	0.81***	0.80***
FRAP (mmol TE/g dm)			1.00	0.90***
CUPRAC (mmol TE/g dm)				1.00

Significance at *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ by ANOVA test ; ns, not significant.

action mechanism : a metal reduction and a free radical scavenging, respectively.

CONCLUSIONS

As a consequence of the increasing use of minor varieties in winemaking to meet consumer demand, the present research provides an overall characterization and comparison of the phenolic and antioxidant potential of the three main winery by-products – pressed pomace, fermented pomace and stems – from red and white landrace minor varieties, as a basis for their integrated exploitation.

In general, all the winery residues constituted a rich source of bioactive compounds with interesting antioxidant properties. In particular, stems presented significantly higher TPC (average 5.57 ± 1.25 g/100 g dm) and TC values (average 10.26 ± 2.10 g/100 g dm) than pomaces ($p < 0.05$). As expected, ANT was almost negligible in stems and white PP, but was important in both PP and FP by-products from red landrace varieties (0.15-0.40 g/100 g dm and 0.18-0.72 g/100 g dm, respectively). Regardless of the grape variety considered, the winery by-products denoted great antioxidant capacity, with values depending on the analytical assay applied.

To the best of our knowledge, comparison between PP and FP by-products from different red grape varieties has not been previously reported in the scientific literature. In contrast to expected behaviour, FP exhibited a higher TPC and antioxidant potential than the corresponding PP for the same grape variety. This trend may be explained by the different composition of the dry matter basis of both pomaces and by the different proportion represented by the phenolic compounds.

Landrace minor varieties also had similar or even greater polyphenol content and antioxidant properties than the reference varieties considered. Specifically, when evaluating FP, both autochthonous Escursac and Sabater varieties exhibited the largest TPC and also the highest ANT and TC values, respectively, among the grape varieties studied. With regard to the stem by-products, the local variety Escursac exhibited the highest TPC and TC values.

These results demonstrate that winery by-products from local minor varieties are promising sources of phenolic concentrates, despite the lack of selection process that the reference varieties have been subjected to as a result of their intensive production over years.

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