Food Research International 176 (2024) 113826

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Effects of grape variety and roasting on the proanthocyanidin oligomers distribution, cyclic proanthocyanidins, and total polyphenol content in grape seed powders

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ARTICLE INFO

Keywords:
Grape seeds powder
Roasting
Cyclic proanthocyanidins
Protein stabilization

ABSTRACT

Grape seeds are a valuable source of natural phenolic compounds, particularly flavan-3-ol derivatives such as condensed tannins. Recent studies have shown that grape seed powder can be applied to reduce the undesirable effects of protein instability in wine. One pretreatment method applied to grape seeds is roasting. Roasting causes the heavier proanthocyanidins (PAC) oligomers to break down, thereby increasing the concentration of smaller oligomers available for interaction with proteins. In addition, roasting can prolong grape seed storage. Among the subclasses of proanthocyanidins, oligomeric macrocyclic proanthocyanidins have also shown potential effects in terms of wine stabilization, particularly by presenting selective interactions with metal cations such as potassium and calcium. However, their composition in grape seed extracts has never been studied. Here, the characterization of condensed tannins according to the degree of polymerization in grape seeds, the profile of cyclic proanthocyanidins and the total polyphenol content were characterized in relation to different grape varieties and the application of roasting. Roasting greatly influenced the distribution of PAC according to the degree of polymerization, increasing the abundance of almost all classes of PAC. However, the overall effect of roasting was highly dependent on grape variety. PAC were analyzed according to the degree of polymerization. Grape seed roasting of red varieties (Croatina and Sangiovese) showed an increase in all classes of PAC except trimers. The white variety (Ortrugo) and the mix of Nebbiolo and Barbera varieties (80% and 20% w/w, respectively) showed no clear effect on the profile of PAC upon roasting. Notably, cyclic procyanidins were identified for the first time in grape seeds: a cyclic tetrameric procyanidin (ESI + m/z 1153) and cyclic pentameric procyanidin (ESI + m/z 1441) were found. The abundances of these cyclic PAC were found to be completely stable upon roasting, also in agreement with the already known stability of these compounds against depolymerizing conditions. Interestingly, the cyclic pentameric procyanidin was significantly more abundant in Ortrugo (white variety), than in Sangiovese and Croatina (red varieties). Besides, no effect of roasting occurred on the profile of cyclic procyanidins in grape seed powder. Finally, the total polyphenol content was evaluated, showing that roasting caused an increase of polyphenolic molecular species potentially available for protein stabilization, but only in GSP of red varieties. Overall, the grape variety was found to be a significant factor in determining how much the roasting would change the PAC profile, providing valuable information for future applications of GSP in enology.

1. Introduction

Condensed tannins (i.e., proanthocyanidins - abbr. PAC) are one of the main components of phenolic molecular classes in grapes and wine. They play a key role in protein interaction (Ribéreau-Gayon et al., 2000), exerting hydrophobic and hydrogen bonding capacity (Haggerman & Butler, 1981). PAC are found in grape skins and seeds, both in different compositions (Bordiga et al., 2011). In this regard, grape seed tannins have been extensively studied for their ability to bind to proteins in wine, contributing to haze formation and instability (Marangon et al.,

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https://doi.org/10.1016/j.foodres.2023.113826
Received 13 August 2023; Received in revised form 29 November 2023; Accepted 2 December 2023
Available online 4 December 2023

2010; Van Sluyter et al., 2015). At the same time, this protein-binding ability has been successfully used for preventive purposes to reduce the risk of protein haze formation thanks to a treatment with grinded grape seeds to get a grape seed powder (GSP) (Romanini et al. 2020; Romanini et al., 2021). In this regard, grape seeds may become a renewable resource available to wineries, instead of being considered as a by-product or a waste (Shi et al., 2003; Dávila et al. 2017). GSP contains flavan-3-ol oligomers, with higher abundances of galloylated derivatives than PAC in grape skins. Galloylation has also been shown to play a key role in protein interaction (Ma et al. 2014; Ma et al. 2016; Siebert, 2006) so to enhance GSP as a promising alternative to bentonite (Romanini et al. 2020; Romanini et al., 2021).

Among PAC, a group of macrocyclic oligomers, namely cyclic proanthocyanidins (abbr. c-PACs), constitute a particular subclass (Longo et al., 2019; Merkytė et al., 2020; Zeng et al, 2019) with interesting and unusual chemical properties, such as higher polarity than their direct noncyclic analogs (Longo et al., 2018a), better resistance to acidic depolymerization conditions, much higher resistance to depolymerization than their noncyclic analogs under low-energy collision-induced dissociation conditions in MS/MS fragmentation experiments, and varying selectivity towards metal binding (Longo et al., 2018b). Considering their resistance to acidic conditions (as in wine with a pH < 4.0), these compounds have been proposed in red and white wine as varietal chemical markers (Longo et al., 2018c; Longo et al., 2019) which may overcome the wine aging. Overall, through the application of NMR (Zeng et al., 2019), LC-MS and modified hydrogen/deuterium exchange (HDX) LC-MS methods (Longo et al., 2018a; Longo et al., 2018d), it was possible to identify up to fifteen c-PACs in wine (Merkytė et al., 2020). Such small number of identified species is believed to be the result of several factors: i) some compounds are present in traces, misleading their detection without prior purification, extraction, and sample concentration (Longo, 2021); ii) most of compounds show a very narrow variability in terms of regio-/diastereoisomers, which usually characterize their noncyclic analogues. For example, the most concentrated cyclic procyanidin and cyclic prodelphinidin in wine (*m/z* 1153 and *m/z* 1169) show only one species each in wine and grape extracts (Longo et al., 2019; Merkytė et al., 2020), highlighting potential selective or even specific synthesis. In contrast, their direct noncyclic analogs (*m/z* 1155 and *m/z* 1171) show a rather wide distribution of isomeric compounds. The cyclic pentameric procyanidin (*m/z* 1441) shows two isomeric forms whose complete structural characterization has not yet been achieved.

In this report, grape seeds were characterized for their PAC content according to their degree of polymerization (DP), total phenolic content (TPC), along with c-PACs. The effect of both roasting and grape variety (Sangiovese, Croatina, Ortrugo, and a mix of the varieties) (Table 1) will be discussed.

2. Material and methods

2.1 Materials

All chemicals and solvents used have been purchased from Merk Life Science S.r.l. (Via Monte Rosa, 93, 20,149 Milano, Italy). Grape seeds were isolated from grape marc sourced from the experimental winery at Università Cattolica del Sacro Cuore (Piacenza, Italy). They were separated by flotation of fresh marc arising from Ortrugo (O), Croatina (C), and Sangiovese (S) from 2019 vintage and then dried in oven at 50 °C till 8% of moisture.

Table 1
List of grape seeds powders. *80%Nebbiolo, 20%Barbera.

VARIETY	Roasted	CODE
Sangiovese	Yes	ST
Sangiovese	No	SC
Croatina	Yes	CT
Croatina	No	CC
Ortrugo	Yes	OT
Ortrugo	No	OC
Mix*	Yes	MT
Mix*	No	MC

2.2 Preparation of grape seed powder (GSP)

Grape seed powder was prepared by either directly grinding (controls, C) into a powder using a mortar and pestle (IKA A11 basic; IKA-Werke GmbH, Staufen, Germany) to increase the surface area of contact or by grinding into powder after first roasting (treatment, T) in an oven (180 °C,10 min).

2.3 Extraction of condensed tannins from grape seed powder

An aliquot (2 g) of each ground grape seed sample was diluted with 10 mL of extraction solvent (700 mL, acetone, 295 mL water and 5 mL glacial acetic acid). Then the samples were vortexed for 1 min, ultrasonicated at 50 °C for 5 min, and then centrifuged at 20 °C at 100 × g for 5 min. Finally, the collected supernatant solution polyphenols was filtered through a 0.22 μm PTFE syringe filter into amber HPLC vials for the analysis. Each grape seed sample was extracted in triplicates.

2.4 Calibration of (−)-epicatechin standard solutions

25 mg of (−)-epicatechin was weighed into a 25-mL volumetric flask and dissolved in a portion of extraction solvent, and then diluted to 25 mL with the same extraction solvent described in the previous paragraph. The resulting concentration of the stock solution was 1 $mg{\cdot}mL^{-1}$. A seven-points calibration curve using (−)-epicatechin as standard was made by diluting the stock solution with the extraction solvent (calibration levels: 0.00, 0.02, 0.20, 0.30, 0.40, 0.50, 0.60, and 1.00 $mg{\cdot}mL^{-1}$). The calibration model (intercept, coefficient, and their standard errors) is reported in Table 2.

2.5 NPLC-FLD quantitation of condensed tannins by degree of polymerization

The separation was carried on a Sepax HP-Diol column (4.6 × 50 mm, 1.8 μm, 120 Å) on a Nexera X2 UHPLC (Shimadzu) with fluorescence detection. The binary gradient consisted of (solvent A) acetonitrile and glacial acetic acid (98 + 2, v/v), and (solvent B) methanol, water, and glacial acetic acid (95% / 3% / 2% v/v/v) (Machonis et al., 2014a; Machonis et al., 2014b). The purity of all used solvents was at least of LC grade or higher. The gradient conditions were [min, $\%_B$]: [0, 7]; [1, 7]; [11.5, 30]; [13, 100]; [14, 100]; [15, 7]; [20, 7]. The analysis time was 15 min with a flow rate of 1.0 $mL{\cdot}min^{-1}$. The injection volume was 1 μL. Detector parameters: excitation wavelength = 230 ± 4 nm; emission wavelength = 321 ± 4 nm; column temperature = 35 °C, autosampler temperature = 5 °C. According to the followed protocol, the quantitation against (−)-epicatechin standards was corrected for a factor considering the relative impact of the degree of polymerization on the fluorescence output, as indicated by the protocol (Machonis et al., 2014a; Machonis et al., 2014b). The applied correction factors are reported in Table 3.

2.6 RPLC-QqQ/MS profile of cyclic proanthocyanidins

The PAC profile analyses have been performed in full scan mode on a UHPLC-DAD-QqQ/MS instrument (Agilent LC/TQ 6465 system) equipped with a 1260 Infinity II UHPLC quaternary pumps system, and a 1260 Infinity II WR DAD detector in series to a AJS ESI QqQ mass analyzer (Dupas de Matos et al., 2020). The separation was carried on a 250 mm × 4.6 mm × 5 μm Knauer Eurosphere II C18 stationary phase (purchased from LabService Analytica, Anzola dell'Emilia, Bologna, Italy). The

Table 2
Calibration model ($r^2 > 0.999$).

Source	Value	Standard error	t	Pr > |t|	Lower bound (95%)	Upper bound (95%)	p-values signification codes
Intercept	−1187.498	126.100	−9.417	<0.0001	−1454.817	−920.178	***
Slope	699107.165	273.699	2554.291	<0.0001	698526.949	699687.381	***

Signification codes: 0 < *** < 0.001 < ** < 0.01 < * < 0.05 <. < 0.1 < ° < 1.

Table 3
Correction factor applied for the quantitation of proanthocyanidins by DP with NPLC-FLD analysis.

DP	Correction Factor
II	0.377
III	0.152
IV	0.146
V	0.146
VI	0.119
VII	0.090
VIII	0.071
IX	0.068
X	0.067

HPLC mobile phase was composed of solvent A (0.1% formic acid in degassed milliQ water) and solvent B (0.1% formic acid in acetonitrile). The gradient method was: 0–2.5 min 1% B, 2.5–50 min 1–25% B, 50–51 min 25–99% B, 51–55 min 99% B, 55–56 min 99–1% B, 56–62 min 1% B. The HPLC flow rate was 0.7 $mL \cdot min^{-1}$. The column was kept at 30 °C throughout the analysis time. The ESI ion source was operated in positive ionization mode. The applied detector parameters were as follows: gas temperature 230 °C, gas flow: 8 $L \cdot min^{-1}$, nebulizer pressure 20 psi, sheath gas heater: 300 °C, sheath gas flow: 10 $L \cdot min^{-1}$. Positive capillary voltage 4000 V (V), nozzle voltage = 1500 V, fragmentor potential: 135 V, cell acceleration: 5 V. For MS/MS analysis, the same setup was employed, but running the instrument in product reaction monitoring mode, applying unit resolution to the first quadrupole and a collision energy of 10, 15, or 20 eV, with the second quadrupole scanning between m/z 100 and the molecular ion mass value + 5 Da, with a scan rate of 2 $spectra \cdot s^{-1}$.

2.7 Total polyphenol content

The total polyphenol content (TPC) was determined with the Folin-Ciocalteu method using a MIURA One automated analyzer (Exacta & Optech, San Prospero, Modena, Italy).

2.8. Statistical analysis

The statistical analysis and the related graphics were performed with XLStat (version 2019.2.2.59417, Addinsoft, Paris, France).

3. Results and discussion

All PAC classes showed a significant effect of both factors and their interaction. The variety played an important role, as GSP extracts of red grape seeds (Sangiovese and Croatina) showed higher amounts than most PAC classes for DP, except for V, VII, IX and X. Roasting caused an increase in almost all classes, except for the trimers (III), which showed higher abundances in the untreated samples.

Some discrepancies from the common trend observed with the varieties can be explained by looking at the interaction term. In general, the highest values were present in the treated GSPs of the red varieties. The trimers (III), on the other hand, were relatively higher in the unroasted GSP from Croatina (C*C), then in the roasted GSP from red varieties (C*T, S*T). Thus, the general trends are well summarized by the PCA model (Fig. 1).

Overall, the PCA model differentiates the samples rather well, with the exception of Ortrugo treated (OT) vs. untreated (OC) samples. The main trend corresponds to the separation of roasted and unroasted samples for the red varieties Sangiovese (S) and Croatina (C) along PC1; the two varieties are separated relatively similarly, indicating that the process was similar for these two varieties. PC2 mainly reflects the separation of these two varieties. Ortrugo is not separated between the roasted (OT) and unroasted (OC) samples along any specific direction in the PCA projection shown. This indicates a substantial resistance of the seeds of this variety to the roasting treatment. As for the variety mix, the samples show some degree of separation between roasted (MT) and unroasted (MC) along PC2, thus in a completely orthogonal direction compared to Sangiovese and Croatina, thus indicating a completely different process. Probably, this is due to the varietal composition of the GSP blend, containing 80% Nebbiolo and 20% Barbera grape seeds.

All classes of PACs, except trimers, were higher in the roasted seed powders of Croatina (CT) and Sangiovese (ST) (red varieties). The trimers (III) seem to mainly influence the separation between the two red varieties (PC2), regardless of roasting application. Trimers also correlate with the separation between the roasted and unroasted GSP blend (MT vs MC), as roasting caused a decrease in trimers content. The anomaly observed for trimers (III) parallels the ANOVA results (Table 4), as the interaction of the two factors (Table 4 - Part C) caused such a different trend for Croatina. Overall, the trimers (III) do not show a trend to higher values in the treated samples (ST and CT).

Nonamers and decamers distinguished Croatina varieties (CC and CT) from Sangiovese varieties (SC and ST) along PC2. Notably, for the red varieties, PC3 also separates them according to roasting, although in a completely anticorrelated manner between each other: as also evidenced by ANOVA (Table 4), trimers (III) increased with roasting in Sangiovese (ST), while they decreased with roasting for Croatina (CT). The varietal mix (treated - MT, or untreated - MC) is at intermediate PC1 and PC2 values, indicating that blending the different varieties (mix = 80% Nebbiolo and 20% Barbera) produced GSP samples with intermediate properties. Its higher resistance to roasting, evidenced by the absence of trends for M in PC1, could be due to a specific varietal contribution, as seen with Ortrugo. PC2 shows a clear separation between the GSP of the treated and the untreated blend (MT and MC), however, this parallels the separation in PC2 between Sangiovese and Croatina, and not the separation of the GSP of the red variety after treatment. This agrees with the ANOVA results (Table 4 - Part C), as only pentamers and heptamers were shown to significantly differentiate MT from MC. To better highlight the variation in DP profile due to roasting and the effect of variety, the profile of the percentage of variation by DP was analyzed (Fig. 2).

The difference between the red varieties and the other samples is made clearer here. Red varieties show an increase in almost all PAC by DP of 60–100%. Ortrugo and the variety mix however showed almost no change for most of the PAC classes. Trimers (III) were clearly shown to be affected differently than other PAC classes in roasted GSP samples of red varieties (C and S). Similarly, nonamers (IX) in Croatina GSP and decamers (X) in Sangiovese GSP increased to a much lesser extent than in the other DPs. In Ortrugo, all DP showed almost no change, apart from the pentamers which were found to decrease during roasting (-20%) and the octamer vice versa (+20%); in the varietal mix, all PAC classes showed a decrease between 0 and 20%, although an anomalous value was found only for heptamers (VII), which increased by 100%. Surprisingly, the variety mix showed a much more homogeneous trend than

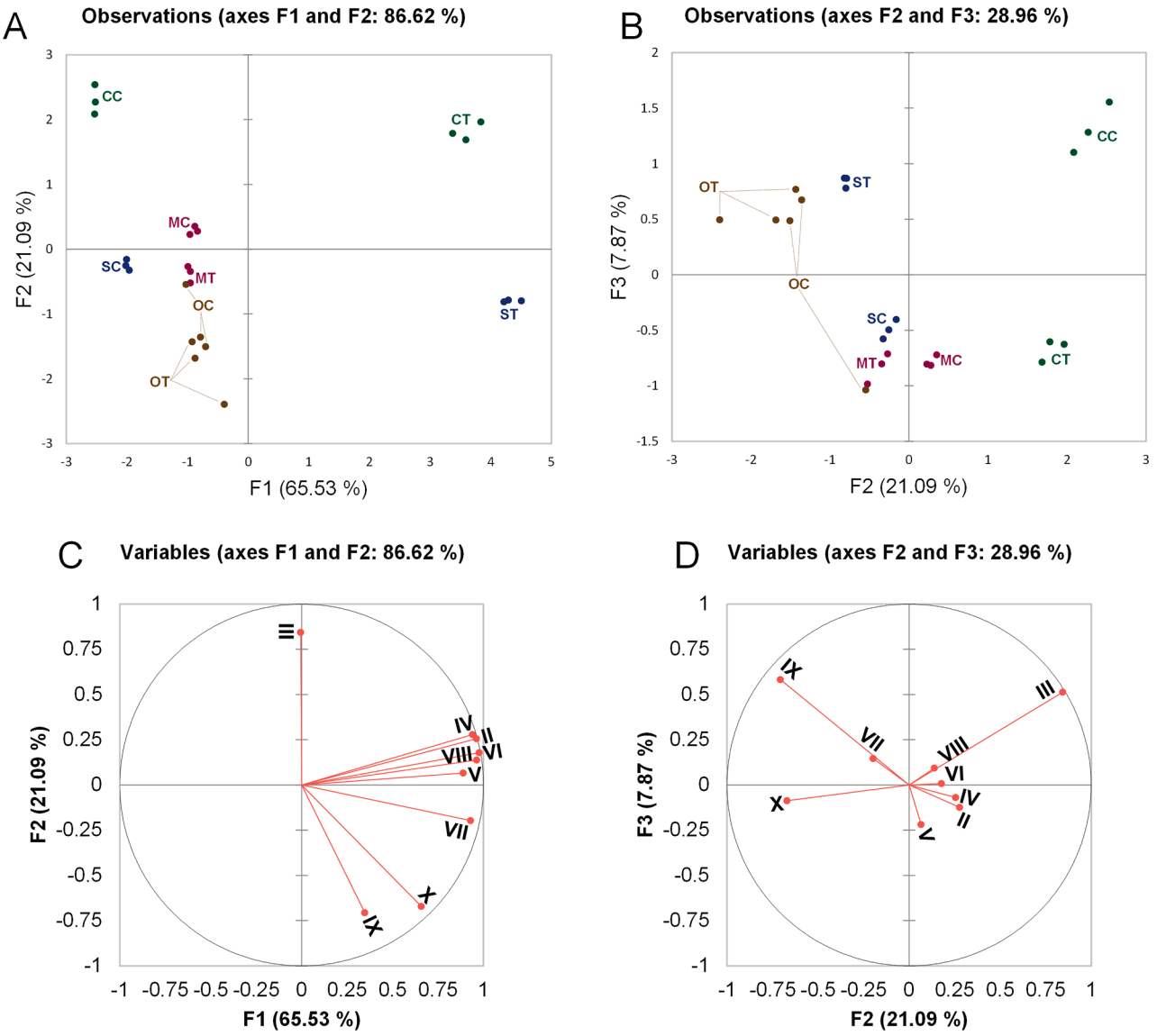

Fig. 1. PCA model built on the PAC profile by degree of polymerization. Scores plot for: A) PC1 *vs* PC2, B) PC2 *vs* PC3; Loadings plots for: C) PC1 *vs* PC2, D) PC2 *vs* PC3.

the other varieties for all DPs except heptamers (VII).

Cyclic proanthocyanidin (c-PAC) profiles from GSP were obtained by RPLC-MS analysis of the extracts. The most abundant cyclic tetrameric procyanidin (ESI + *m/z* 1153) and the most abundant cyclic pentameric procyanidin (ESI + *m/z* 1441) usually found in wine and grape skins, has also been identified in GSP. The associated chromatographic traces and the associated MS/MS fragmentation spectra obtained at CE = 10 and 20 eV are reported in the Supporting Information (Fig. SI2 and Fig. S2 for the c-tetramer and the c-pentamer, respectively). The tetramer showed low degree of fragmentation up to 15 eV, whereas it broke down completely at 20 eV (Fig. SI1B-D). The pentamer could not be fragmented at any of the three energies, and only showed the peak associated to the intact molecular ion (*m/z* 1441). Resistance to low-energy fragmentation conditions was a key element used in differentiating these compounds from their non-cyclic analogues, especially the isomeric A-type analogues (Longo et al., 2018a,d).

No other c-PAC species was observed in the LC-MS traces. The results (relative abundances) for these two species were tested by 2-way ANOVA and Tukey's *post-hoc* HSD test (summary in Fig. 3). Only the grape variety resulted significant in influencing the c-PAC distributions, with no significant effect from the application of roasting or the interaction term.

c-PACs are differentiated by grape variety, even after the roasting treatment of the initial raw material, as no significant difference was found for this factor. Unlike previous studies, here c-PACs were analyzed in the seeds, and not in the grape skins. As far as we know, this is the first time these compounds have been identified in grape seeds. In particular, the highest relative abundance of the pentameric cyclic procyanidin (ESI + *m/z* 1441) was found in the seeds of the Ortrugo grape. In previous studies, it has been shown that white varieties often have higher relative abundances of specific cyclic proanthocyanidins in grape skin compared to their related non-cyclic congeners with identical monomer composition (Longo et al., 2018c; Longo et al., 2019), unlike what was observed in red varieties. However, compared to red varieties, white varieties usually have a lower relative abundance of cyclic and non-cyclic proanthocyanidins in grape skins. Furthermore, excluding the GSP mix (M), tetrameric cyclic procyanidin is also significantly higher for Ortrugo, although not significantly different from Croatina (C). The higher abundance of cyclic procyanidins in Ortrugo could be partially related to such a small effect of roasting on the distribution of PAC by DP for this variety (ANOVA, Table 4). These cyclic oligomers are known to be more resistant to depolymerization conditions (e.g. acidic conditions

Table 4
LS means ($mg \cdot g^{-1}$) of PAC in GSP by degree of polymerization (DP). Part A: Summary (LS Means) for factor Variety (S = Sangiovese; C = Croatina; O = Ortrugo; M = varietal mix); Part B: Summary (LS Means) for factor Roasting (T = treatment applied; C = control); Part C: Summary (LS Means) for the two-factors' interaction.

PART A (LS means) - **Variety**

	II	III	IV	V	VI	VII	VIII	IX	X
S	0.863c	0.218b	1.040c	0.025 a	0.808 d	0.015c	0.462c	0.005b	0.015c
C	1.160 d	0.328c	1.185 d	0.030b	0.740c	0.010b	0.358b	0.000 a	0.005 a
O	0.405 a	0.180 a	0.323 a	0.025 a	0.225 a	0.010b	0.085 a	0.008b	0.012 bc
M	0.587b	0.200 ab	0.520b	0.025 a	0.340b	0.005 a	0.123 a	0.000 a	0.010b
Pr > F(V)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

PART B (LS means) - **Roasting**

	II	III	IV	V	VI	VII	VIII	IX	X
T	1.133b	0.216 a	1.180b	0.032b	0.808b	0.017b	0.411b	0.005b	0.013b
C	0.374 a	0.248b	0.354 a	0.020 a	0.249 a	0.003 a	0.103 a	0.002 a	0.008 a
Pr > F(T)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.001	<0.0001
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

PART C (LS means) - **Interaction**

	II	III	IV	V	VI	VII	VIII	IX	X
S*T	1.497 e	0.237 cd	1.817 d	0.040 d	1.397 e	0.030 d	0.803 d	0.010b	0.020c
C*T	2.020f	0.273 d	2.107 e	0.050 e	1.297 d	0.020c	0.630c	0.000 a	0.010b
O*T	0.447c	0.177 a	0.327 ab	0.020b	0.230 ab	0.010b	0.103 ab	0.010b	0.013b
M*T	0.570 d	0.177 a	0.470 bc	0.020b	0.307 bc	0.010b	0.107 ab	0.000 a	0.010b
S*C	0.230 a	0.200 abc	0.263 a	0.010 a	0.220 a	0.000 a	0.120 ab	0.000 a	0.010b
C*C	0.300 ab	0.383 e	0.263 a	0.010 a	0.183 a	0.000 a	0.087 ab	0.000 a	0.000 a
O*C	0.363 bc	0.183 ab	0.320 a	0.030c	0.220 a	0.010b	0.067 a	0.007b	0.010b
M*C	0.603 d	0.223 bc	0.570c	0.030c	0.373c	0.000 a	0.140b	0.000 a	0.010b
Pr > F(V*T)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.002	0.001
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

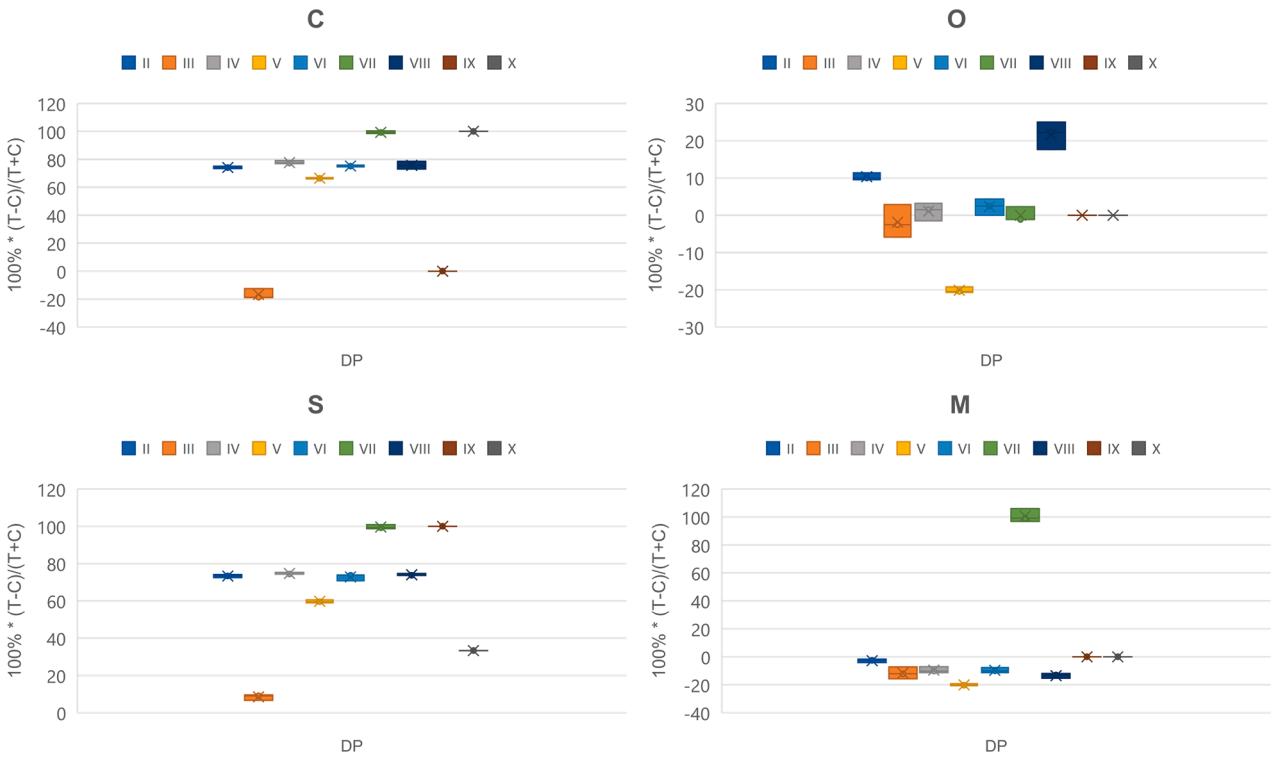

Fig. 2. Relative change (%) of PAC classes by DP depending on the variety.

and/or applied collision energy in mass spectrometry fragmentation experiments) than their non-cyclic analogues (Longo et al., 2018a; Zeng et al., 2019) might not affect the cyclic species so much as the non-cyclic congeners. Thus, GSP rich in cyclic oligomers might result less influenced by roasting. However, considering the whole PAC profile, this hypothesis might represent just a marginal effect, as the overall scarce

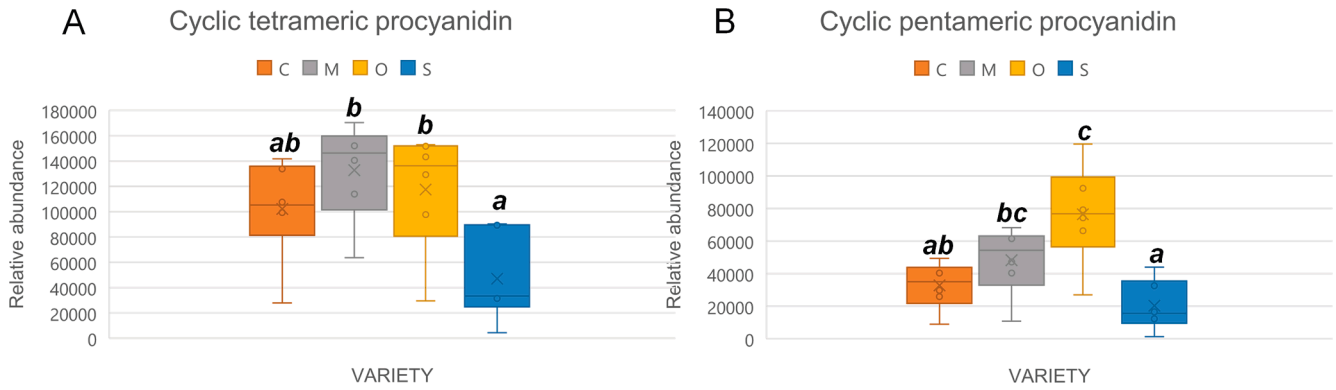

Fig. 3. c-PAC relative abundances distribution in GSP extracts according to the grape variety, reported as box plots. Italic fonts correspond to the grouping obtained from the Tukey's *post-hoc* HSD test for variety. C = Croatina, M = variety mix, O = Ortrugo, S = Sangiovese.

effectiveness of roasting on Ortrugo seeds might be due to entirely different reasons, related to the physico-chemical properties of these seeds in the first place.

These results are in sharp contrast to those obtained for the analysis of PAC by DP (Table 4). Roasting and the interaction between roasting and variety had a strong effect on almost all PAC classes (by DP), whereas no effect was present for c-PAC species. The higher resistance of c-PAC to depolymerizing conditions have been demonstrated in several studies (Longo et al., 2018a,b; Longo et al., 2019; Zeng et al., 2019) and here have been again confirmed. Finally, the total polyphenol content in these grape seed powder samples was investigated (Table 5).

The results in Table 5 highlight the overall effects of variety and roasting on the different samples. As expected, the red varieties had the highest TPC, followed by the mix and the white variety (Ortrugo). Roasting caused an increase in TPC, mirroring the observed breakdown of larger molecules with an increase in smaller molecular species. However, the interaction term was again significant due to the effects on Ortrugo seeds and mixed variety seeds. While the red varieties showed a marked increase in TPC with roasting, the Ortrugo seeds did not show any significant effect (if not even an opposite trend) from roasting (interaction term, OC vs OT), and the mix seeds showed even a significant decrease in TPC. This is in line with observations made on PAC profiles from DP and cyclic procyanidin profiles.

4. Conclusions

This study shows an account of the effects of roasting treatment and grape variety on the condensed tannin profile and total polyphenol content in grape seed powder. Roasting is a viable tool to potentially increase the effectiveness of GSP in removing proteins from wine, as it has been shown to produce a breakdown of larger condensed tannin molecules, therefore with an increase in smaller molecular species potentially available for protein binding. Overall, an actually higher PAC content in roasted GSP, largely due to the breakdown process, was only demonstrated for GSP from red grape varieties. A non-obvious or even opposite effect was observed for Ortrugo grape seeds and for grape seeds from a mix of Nebbiolo and Barbera grape varieties (80 and 20%, respectively). Herein, cyclic procyanidins were identified for the first time in grape seed powder. No significant effect of roasting was observed on their relative abundance, although grape variety was a factor influencing their profile. This evidence confirms previous findings on the greater resistance of cyclic PAC compared to their non-cyclic analogues towards chemical and physical depolymerization conditions. In particular, the cyclic pentameric procyanidin was found to have the highest relative abundance in Ortrugo GSP (a white grape variety). Finally, roasting increased the total amount of phenolic molecular species (measured by the Folin assay), which agrees with the known cleavage effect that roasting produces on larger oligomers, but only for red varieties. Once again, Ortrugo showed a completely opposite trend. Roasting has been shown to effectively increase the concentration of potentially active flavan-3-ol oligomers suitable for protein binding, especially in seeds of red varieties. The grape variety proved to be a significant factor in determining the effectiveness of the roasting process. According to the results of this study, the grape variety of the seeds proved to be an important factor in modulating the profile of condensed tannins in the seeds, in influencing the effects of the roasting treatment on the PAC, and potentially in determining how much GSP would be effective in winemaking. Therefore, the grape seed variety is an important element in deciding whether roasting should be considered or not as well as the outcomes of this study could be very useful in facilitating the application of GSP in winemaking.

Table 5
LS means ($mg \cdot g^{-1}_{eq.\ gallic\ acid}$) for TPC. Legend: S = Sangiovese; C = Croatina; O = Ortrugo; M = mix, T = treatment applied; C = control, V*T = interaction factor.

Summary (LS means) Variety		Summary (LS means) Roasting		Summary (LS means) Interaction	
Samples group	TPC ($mg \cdot L^{-1}$ eq. gallic acid)	Samples group	TPC ($mg \cdot L^{-1}$ eq. gallic acid)	Samples group	TPC ($mg \cdot L^{-1}$ eq. gallic acid)
S	6440 d	T	5382b	S*T	8039 d
C	5748c	C	3719 a	C*T	7910 d
O	2775 a			O*T	2699 a
M	3238b			M*T	2880 a
				S*C	4841c
				C*C	3787b
				O*C	2851 a
				M*C	3597b
Pr > F(V)	<0.0001	Pr > F(T)	<0.0001	Pr > F (V*T)	<0.0001
Significant	Yes	Significant	Yes	Significant	Yes

CRediT authorship contribution statement

Edoardo Longo: Conceptualization, Methodology, Formal analysis, Writing – original draft, Supervision. **Vakarė Merkytė:** Methodology, Software, Validation, Investigation, Writing – review & editing, Visualization. **Elia Romanini:** Conceptualization, Methodology, Investigation. **Milena Lambri:** Methodology, Validation, Formal analysis, Investigation, Writing – review & editing, Supervision, Funding acquisition. **Emanuele Boselli:** Resources, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2023.113826.

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