HIGH-TEMPERATURE DRYING OF RED GRAPE POMACE: EFFECTS ON THE POLYPHENOLIC COMPOSITION OF SKINS AND SEEDS

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Abstract

The management and disposal of large amounts of grape pomace (GP) annually generated by the wine industry represent a serious economic and environmental problem. On the other hand, GP can be profitably exploited for many possible purposes as a source of natural phenolic compounds.

GP is highly perishable, and it is produced in large amounts during a short period of time: a rapid stabilization by drying is needed to prolong its availability for any further processing. In the case of large amounts of GP to be dried at an industrial level, the balance between costs and final quality of the dried product must be considered.

The effect of high-temperature treatments (160-200°C) for short duration on the polyphenolic composition of GP, and on its stability over time during storage, were studied separately for skins and seeds. The high-temperature treatments caused the enrichment of the polyphenolic content of the skins and the contemporary decrease in that of the seeds. Overall, the dried GP retained over time the polyphenolic composition and the anti-radical properties determined at the end of the drying process. This result is of particular interest in view of the use of dried GP throughout the year for any further processing.

Keywords: grape pomace, drying, polyphenols, anti-radical capacity, shelf life

1. Introduction

The wine industry generates annually more than 10 Mt of grape pomace (GP) in the world (FAOSTAT, 2019), whose management and disposal represent a serious economic and environmental problem due to its seasonality and polluting characteristics. On the other hand, among agro-industrial byproducts GP is one of the richest sources of natural phenolic compounds, that are widely studied as biologically active substances in the medicine, pharmacology, and crop protection, as food preservatives to replace synthetic antioxidants, as nutraceuticals, and for many other industrial applications (natural colorants, cosmetics, etc.) [1].

GP is highly perishable due to its high moisture content and to the possible presence of residual sugars, and it is produced in large amounts during a short period of time, therefore it needs to be rapidly stabilized to prolong its availability for any further processing.

Drying can inhibit the biological activity and the chemical and physical changes that occur during storage. Given that open air and sun drying can result as time-consuming and detrimental for the final quality of dried GP, due to the high sensitivity of polyphenols to oxygen and UV light exposure [2], the drying of GP has been widely studied on a laboratory scale, with different methods: forced air oven [3], vacuum belt drying [4], infrared, convective and combined drying [5], tray drier [6], electro-hydrodynamic drying [7], heat pump drying [8].

The effect of heat drying on the polyphenolic composition of GP is not univocal. Polyphenols are heat labile, and prolonged heat treatments can irreversibly modify their chemical structure and affect their antioxidant capacity [3, 6, 9]. On the other hand, drying can increase the extractability of polyphenols from GP due to the breakdown of cellular constituents and covalent bonds in the matrix [10], and a positive effect of heating on the

extractability and antioxidant capacity of GP polyphenols was observed by other Authors [11-13]. Indeed, the time–temperature combinations of the drying process are fundamental for the preservation of polyphenols, and this topic has been widely studied [14-17].
Regarding the storage of dried GP, few studies have been published to date in the literature: [18] reported significant losses of bioactive compounds during 16 weeks of storage at 15 °C; [19] observed the increase of gallic acid content related to hydrolytic reactions, and the decrease of catechin and epicatechin contents, at either relative humidity level for long storage times.
The present research aimed at evaluating the effect of industrial-scale drying at high-temperatures for short duration on the polyphenolic composition of GP, in comparison with a lab-scale drying procedure at low temperature. Furthermore, the stability over time during storage (shelf life) of the polyphenolic composition of dried GP was assessed. The relationship between the polyphenolic content of dried skins and seeds and their antioxidant capacity was studied with the DPPH test.

2. Materials and methods

GP derived from organic Barbera grapes harvested from the same vineyard and processed with the same winemaking protocol during two consecutive vintages (2015 and 2016) at *Tre Secoli* winery (Mombaruzzo, AT, Italy). GP was sampled at the end of fermentative maceration (racking off), after soft pressing (0.5 bar).

2.1 Industrial scale drying trials

The industrial scale drying trials (ISD trials) were performed at *Geovita Srl* with a two-stage rotary paddle dryer. The two drying stages were heated by heat transfer from a diathermic liquid circulating in a heating jacket that surrounds the two cylinders. The transfer of thermal energy to the product occurred by direct contact with the hot wall maintained at a controlled temperature. Inside the two heated cylinders, the product was kept in linear and constant movement and in continuous contact with the hot wall by a rotating paddle shaft.
The heating conditions were chosen by *Geovita* among those used to dry cereal products and by-products. The process was divided into two drying stages, and temperature was applied in both drying stages, to obtain an acceptable final level of humidity/dry matter (humidity < 14%, dry matter > 86%). The % humidity was determined according to the method ISO 711:1985. The residence time of the product in the machine was approximately 2 + 2 minutes (1st and 2nd stage). The description of the different drying trials is reported in Table 1.

		Temperature (°C)		Humidity (%)	
		Stage 1	Stage 2	Inlet	Outlet
1st year	M	35		53.6	9.5
	A1	160		53.6	32.0
	A2		180	32.0	9.0
	A3	190		53.6	30.1
	A4		170	30.0	6.5
	A5	170	170	53.6	10.1
2nd year	M	35		56.4	9.5
	B1	180		56.4	36.9
	B2		160	30.0	8.0
	B3	200		55.5	30.0
	B4		160	26.5	6.5

M = lab-scale drying procedure at low temperature: air-drying in ventilated oven at 35 °C for 48 h.

ISD trials:
First year: A1 = First experiment, first stage; A2 = First experiment, second stage; A3 = Second experiment, first stage; A4 = Second experiment, second stage; A5 = Third experiment, first + second stages.
Second year: B1 = First experiment, first stage; B2 = First experiment, second stage; B3 = Second experiment, first stage; B4 = Second experiment, second stage.

Table 1. *Temperatures of the drying treatments, and average humidity values measured before and after each drying treatment.*

In the first year, the total mass of GP (2.5 tons) was divided into 3 aliquots, which were dried at different temperatures. For the first drying experiment, the two drying stages were performed at 160 °C and 180 °C, and GP was sampled at the end of each stage (trials A1 and A2). For the second experiment, the two drying stages were performed at 190 °C and 170 °C (trials A3 and A4). For the third experiment, drying was performed at the same temperature (170 °C) in the first and second stages, and only the final dried mass was sampled at the end of the second stage (trial A5).
In the second year, a total mass of 2.0 tons was treated. Two drying experiments were performed with different temperatures for the first stage — 180 °C (B1) and 200 °C (B3) —, while the temperature of the second stage was 160 °C for both experiments. The treated GP was sampled at the end of the first (trials B1 and B3) and second stages (trials B2 and B4).
The final dried product was averagely 40 % by weight of the wet GP. The processing capacity of the wet product was averagely 150 kg/h. The production capacity of the final dry product was averagely 55 kg/h. The dried GP was packaged in food-grade polyethylene big bags (1000 kg) and stored in a warehouse in normal environmental conditions (average temperature 25 °C, relative humidity 60-80%), away from heat and sunlight.
The ISD trials were compared to a lab-scale drying procedure at low temperature, performed with the same modalities in the two years: air-drying in ventilated oven at 35 °C for 48 h (M trials).

2.2 Extraction of polyphenols from GP, and characterization of the polyphenolic extracts

Dried GP was manually processed to separate skins from seeds and to obtain the respective flours by grinding (coffee grinder, 1 min). The extraction of polyphenols with ethanol:water (1:1) was performed according to a previous work [20], and the polyphenolic extracts were finally vacuum dried at 35 °C. All extractions were performed in triplicate.
The polyphenolic profile of the extracts was determined by UV-VIS spectrophotometry, with the same methods as reported by [21]. The total condensed tannins content, their mean degree of polymerization (mDP) and the percentage of each constitutive unit were determined with the phloroglucinolysis method [22], as reported by [21]. Monomer flavan-3-ols — (+)-catechin, (-)-epicatechin and (-)-epicatechin-3-*O*-gallate — were determined with the same HPLC method used for the phloroglucinolysis method, excluding the reaction with phloroglucinol.
As regards the radical scavenging activity, the EC_{20} parameter (DPPH test) was calculated according to the method proposed by [23], as reported by [21]. The data were expressed as Efficient Concentration EC_{20}, which indicates the weight of skins or seeds flour (expressed as mg DW/mg DPPH) needed to reduce by 20% the DPPH content: EC_{20} is therefore inversely proportional to the antioxidant capacity of the sample.

2.3 Shelf life of dried GP

In the first year, the stability of the polyphenolic composition and antiradical capacity of dried GP (trials M and A4) was monitored over time: the analyses were performed after the drying process (time zero) and after three and six months of storage.

In the second year, the study was limited to verifying the stability of the polyphenolic composition of dried GP after one year of storage. The stored big bags containing the dried GP (trials B2 and B4) were averagely and randomly sampled of the same amount of product; the samples were mixed and sieved to separate seeds from skins, and the two fractions were separately milled and analyzed. The results were compared to the average data of the first control of the trials B2 and B4 performed after the drying process (time zero).

3. Results

3.1 First year - Skins

At the end of each drying stage the final humidity of the samples varied according to the adopted procedure (Table 1): A1 and A3, being respectively the first drying stages of A2 and A4, still had a high moisture content at the end of the treatment. Therefore, the data regarding the polyphenolic composition of the skin flours were reprocessed referring to the flour weight at a constant 10 g/100 g humidity for all samples. Table 2 reports the normalized polyphenolic composition of the skins sampled at the end of each drying treatment: this normalization allowed to better evaluate the changes in polyphenolic content of the skins between the first and second drying stages, the latter being performed at different temperatures (180 °C for A2 and 170 °C for A4).

First year		Skins								Seeds							
		M	A1	A2	A3	A4	A5	F	sig	M	A1	A2	A3	A4	A5	F	sig
Total flavonoids (mg/g)		14.0 a[1]	33.0 b	51.1 cd	41.9 cd	54.9 d	39.4 bc	31.0	***[2]	19.5 b	12.2 ab	8.3 a	15.5 ab	9.5 a	13.7 ab	7.2	*
Proanthocyanidins (mg/g)		7.5 a	13.1 ab	14.7 bc	13.7 b	19.7 cd	21.0 d	23.3	**	16.7 b	5.4 a	3.1 a	6.5 a	3.7 a	5.1 a	51.5	***
Total polyphenols (GAE) (mg/g)		10.3 a	22.8 b	24.5 bc	26.6 bc	26.8 bc	29.0 d	48.0	***	15.6 b	6.1 a	4.6 a	7.7 a	5.2 a	6.8 a	50.2	***
Total anthocyanins (mg/g)		4.5 a	7.5 c	5.3 b	7.9 c	4.4 a	4.6 ab	131	***	0.4 ab	0.4 ab	0.4 ab	0.6 b	0.3 a	0.3 a	6.3	*
Condensed tannins (mg/g)		2.6 a	6.8 b	9.8 c	7.0 b	9.3 bc	8.6 bc	40.0	***	9.5 d	3.9 b	2.9 a	4.6 c	3.1 a	3.1 a	1378	***
mDP		6.0 c	4.6 ab	4.5 ab	5.1 b	4.6 ab	4.4 a	33.6	***	4.6 d	4.3 c	3.3 a	4.2 c	3.6 b	3.6 b	256	***
G (%)		16.9 a	16.2 a	18.9 b	16.0 a	19.5 b	19.7 b	46.1	***	22.6 e	20.1 d	18.1 a	19.5 c	19.0 b	18.8 b	426	***
Total monomer units (%)	C	28.6	27.9	24.6	26.6	23.9	25.4	4.6	ns	23.8 a	29.7 bc	32.8 d	27.8 b	31.6 cd	32.3 d	72.9	***
	EC	71.4	72.1	75.4	73.4	76.1	74.6	4.6	ns	76.2 d	70.3 bc	67.2 a	72.2 c	68.4 ab	67.7 a	72.9	***
	EGC	1.9 b	1.3 ab	1.0 a	1.7 b	0.9 a	0.9 a	12.4	***	0.4 a	0.7 b	0.9 c	0.6 b	0.9 c	0.9 c	35.4	***
Monomer flavan-3-ols (µg/g)	C	24.6 a	152.0 c	228.0 e	113.0 b	210.0 de	191.0 d	134	***	344.9 d	155.6 a	223.6 c	183.2 b	173.1 b	169.0 ab	522	***
	EC	41.5 a	99.2 b	147.9 c	75.8 b	140.7 c	128.7 c	98.0	***	250.3 c	121.4 a	161.7 b	136.9 a	129.6 a	135.1 a	196	***
	ECG	9.6 a	24.3 b	31.0 bc	30.2 bc	35.0 c	32.2 c	55.7	**	23.0 b	14.5 a	17.7 a	18.5 ab	18.0 a	15.9 a	11.9	**
EC_{20}		2.38 d	1.56 b	1.25 ab	1.91 c	1.24 ab	0.93 a	82.6	***	0.46 a	4.50 b	5.58 cd	4.12 b	5.81 d	5.33 c	670	***

Table 2 *Polyphenolic composition of the hydro-alcoholic extracts of GP skins and seeds sampled at the end of each drying treatment in the first year of the experiment. All values are referred to the dry weight (DW) of skins or seeds flour.*

C = (+)-catechin; EC = (-)-epicatechin; ECG = (-)-epicatechin-3-O-gallate; EGC = (-)-epigallocatechin.

[1] Different letters along the line discriminate the treatments significantly different from one another ($p < 0.05$, Tukey's test).

[2] Significance: *, **, *** and ns represent significance at $p \leq 0.05$, 0.01, 0.001 and not significant, respectively.

Significant differences were observed for all parameters. M had a significantly lower content in polyphenolic compounds (total polyphenols, total flavonoids and proanthocyanidins) than all ISD trials. As regards the ISD trials, at the end of the second drying stage no significant

variation of the total polyphenols content was observed with respect to the first drying stage (comparison between A1 and A2, and between A3 and A4). A5, whose first drying stage was performed at lower temperature than the first stage of A4, had a significantly higher content in total polyphenols than A4. Anthocyanins were markedly affected by the duration of drying, whatever the temperature: both the slow drying at low temperature (M) and the second ISD stages at high temperatures (A2 and A4) were particularly detrimental for the final anthocyanins content.
The most marked differences between the low and high temperature drying modes (M vs ISD trials) concerned the condensed tannins content of the skins: M had a very low concentration in condensed tannins, equal to 23-38% of the values reported for ISD trials. The condensed tannins content increased significantly in the skins sampled after the second ISD drying stage (A2 and A4).
Regarding the composition of condensed tannins, M and the first stage ISD trials A1 and A3 had a similar galloylation degree (G%), significantly lower than the values observed for A2 and A4, that were subjected to prolonged heating at high temperature. The mean degree of polymerization (mDP) dropped with increasing drying temperatures: M had a significantly higher mDP than all ISD trials. The percentage content of (-)-epigallocatechin (EGC) in the condensed tannins was very low and it dropped with prolonged heating (A2 and A4). No significant differences in the content and composition of condensed tannins were observed between the three complete ISD trials (A2, A4 and A5).
The concentrations of monomer flavan-3-ols present in free form in the skins (C, EC, ECG) were significantly higher after prolonged heating (A2 and A4); an intermediate content was observed after the first stage at high temperature (A1 and A3), while the lowest content was observed in M. C was the prevalent flavan-3-ol in all ISD trials, while EC prevailed in M. The C/EC ratio was similar for all ISD trials (between 1.48 and 1.54), while it dropped to 0.59 for M.
The EC_{20} parameter (DPPH test) was influenced by the polyphenolic content of the extracts: it was significantly higher in M than in ISD trials and, among these, it decreased with the duration of drying (with significant difference between A3 and A4). EC_{20} resulted similar for the three complete drying processes (A2, A4 and A5). EC_{20} is inversely proportional to the antioxidant capacity of the sample: a highly significant negative correlation was observed between EC_{20} and the polyphenolic content, particularly proanthocyanidins, total polyphenols (GAE) and condensed tannins (data not reported).

3.2 First year - Seeds

The highest concentrations of polyphenolic compounds were observed in the seeds derived from GP dried at low temperature (M) (Table 2). M had a significantly higher content of proanthocyanidins and total polyphenols than all ISD trials, among which no significant differences were observed for these parameters. M had the highest total flavonoids content, significantly different only from A2 and A4 (lowest concentrations), and the highest condensed tannins content. In contrast to what was observed for the skins, the condensed tannins content of the seeds dropped significantly with high temperatures and in proportion to the duration of drying (A1>A2 and A3>A4). Conversely, no significant differences were observed in the content of total flavonoids, total polyphenols, proanthocyanidins and condensed tannins of the seeds subjected to the three complete drying processes (A2, A4 and A5). As regards the composition of condensed tannins, M had the highest G% value, followed by A1 and A3 (one drying stage), and then by A4 and A2 (two drying stages: longer permanence at high temperatures). M had the significantly highest mDP, followed in decreasing order by A1 and A3 (first drying stages), A4 and A5 (two drying stages), and finally A2 with the lowest value (two drying stages). EC was the main monomer unit of

condensed tannins, and it was higher in M and in the ISD trials sampled after the first drying stage. The conditions of the drying process caused modest but significant variations in the composition of condensed tannins, in particular G% and mDP: both parameters were lower in A2, subjected to a second drying stage at higher temperature than the other two trials (A4 and A5).

Modest concentrations of total anthocyanins and EGC — a monomer unit only present in skins tannins — were observed in the seeds: these molecules were absorbed by seeds during fermentative maceration.

Regarding monomer flavan-3-ols, the concentrations of C, EC, and ECG were significantly higher in M than in ISD trials. C was the most abundant flavan-3-ol and the C/EC ratio was constant (1.3-1.4), regardless of the drying method adopted. Among the three drying processes, the flavan-3-ols content was significantly higher in A2, whose condensed tannins had the lowest mDP, than in A4 and A5.

As regards the EC_{20} parameter (DPPH test), it was significantly lower for M than for ISD trials, among which it tended to increase significantly with the duration of the drying process (significant differences between A1 and A2 and between A3 and A4). As observed for skins, a highly significant negative correlation was observed between EC_{20} and the polyphenolic content of the seed extracts (data not reported). The absolute values of the correlation coefficients between EC_{20} and the total polyphenols, proanthocyanidins and condensed tannins content of the seeds were higher than those observed for the skins. According to our previous work [24], the highest correlation coefficient was observed between EC_{20} and the condensed tannins content (r = 0.99).

3.3 Second year - Skins

Table 3 reports the polyphenolic composition of the skins sampled at the end of each drying stage. As for the first year, the data regarding the polyphenolic composition of the skin flours were normalized at a constant 10% humidity for all samples.

Second year		Skins							Seeds						
		M	B1	B2	B3	B4	F	sig	M	B1	B2	B3	B4	F	sig
Total flavonoids (mg/g)		18.5 a[1]	30.9 b	34.1 b	30.2 b	48.3 c	173	***[2]	30.6 c	7.9 ab	6.0 a	9.7 b	6.3 a	135	***
Proanthocyanidins (mg/g)		8.6 a	14.0 b	15.6 b	13.6 b	17.8 c	282	***	18.7 d	5.1 b	4.1 a	6.6 c	4.1 a	153	***
Total polyphenols (GAE) (mg/g)		11.6 a	17.8 b	19.0 ab	19.2 bc	21.8 c	100	***	17.6 b	7.0 a	4.9 a	7.4 a	5.3 a	57	***
Total anthocyanins (mg/g)		6.0 a	9.8 c	7.2 b	8.3 b	7.1 ab	45.6	***	0.3 b	0.1 a	0.1 a	0.1 a	0.1 a	51.1	***
Condensed tannins (mg/g)		2.1 a	4.5 b	4.8 b	4.6 b	5.9 c	647	***	9.0 c	3.7 b	2.1 a	3.8 b	2.3 a	66	***
mDP		5.0 a	5.7 c	5.3 b	5.2 ab	5.5 bc	34.4	***	4.8 c	3.6 b	3.1 a	3.8 b	3.3 a	146	***
G (%)		15.7 a	17.1 b	17.1 b	17.7 b	19.0 c	28.9	***	21.3 c	18.1 b	15.9 a	18.4 b	16.4 a	60.7	***
Total monomer units (%)	C	29.9	28.3	29.5	30.8	28.4	1.5	ns	24.4 a	30.1 b	32.6 c	29.6 b	32.6 c	80.7	***
	EC	70.1	71.7	70.5	69.2	71.6	1.5	ns	75.6 c	69.9 b	67.4 a	70.4 b	67.4 a	80.7	***
	EGC	1.1 ab	1.3 b	0.9 ab	0.8 ab	0.7 a	8.4	*	0.2 a	0.4 bc	0.4 c	0.3 b	0.4 bc	39.1	***
Monomer flavan-3-ols (μg/g)	C	17.6 a	94.5 b	95.0 b	105.6 b	125.6 b	42.6	***	193.3 b	195.8 b	143.4 a	171.8 ab	151.9 a	11.2	**
	EC	35.6 a	71.0 b	82.0 b	75.0 b	88.1 b	43.3	***	225.0 d	190.1 c	139.3 a	173.9 bc	145.7 ab	39.7	***
	ECG	traces							17.4 b	15.5 ab	12.9 a	18.2 b	15.7 ab	13.3	**

Table 3 *Polyphenolic composition of the hydro-alcoholic extracts of GP skins and seeds sampled at the end of each drying treatment in the second year of the experiment. All values are referred to the dry weight (DW) of skins or seeds flour.*

C = (+)-catechin; EC = (-)-epicatechin; ECG = (-)-epicatechin-3-O-gallate; EGC = (-)-epigallocatechin.

[1] Different letters along the line discriminate the treatments significantly different from one another ($p < 0.05$, Tukey's test).

[2] Significance: *, **, *** and ns represent significance at $p \leq 0.05$, 0.01, 0.001 and not significant, respectively.

M had significantly lower contents of total anthocyanins, total polyphenols (GAE), total flavonoids and proanthocyanidins than ISD trials. The anthocyanins content was significantly higher in the first stage ISD trials (B1 and B3), while it dropped significantly (B2) and not significantly (B4) after the second drying stage. The observed trend was the same as in the first year.
The ranking of the trials based on the condensed tannins content was the same as observed for the other polyphenolic indices. The differences between M and the other trials were quantitatively marked: M contained the 36-47% of the tannins content measured in ISD trials.
B4 skins, subjected to higher temperature during the first drying stage, had a higher content in total polyphenols, proanthocyanidins and condensed tannins than B2 skins. As regards the composition of condensed tannins, G% was significantly higher in ISD trials, particularly in B4, than in M; statistically significant but quantitatively small differences were also observed for mDP, which was lower in M. Conversely, no significant differences were observed in the percentage weights of the main monomer units (C and EC) of condensed tannins.
Monomer flavan-3-ols (C and EC; ECG was only present in traces) were significantly more abundant in the second stage ISD trials than in the first stage ones (B2 > B1, and B4 > B3), while M had the lowest concentration, as observed in the first year. C was prevalent in all trials except M. The C/EC ratio ranged between 1.16 and 1.43 for the ISD trials, while it dropped to 0.50 for M.

3.4 Second year - Seeds

The seeds dried at low temperature (M) had higher concentrations of polyphenolic compounds (total polyphenols, proanthocyanidins, total flavonoids and condensed tannins) than the seeds of all ISD trials (Table 3). No significant differences in polyphenolic composition were observed between the two complete ISD processes (B2 and B4).
The proanthocyanidins and condensed tannins content decreased significantly with prolonged heating (B1 > B2 and B3 > B4). The same trend was observed for the total polyphenol content (GAE), but without statistical significance.
Regarding the composition of condensed tannins, M had the significantly highest mDP and G%. In ISD trials, mDP and G% dropped with prolonged heating. Highly significant differences were observed for the percentage weight of the monomer units of condensed tannins. The prevailing monomer unit in all trials was EC, whose percentage weight dropped at high temperatures and with prolonged heating; the opposite trend was observed for C. The percentage content of ECG increased with high temperatures and with prolonged heating. As already reported for the first year, modest concentrations of total anthocyanins and EGC, absorbed by seeds during fermentative maceration, were observed.
The monomer flavan-3-ols content in the seeds was higher than in the skins. The average concentration of C, EC and ECG was lower in B2 and B4, intermediate in B3, and higher in M and B1. The content of C and EC was equivalent in all ISD trials (C/EC ratio = 1.0), while in M slightly prevailed EC (C/EC ratio = 0.9).

3.5 Shelf life of dried GP

First year. The effect of storage on the polyphenolic composition of dried GP was studied with one-factor ANOVA and Tukey's test. The results are reported in Table 4.

		Skins								Seeds							
		M			A4					M			A4				
		Time 0	3 months	6 months	Time 0	3 months	6 months	F	Sig	Time 0	3 months	6 months	Time 0	3 months	6 months	F	Sig
Total flavonoids (mg/g)		14.0 a[1]	16.4 ab	17.8 b	54.9 d	34.9 c	33.1 c	943	***[2]	19.5 b	32.3 c	28.6 c	9.5 a	5.2 a	5.6 a	143	***
Proanthocyanidins (mg/g)		7.5 a	8.8 a	9.5 a	19.7 b	21.3 b	21.9 b	146	***	16.7 b	20.4 c	19.7 bc	3.7 a	3.1 a	4.4 a	206	***
Total polyphenols (GAE) (mg/g)		10.3 a	13.2 b	14.5 b	26.8 c	27.8 c	25.5 c	281	***	15.6 b	22.6 c	20.6 c	5.2 a	5.2 a	5.7 a	298	***
Total anthocyanins (mg/g)		4.5 a	6.0 b	6.2 b	4.4 a	4.5 a	4.5 a	196	***	0.4	0.5	0.4	0.3	0.2	0.2	3.7	ns
Condensed tannins (mg/g)		2.6 a	2.8 a	3.4 a	9.3 b	10.3 b	9.9 b	86.1	***	9.5 b	9.3 b	11.1 c	3.1 a	2.6 a	2.4 a	975	***
mDP		6.0 b	4.9 a	6.2 b	4.6 a	4.6 a	4.9 a	56.9	***	4.6 e	3.9 d	4.0 d	3.6 c	3.0 b	2.5 a	589	***
G (%)		16.9 b	18.3 c	12.5 a	19.4 cd	19.5 cd	20.4 d	146	***	22.6 d	19.4 b	20.5 c	19.0 b	16.5 a	15.8 a	258	***
Total monomer units (%)	C	28.6 bc	29.5 c	29.0 c	23.9 ab	23.6 a	23.0 a	13.6	**	23.8 a	26.0 ab	26.8 b	31.6 c	35.6 d	39.3 e	161	**
	EC	71.4 ab	70.5 a	71.0 a	76.0 bc	76.4 c	77.0 c	13.6	**	76.2 e	74.0 de	73.2 d	68.4 c	64.4 b	60.7 a	161	***
	EGC	1.8 bc	2.4 cd	3.0 d	0.9 a	1.0 a	1.3 ab	57.9	***	0.3 b	0.5 c	0.2 a	0.9 e	1.4 f	0.7 d	1127	***
Monomer flavan-3-ols (μg/g)	C	24.6 a	23.8 a	26.1 a	209.9 d	140.7 c	91.2 b	106	***	344.9 d	309.2 c	352.5 d	173.1 a	188.2 a	252.0 b	168	***
	EC	41.5 a	35.5 a	31.5 a	140.7 c	150.5 c	100.0 b	139	***	250.3 c	295.4 d	321.8 d	129.6 a	146.0 ab	175.2 b	173	***
	ECG	9.6 a	10.5 a	50.0 c	35.0 b	26.2 b	29.6 b	38.0	**	23.0 b	26.1 b	35.1 c	18.0 a	16.2 a	25.1 b	58.3	***
EC_{20}		2.40 b	2.97 c	2.23 b	1.24 a	1.21 a	1.00 a	84.3	***	0.46 a	1.14 a	1.11 a	5.60 b	5.44 b	5.70 b	303	***

Table 4 *Polyphenolic composition of skins and seeds extracts during six months of storage in the first year of the experiment. All values are referred to the dry weight (DW) of skins or seeds flour.*

C = (+)-catechin; EC = (-)-epicatechin; ECG = (-)-epicatechin-3-O-gallate; EGC = (-)-epigallocatechin.

[1] Different letters along the line discriminate the treatments significantly different from one another ($p < 0.05$, Tukey's test).

[2] Significance: *, **, *** and ns represent significance at $p \leq 0.05$, 0.01, 0.001 and not significant, respectively.

As regards skins, the overall polyphenolic content (total flavonoids, proanthocyanidins and GAE) remained higher in A4 than in M. In the case of A4 most of the parameters remained unchanged, excluding a decrease in total flavonoids observed only after the first sampling, possibly due to the influence of other molecules than polyphenols with an absorption at 280 nm (e.g., oxidized polymers without a specific maximum wavelength). In the case of M, a higher variability in the polyphenolic profile was observed over time: the total anthocyanins and flavonoids content increased, and the trend of G% was not univocal, probably due to the heterogeneity of composition of the stored samples.

As regards seeds, the overall polyphenolic content (total flavonoids, proanthocyanidins and GAE) remained higher in M than in A4. No losses in polyphenolic compounds during storage were observed in both trials. As observed for skins, in the case of A4 most of the parameters remained unchanged, while a higher variability was observed for M.

The condensed tannins content remained stable over time in skins and seeds of both trials. Modest but significant differences were observed in the monomer composition of condensed tannins. The mDP of seeds tannins tended to decrease, particularly in the case of A4.

As regards the monomer flavan-3-ols content (C and EC), a significant decrease over time was observed only for A4 skins, while in the case of seeds it tended to increase for both trials.

EC_{20} (DPPH test) was higher for A4 skins than for M skins; the opposite was observed for seeds. The EC_{20} values remained stable during storage, excluding some modest variations limited to M skins, probably due to the variability of the plant material.

Second year. Overall, modest changes were observed in the polyphenolic profile of dried GP after one year of storage (Table 5).

		Skins				Seeds			
		T0	T12	F	sig	T0	T12	F	sig
Total flavonoids (mg/g)		41.2 a[1]	31.3 a	2.5	ns[2]	6.1 a	5.4 a	5.0	ns
Proanthocyanidins (mg/g)		16.7 b	12.1 a	20.3	*	4.1 b	2.9 a	56.7	**
Total polyphenols (GAE) (mg/g)		20.4 a	18.9 a	1.5	ns	5.1 a	5.0 a	0.2	ns
Total anthocyanins (mg/g)		7.2 b	6.1 a	54.5	**	0.1 a	0.3 b	157	***
Condensed tannins (mg/g)		5.4 a	5.5 a	0.06	ns	2.2 a	2.2 a	0.00	ns
mDP		5.4 b	5.1 a	13.5	*	3.2 a	3.3 a	1.6	ns
G (%)		18.1 a	17.1 a	1.4	ns	16.2 b	15.4 a	8.3	*
Total monomer units (%)	C	28.9 b	25.9 a	13.6	*	32.6 a	32.8 a	0.5	ns
	EC	71.1 a	74.1 b	13.6	*	67.4 a	67.2 a	0.5	ns
	EGC	0.8 a	2.9 b	404	***	0.4 a	4.7 b	288	***
Monomer flavan-3-ols (μg/g)	C	110.3 b	49.7 a	190	*	147.6 b	128.8 a	19.6	*
	EC	85.0 b	69.4 a	31.0	**	142.5 b	113.6 a	62.9	**
	ECG	traces				14.3 b	7.2 a	35.0	**

Table 5. *Polyphenolic composition of skins and seeds extracts at time zero and after one year of storage in the second year of the experiment. All values are referred to the dry weight (DW) of skins or seeds flour.*

T0 = average data of the first control of the trials B2 and B4 performed after the drying process (time zero).
T12 = trials B2 and B4 averagely and randomly sampled of the same amount of product after 12 months of storage.
C = (+)-catechin; EC = (-)-epicatechin; ECG = (-)-epicatechin-3-O-gallate; EGC = (-)-epigallocatechin.

[1] Different letters along the line discriminate the treatments significantly different from one another ($p < 0.05$, Tukey's test).

[2] Significance: *, **, *** and ns represent significance at $p \leq 0.05$, 0.01, 0.001 and not significant, respectively.

As regards skins, a significant decrease was observed in the content of proanthocyanidins, total anthocyanins and flavan-3-ols. The condensed tannins content remained unchanged; regarding their composition, a modest but significant decrease in the average size (mDP) and in the percentage weight of some monomer units was observed.
As regards seeds, the content of proanthocyanidins, total anthocyanins and flavan-3-ols significantly decreased. The condensed tannins content remained unchanged, as observed for the skins, and a significant decrease of G% was observed.

4. Discussion

The polyphenolic composition of dried GP was strongly influenced by the different drying conditions. The mild effect of the slow and low-temperature drying procedure (M) preserved the proportions between skins and seeds polyphenols generally observed in unfermented grapes, as observed in a previous work [24], similarly to the mild effect of freeze-drying reported by other authors [25, 26]: dried seeds resulted averagely richer in total polyphenols, proanthocyanidins, condensed tannins and flavan-3-ols than dried skins, while skins had higher concentrations of anthocyanins and higher mDP of condensed tannins.
Conversely, the effect of high-temperature drying on the polyphenolic composition of skins and seeds was not univocal. In the case of skins, all high-temperature treatments (ISD trials) caused a general increase in polyphenolic content compared to the low-temperature treatment (M). The positive effect of heating on the polyphenolic content of skins was evident and repeatable over two years. Regarding the composition of skins tannins, G% increased with prolonged heating, while minor variations were observed in the percentage weight of the monomer unities. Differently, the effect of heating on the mDP of the skins tannins was not univocal in the two years: compared to M, a significant decrease of mDP in the ISD trials

was observed in the first year (-23%, averagely), while a slight increase was observed in the second year (+8%, averagely).
The positive effect of drying at high temperature for short duration on the extractability of polyphenols from GP was already observed by other Authors. In a study on the Accelerated Solvent Extraction (ASE) of phenolic compounds from GP, [13] observed that high temperatures enhanced the extraction of phenolics; moreover, a drying pretreatment at 45 °C increased the antiradical activity of the extracts with respect to the wet pomace: highly bioactive polyphenols were extracted due to the drying effect on GP, and to the high temperature ASE process. The heat-induced extraction of bioactive high MW polyphenols from grape seeds was observed by [12] at temperatures > 120 °C; conversely, temperatures ≥ 180 °C and longer heating times caused significant decreases in polyphenols content and antioxidant activity. [14] reported that mild thermal treatments (50-100 °C for a short duration) may enhance the extractability of phenolics from grape seeds and the antioxidant activity of the extracts. [16] reported that 90% of the active compounds may be preserved up to 150 °C if the processing time does not exceed 1 min at this temperature. [11] studied the extrusion processing of GP to enhance the monomer and dimer procyanidins contents at the expense of high MW procyanidin oligomers and polymers in grape seed and pomace: the highest increase in monomers content was observed at 170 °C.
In the present work, we observed in the ISD skins of both years a marked increase in condensed tannins (as well as proanthocyanidins, measured by spectrophotometry) and monomer flavan-3-ols compared to M skins. As reported by other Authors [3, 11, 14], this result may be due to the contemporary action of two different effects of heating: the increased extractability of polyphenols due to the disruption of the GP matrix on one side, and the depolymerization of high MW tannins on the other side. In other words, the thermal degradation of some phenolics compounds may be balanced by the release of bound phenolic compounds from the matrix. In our case, the depolymerizing effect may be confirmed by the increase in flavan-3-ols content, rather than by the variation of mDP of the skins tannins which was not univocal in the two years.
The galloylation degree (G%) of skins tannins increased significantly with heating: in both years, G% was significantly higher in the second stage ISD trials than in M. Considering that skins tannins have generally a lower proportion of galloylated subunits than seeds tannins [27], the observed increase could be due to a migration of polyphenols from seeds to skins, due both to the disruption of the seeds matrix and to the higher adsorbing surface of the skins. Given that condensed tannins are the prevalent polyphenols in the seeds, the same conclusion can be reached considering that the complete two-stages high-temperature treatments caused in the skins a more marked increase in flavan-3-ols and condensed tannins than in other polyphenolic compounds.
As regards anthocyanins, the observed trend was the opposite with respect to the other polyphenolic compounds. Anthocyanins were particularly affected by drying, whatever the temperature: in both years, the lowest anthocyanins concentrations were observed in the slow drying trial at low temperature (M) and in the second stage ISD trials at high temperatures. In all ISD trials the anthocyanins content was significantly higher after the first drying stage, and it dropped after the second drying stage. In the second year the highest anthocyanins concentration was observed in B1 (performed at lower temperature than B3). The reasons for this evidence may be two-fold: a possible prolonged effect of non-inactivated polyphenol oxidase (PPO) in the case of M, and an excessive exposure to high temperatures in the case of the second stage ISD trials. In general, 60 °C are sufficient to inactivate the residual PPO [17, 28], but M trial was dried at 35 °C, allowing PPO to prolong their effect during drying. Regarding ISD trials, the thermal instability of anthocyanins is widely reported in literature: various Authors indicate a threshold

temperature of 40-60 °C, over which anthocyanins are subjected to rapid degradation [3, 9, 29].
As regards seeds, the observed trend was the opposite of what reported for skins: the highest concentrations of polyphenolic compounds were observed in M seeds, and the repeatability of the results was better than in the case of skins. In both years, M seeds had significantly higher concentrations of proanthocyanidins, total polyphenols, total flavonoids, condensed tannins and flavan-3-ols than all ISD trials. Among ISD trials, the polyphenolic content decreased during the process: the lowest values were observed at the end of the second drying stage. Regarding the composition of seeds tannins, M had the significantly highest mDP and G%. In ISD trials, mDP and G% dropped with prolonged heating.
Considering ISD trials overall, the polyphenolic content was averagely lower in the seeds than in the skins: this difference from the proportions between skins and seeds polyphenols generally observed in grapes may confirm the hypothesized migration of polyphenols from seeds to skins due to high-temperature drying. It is interesting to notice that for both skins and seeds, in both vintages, the ISD tannins had a lower mDP than M tannins, regardless of the overall polyphenolic content.
To our knowledge, the behavior of grape skins and seeds subjected together to high-temperature drying observed in this work has not been reported to date in the literature. Generally, GP is always dried and milled as a whole, or only the seeds are considered. In our case, skins and seeds were dried together, then they were processed separately. This approach allowed to observe this migration of polyphenols from seeds to skins due to the combined effect of high temperature (enhanced extraction, depolymerization) and higher adsorbing surface of the skins.
The available results do not allow us to define the optimal thermal conditions for high-temperature drying, which was not the purpose of the experience. Only in some cases, particularly during the second year, an increase in the polyphenolic content of the skins was observed when the first drying stage was performed at higher temperatures. Having verified the effectiveness of high-temperature drying on the extraction of phenolic compounds from GP, the optimization of the thermal conditions of the process will be the focus of further research.
Compared to low-temperature drying, the high-temperature treatments did not cause any appreciable variations in the color and odor of GP which, in both cases, were reminiscent of those of raisins. The treatment with high temperatures also led to a modification of the consistency of GP, which resulted more friable, allowing the easier separation of the skins from the seeds.
As regards the compositional stability of dried GP during storage, both skins and seeds retained a large part of the initial polyphenolic content, with few exceptions: a significant loss of flavan-3-ols was observed in both years in ISD skins, and a significant loss of proanthocyanidins and flavan-3-ols was observed only in the second year in ISD seeds. These differences were probably due to residual oxidation or hydrolysis reactions occurred in the stored plant material. Conversely, EC_{20} (DPPH test) did not significantly vary during storage. [15] reported that the changes in polyphenolic content observed after thermal treatments of grape seed extracts and GP were not related with changes in antioxidant activity.

5. Conclusions

Given the pros and cons of heat drying, the industrial-scale drying of GP should consider costs, benefits, and final quality of the dried product, taking into account the target polyphenols to be preserved: the process yield (amount of treated product vs duration of the

treatment) depends on the balance between the maximum possible operating temperature and the respect of the bioactive compounds.
In our study, the high-temperature treatments caused the enrichment of the polyphenolic content of the skins and the contemporary decrease in that of the seeds, compared to the trials dried at low temperature, and this may suggest that high temperature favored the extraction and the following adsorption on the skins of a part of the polyphenolic content of the seeds. This result is original because to our knowledge it has never been reported in the literature.
Overall, the dried GP retained over time the polyphenolic composition and anti-radical properties determined at the end of the drying process. Considering that the production of GP is concentrated in a period of about 30-40 days close to the harvest and winemaking period, this result is of particular interest in view of the use of dried GP throughout the year for any further processing.

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