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Winemaking by-products as a source of phenolic compounds: Comparative study of dehydration processes

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ABSTRACT

The circular bioeconomy for the production of high-value products has gained attention due to new policies for the reuse and sustainable valorisation of locally available underutilised raw material in several countries. This study is focused to explore the potential of the by-products of winemaking through direct dehydration processes. The phenolic composition (anthocyanins and uncolored phenols) and their quantitative pattern in the skin, seeds and stems were determined by ultra-high performance liquid chromatography with tandem mass spectrometry (UHPLC-QqQ-MS/MS). The results showed that lyophilisation and mainly the Spiral Flash dryer, as the most feasible system for industrial application, could be a promising process for producing high added-value ingredients with retention of bioactive phenolic compounds. The skin fraction is a rich source of anthocyanins. In a complementary way, the seeds are an important source of hydroxybenzoic acids, procyanidins and lignans, a family of phenolic compounds little studied in grapes and wine. Regardless of the dehydration method, the stems are an important source of a wide range of phenolic compounds, mainly proanthocyanidins. The present study established that the winemaking process provides an excellent source of raw materials for the recovery of ingredients rich in valuable phenolic compounds, thus contributing to the circular bioeconomy.

1. Introduction

In recent years, the circular bioeconomy for the production of high-value products has gained attention due to new policies on the reuse and sustainable valorisation of locally-available underutilised raw materials in several countries (Spekreijse et al., 2019). In addition, the demand for natural ingredients has led manufacturers to search for cheap and easy technologies to stabilize raw materials. World wine production in 2020 was around 260 million hectolitres (mhl). In Europe, the production of Italy (49.1 mhl), France (46.6 mhl) and Spain (40.7 mhl) together accounts for 53% of the wine produced worldwide, with a strong increase over 2019 (OIV, 2021). During the wine processing chain, about 9 million tonnes of waste are generated, the disposal of which has great environmental impact. These wastes are basically vine shoots, stems, grape pomace, lees and spent filter cakes (Beres et al., 2017). In the first stage of winemaking, the residues produced during the destemming and crushing processes are the grape stems and the grape pomace, respectively, the latter composed of a mixture of skin and seeds and residual stems (Spigno et al., 2017). Grape pomace is the main fraction of the solid waste from winemaking, being up to 60% by weight and 20%–25% of the grapes received, while grape stems represent about 14% by weight of the total solid waste (Hogervorst et al., 2017).

The use of the by-products from winemaking is an urgent issue in Europe. Due to the lack of alternative uses with economic benefits, these products have long been undervalued. Conventionally, part of the waste was destined for use as fertilizer or animal feed (García-Lomillo & González-San José, 2017). Other alternatives have led to the use of wine pomace in distilleries to produce a wide range of products including ethanol, anthocyanins, tartrate and grape seed oil (Maier et al., 2009; Rondeau et al., 2013). Over recent years, research related to the treatment and disposal of wine making waste has led to valuable by-products being obtained. Recent studies have drawn attention to these by-products as a good opportunity for the recovery of value-added antioxidant compounds, with potential applications as nutraceuticals and functional food ingredients (Alonso et al., 2002; Barcia et al., 2014; Beres et al., 2017; Silva et al., 2018).

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Table 1
Impact of drying processes on the anthocyanin and non-coloured phenols contents of the grape skins from by-products obtained from conventional fermentation (CF) and carbonic maceration (CM) winemaking, respectively. Results are expressed as mean (mg/kg) ± standard deviation ($n = 10$).

Compound (mg/kg)[a]	Grape skin from conventional fermentation (CF) winemaking					Grape skin from carbonic maceration (CM) winemaking				
	Fresh (dry weight)	Lyophilisation	Air-drying 40 °C	Air-drying 60 °C	Spiral Flash	Fresh (dry weight)	Lyophilisation	Air-drying 40 °C	Air-drying 60 °C	Microwave
Total Malvidins	1936 ± 157 a	1973 ± 109 a	1798 ± 3.24 a	865 ± 63.0 b	1929 ± 69.1 a	2129 ± 89.2 a	1898 ± 0.99 b	1519 ± 33.8 c	1104 ± 10.0 d	301 ± 11.9 e
Total Petunidins	365 ± 2.84 a	312 ± 12.1 b	304 ± 1.52 b	120 ± 0.37 c	334 ± 34.6 ab	298 ± 28.3 ab	317 ± 31.7 a	263 ± 1.10 b	157 ± 8.99 c	42.0 ± 2.09 d
Total Delphinidins	456 ± 13.9 b	427 ± 24.1 bc	403 ± 8.89 c	125 ± 3.78 d	494 ± 3.27 a	404 ± 19.3 b	481 ± 6.18 a	298 ± 4.90 c	171 ± 2.80 d	51.9 ± 2.09 e
Total Peonidins	282 ± 0.79 a	176 ± 4.17 c	204 ± 2.53 b	91.2 ± 0.15 d	94.4 ± 2.19 d	538 ± 63.0 a	458 ± 12.6 b	307 ± 0.45 c	294 ± 10.9 c	87.5 ± 5.08 d
Total Cyanidins	57.5 ± 3.41 a	53.4 ± 1.55 a	55.4 ± 1.04 a	18.6 ± 0.25 c	25.8 ± 0.22 b	60.8 ± 6.82 b	92.0 ± 0.50 a	51.4 ± 0.73 c	41.5 ± 0.44 d	10.3 ± 0.40 e
Pelarg-3-G-6-Gluc	0.68 ± 0.02 a	0.32 ± 0.01 b	0.32 ± 0.01 b	0.18 ± 0.01 c	0.06 ± 0.00 d	1.33 ± 0.23 a	1.34 ± 0.07 a	0.95 ± 0.05 b	0.87 ± 0.09 b	0.37 ± 0.04 c
Total Vitisins	10.0 ± 0.25 c	14.3 ± 0.88 a	12.3 ± 0.16 b	9.82 ± 0.78 c	9.51 ± 0.25 c	9.13 ± 1.38 b	11.6 ± 0.14 a	9.59 ± 0.07 b	7.49 ± 0.07 c	2.40 ± 0.23 d
Total Anthocyanins	**3107 ± 138 A**	**2955 ± 152 AB**	**2777 ± 10.9 B**	**1229 ± 68.4 C**	**2887 ± 29.1 AB**	**3441 ± 68.2 A**	**3259 ± 26.6 B**	**2449 ± 39.6 C**	**1776 ± 33.3 D**	**495 ± 6.10 E**
Total Hydroxycinnamic acids	36.1 ± 0.79 c	73.0 ± 3.21 b	66.5 ± 0.68 b	67.9 ± 0.64 b	134 ± 11.3 a	37.0 ± 1.57 e	73.3 ± 1.93 b	60.3 ± 0.17 d	68.4 ± 2.65 c	97.6 ± 1.76 a
Total Hydroxybenzoic acids	118 ± 0.12 e	132 ± 3.76 d	238 ± 2.99 c	307 ± 1.00 a	270 ± 9.72 b	117 ± 5.07 e	132 ± 2.73 d	218 ± 3.55 c	302 ± 6.54 b	378 ± 0.90 a
Total Phenolic acids	154 ± 0.90 E	205 ± 6.97 D	305 ± 2.31 C	375 ± 1.64 B	404 ± 21.0 A	154 ± 6.64 E	206 ± 0.80 D	278 ± 3.71 C	371 ± 9.19 B	476 ± 2.66 A
Total Phenyl alcohols	2.88 ± 0.11 C	3.89 ± 0.11 A	2.88 ± 0.05 C	2.80 ± 0.05 C	3.42 ± 0.06 B	2.61 ± 0.10 C	3.41 ± 0.11 A	3.14 ± 0.15 AB	2.87 ± 0.08 BC	3.19 ± 0.10 A
Total Isorhamnetin	23.8 ± 0.04 b	22.2 ± 0.17 c	24.1 ± 0.06 b	16.7 ± 0.18 d	32.0 ± 0.65 a	31.0 ± 1.14 a	27.9 ± 0.22 b	27.0 ± 0.60 b	27.4 ± 0.98 b	21.4 ± 0.09 c
Total Kaempferol	42.2 ± 0.74 ab	42.7 ± 0.90 a	39.0 ± 2.65 bc	38.0 ± 0.13 c	38.1 ± 1.00 c	38.2 ± 0.42 b	41.5 ± 1.53 a	40.3 ± 0.33 ab	41.5 ± 1.05 a	33.3 ± 1.22 c
Total Myricetin	53.1 ± 1.08 c	64.9 ± 3.55 b	64.5 ± 1.45 b	48.1 ± 2.58 c	129 ± 5.18 a	56.8 ± 1.13 bc	73.4 ± 0.59 a	57.4 ± 0.85 b	54.8 ± 0.96 bc	54.5 ± 1.48 c
Total Quercetin	367 ± 16.4 c	415 ± 5.01 b	420 ± 1.88 b	329 ± 5.88 d	578 ± 26.8 a	377 ± 7.60 b	413 ± 8.18 a	420 ± 11.7 a	407 ± 2.41 a	361 ± 17.0 b
Total Laricitrin	16.4 ± 0.07 d	19.7 ± 1.06 bc	20.5 ± 0.43 b	18.2 ± 1.05 cd	24.8 ± 0.56 a	16.5 ± 0.52 b	18.7 ± 0.38 a	20.5 ± 0.24 a	19.4 ± 0.83 a	15.8 ± 1.48 b
Total Syringetin	27.3 ± 0.25 d	35.6 ± 2.02 b	32.7 ± 0.66 c	35.5 ± 1.15 bc	42.6 ± 0.41 a	31.4 ± 0.26 c	29.6 ± 0.49 c	36.8 ± 0.31 ab	39.0 ± 2.11 a	34.7 ± 0.22 b
Total Astilbins	1.12 ± 0.04 b	1.49 ± 0.15 b	2.08 ± 0.24 a	1.88 ± 0.03 a	1.26 ± 0.14 b	1.20 ± 0.16 d	2.64 ± 0.05 b	2.33 ± 0.19 b	3.32 ± 0.24 a	1.78 ± 0.16 c
Total Flavonols	531 ± 18.1 C	602 ± 12.9 B	603 ± 0.62 B	487 ± 1.44 C	845 ± 34.7 A	552 ± 8.64 B	607 ± 9.29 A	604 ± 13.4 A	592 ± 4.21 A	523 ± 18.5 B
Total Catechin derivates	18.8 ± 2.34 d	35.2 ± 1.33 c	191 ± 2.44 b	273 ± 2.45 a	187 ± 8.72 b	12.0 ± 0.50 e	34.2 ± 1.57 d	164 ± 0.56 c	220 ± 2.80 b	368 ± 3.91 a
Total Procyanidins	67.8 ± 6.45 c	66.2 ± 0.82 c	237 ± 11.4 b	298 ± 11.6 a	220 ± 8.38 b	47.2 ± 0.62 c	62.9 ± 4.54 c	172 ± 0.12 b	253 ± 22.6 a	237 ± 15.2 a
Total Proanthocyanidins	86.6 ± 8.79 C	101 ± 0.50 C	428 ± 9.30 B	571 ± 14.1 A	406 ± 17.1 B	59.2 ± 1.13 E	97.1 ± 2.97 D	336 ± 0.43 C	473 ± 25.4 B	605 ± 19.1 A
Total Stilbenes	13.6 ± 8.67 AB	8.62 ± 0.24 B	16.0 ± 0.53 AB	9.54 ± 1.59 B	22.7 ± 2.25 A	7.74 ± 0.41 B	24.3 ± 0.47 A	21.3 ± 3.58 A	25.9 ± 7.75 A	18.0 ± 0.24 A
Total Lignans	34.5 ± 3.48 C	35.2 ± 1.63 C	74.2 ± 0.46 A	81.0 ± 4.47 A	60.8 ± 7.85 B	30.8 ± 2.44 D	30.8 ± 2.35 D	60.9 ± 4.86 C	72.0 ± 0.41 B	86.8 ± 4.62 A
Total non-coloured phenols	**823 ± 13.9 D**	**955 ± 21.3 C**	**1429 ± 12.0 B**	**1527 ± 20.3 B**	**1743 ± 83.0 A**	**805 ± 19.2 E**	**968 ± 7.30 D**	**1303 ± 0.62 C**	**1537 ± 47.0 B**	**1711 ± 6.31 A**

For each row in CF and CM independently, values not displaying the same letter are significantly different (one-way ANOVA, Fisher's test between all means, $p < 0.05$).
[a] The average concentrations of the individual compounds for each phenolic group can be consulted in Supplementary Table 3S.

Research with grape pomace has shown the possibility of preparing extracts rich in polyphenols from the skin and seed fractions (Brahim et al., 2014; Caldas et al., 2018; Maier et al., 2009; Medouni-Adrar et al., 2015) and this by-product also can be used as a source of polysaccharides and fibres (Beres et al., 2019; Mendes et al., 2013; Rondeau et al., 2013). The stems, obtained from destemming the bunches, also show up as an important source of phenolic compounds (Anastasiadi et al., 2012; Barros et al., 2014; González-Centeno et al., 2012; Spatafora et al., 2013), particularly stilbenes (Ewald et al., 2017; Piñeiro et al., 2013; Prozil et al., 2012).

Several *in-vivo* and *in-vitro* studies with the phenolic compounds present in wine and pomace waste have demonstrated important health-promoting effects, such as protection of neurons preventing cognitive and psychiatric disorders (Gomez-Pinilla & Nguyen, 2012; Zorraquín-Peña et al., 2019), a reduction of cholesterol (Oliveira et al., 2017), the prevention of cardiovascular diseases (Chacar et al., 2019; Herrera-Bravo et al., 2021), a reduction of insulin resistance (Costa et al., 2017) and antiproliferative activity against cancer cells (Pino-García et al., 2017).

From a perspective of resource recovery, obtaining ingredients rich in valuable phenolic compounds for application in the food, cosmetic and pharmaceutical industries not only contribute to a lower

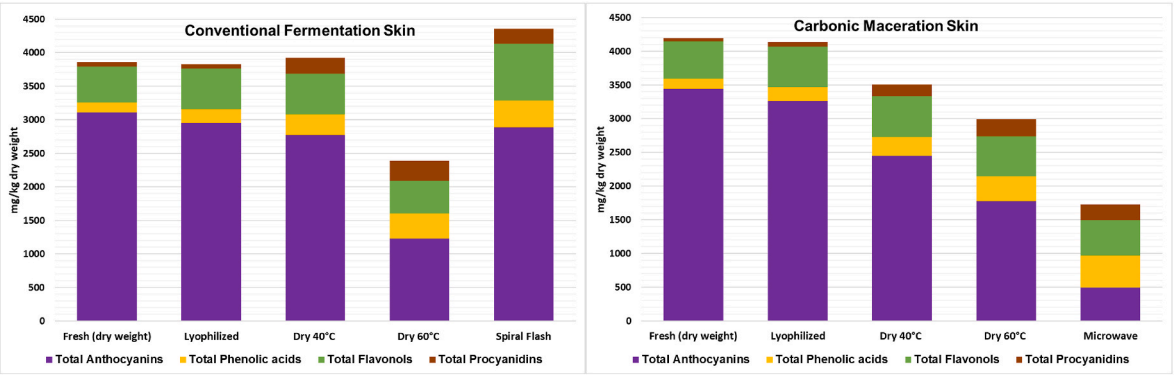

Fig. 1. Impact of dehydration technology on major polyphenols present in the skin (grape pomace) from conventional (CF) and maceration carbonic (MC) winemaking.

environmental impact by the wine industry, but also helps to reduce costs and increase efficiency and valorisation of the product. Most of the plant-based functional ingredients currently available on the market are obtained by extraction processes with aqueous or alcoholic solvents (Anastasiadi et al., 2012; Brahim et al., 2014; Caldas et al., 2018; Costa et al., 2017; Esparza et al., 2020) and most recently, by supercritical CO_2 extraction (Barajas-Alvarez et al., 2021; Silva et al., 2021). However, these extraction processes present technical difficulties and are costly for industrial scale-up. Given the problem of waste from wine production and searching for sustainable reuse viable for industry, this study is focused on exploring the potential of using by-products for winemaking to obtain ingredients with an enhanced functional value through direct dehydration processes, avoiding unnecessary and expensive extraction procedures.

Thus, the aim of this study was to search for the optimal dehydration conditions to obtain products with a high shelf-stability while maintaining high concentrations of the valuable phenolic compounds. Wine industry by-products obtained from two different winemaking processes were studied, these being a conventional process of destemming and crushing grape bunches prior to conventional alcoholic fermentation (CF), and a carbonic maceration (CM) process. The by-products used in this study were stems and grape pomace with subsequent separation into skin and seeds. The phenolic composition (anthocyanins and non-coloured phenols) and their quantitative pattern in skin, seeds and stems were determined using a targeted chromatographic approach based on ultra-high performance liquid chromatography with tandem mass spectrometry (UHPLC-QqQ-MS/MS).

2. Materials and methods

2.1. Chemicals and reagents

Cyanidin-3-glucoside, delphinidin-3-glucoside, malvidin-3-glucoside, peonidin-3-glucoside, petunidin-3-glucoside, isorhamnetin-3-glucoside, syringetin-3-glucoside, quercetin, quercetin-3-glucuronide *trans*-resveratrol, *trans*-resveratrol glucoside, (−)-epicatechin, dimer B1 and B2 and quercetin were purchased from Extrasynthese (Genay, France). (+)-Catechin, *p*-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid (protocatechuic acid), *p*-coumaric acid, gallic acid, caffeic acid, ferulic acid, vanillic acid, syringic acid, matairesinol and secoisolariciresinol were acquired from Sigma-Aldrich (St. Louis, USA). Caftaric acid and kaempferol-3-glucoside were purchased from Purifa -Cymit (Barcelona, Spain). Narigerin and coutaric acid were purchased from Fluochem (Hadfield, England) and Phytolab (Madrid, Spain), respectively. The solvents methanol (HPLC grade), acetonitrile (HPLC-MS grade) and formic acid (HPLC grade) were purchased from Scharlab Chemie (Sentmenat, Catalonia, Spain). The ultrapure water was supplied from a MilliQ system (Millipore Corp, Bedford, MA, USA). Stock solutions of standard compounds were prepared by dissolving each compound in methanol at a concentration of 1000 mg/L, and storing them in dark flasks at − 20 °C.

2.2. Plant material

This study was carried out with the by-products from *Vitis vinifera* L. cv. 'Tempranillo' during the 2020 vintage from the experimental winery in the ICVV (Instituto de Ciencias de la Vid y del Vino-ICVV, La Rioja, Spain) obtained from two different vinification processes: a conventional process of destemming and crushing (CF) grape bunches, and a carbonic maceration (CM) process (Fig. S1 of Supplementary Material). Ten different lots of winemaking by-products from Tempranillo variety were obtained from CF (n = 5) and CM (n = 5) processes, respectively, in the period between October and November 2020.

In the conventional winemaking (CF), the bunches were destemmed and crushed. The must was inoculated with commercial *Saccharomyces cerevisiae* yeast strains Uvaferm VRB® (Lallemand, St Simon, France) (20 g/hL) following the manufacturer's instructions. The musts were fermented in a temperature-controlled room (~20 °C) for 9 days and the cap was plunged down every day. When the fermenting wines reached a density value of approximately 990–1000 g/L, they were pressed in a small water bag press (~1000 kPa) and transferred to glass flagons for malolactic fermentation. The stem samples were collected immediately after the destemming and crushing process, and the pomace samples were obtained after pressing. All the samples were immediately frozen and stored at −20 °C (Fig. S1 of Supplementary Material).

In the carbonic maceration (CM) winemaking, the intact grape bunches, without destemming or crushing, were placed in a closed tank with an atmosphere rich in carbon dioxide. Under anaerobic conditions, intracellular fermentation occurred inside the whole grapes triggering the production of alcohol, the degradation of malic acid, pectolytic and proteolytic phenomena, the formation of volatile compounds and the diffusion of phenolic compounds from the skin to the pulp (Tesniere & Flanzy, 2011). After this first phase of carbonic maceration (5 days), racking was done by drawing off a free-run, partly fermented wine, and the grapes that remained whole were pressed releasing a higher-density must. Sampling of stem and grape pomace was done after this first phase (Fig. S1 of Supplementary Material). This partly fermented must was inoculated with commercial *Saccharomyces cerevisiae* yeast strains Uvaferm VRB® (Lallemand, St Simon, France) (20 g/hL) following the manufacturer's instructions and fermented in a temperature-controlled room (~20 °C) until a density value of approximately 990–1000 g/L was reached. It was then transferred to glass flagons for malolactic fermentation by lactic acid bacteria.

Table 2
Impact of drying processes on the anthocyanin and non-coloured phenols contents of the seeds from by-products obtained from conventional fermentation (CF) and carbonic maceration (CM) winemaking, respectively. Results are expressed as mean (mg/kg) ± standard deviation ($n = 10$).

Compound (mg/kg)[a]	Seeds from conventional fermentation (CF) winemaking					Seeds from carbonic maceration (CM) winemaking			
	Fresh (dry weight)	Lyophilisation	Air-drying 40 °C	Air-drying 60 °C	Spiral Flash	Fresh (dry weight)	Lyophilisation	Air-drying 40 °C	Air-drying 60 °C
Total Malvidins	73.6 ± 21.6 a	38.8 ± 2.03 bc	62.8 ± 9.45 ab	27.7 ± 0.12 c	20.3 ± 2.55 c	141 ± 4.82 a	44.9 ± 1.89 b	41.5 ± 4.09 b	51.8 ± 3.74 b
Total Petunidins	7.61 ± 4.11 a	3.92 ± 0.15 ab	4.96 ± 0.34 ab	1.83 ± 0.26 b	2.25 ± 0.29 b	10.6 ± 1.42 a	3.54 ± 0.30 b	2.40 ± 0.26 b	3.61 ± 0.39 b
Total Delphinidins	6.44 ± 2.63 a	4.03 ± 0.18 abc	5.03 ± 0.33 ab	1.66 ± 0.03 c	2.92 ± 0.32 bc	9.07 ± 0.51 a	3.36 ± 0.44 b	2.07 ± 0.09 c	3.37 ± 0.30 b
Total Peonidins	8.79 ± 1.35 a	3.69 ± 0.04 c	6.90 ± 0.73 b	2.75 ± 0.27 c	0.94 ± 0.13 d	35.1 ± 0.72 a	10.4 ± 1.13 b	7.13 ± 0.00 b	10.8 ± 2.40 b
Total Cyanidins	1.10 ± 0.11 a	0.64 ± 0.03 b	0.80 ± 0.10 b	0.37 ± 0.02 c	0.23 ± 0.03 c	2.88 ± 0.02 a	1.14 ± 0.16 b	0.65 ± 0.04 c	0.80 ± 0.15 c
Vitisin B	0.27 ± 0.00 ab	0.27 ± 0.04 ab	0.36 ± 0.07 a	0.26 ± 0.03 b	0.12 ± 0.02 c	0.62 ± 0.07 a	0.20 ± 0.01 c	0.24 ± 0.01 c	0.42 ± 0.05 b
Total Anthocyanins	**97.9 ± 29.8 A**	**51.4 ± 2.03 BC**	**80.8 ± 11.0 AB**	**34.6 ± 0.44 C**	**26.8 ± 3.32 C**	**199 ± 7.55 A**	**63.6 ± 3.92 BC**	**54.1 ± 4.48 C**	**70.8 ± 7.03 B**
Total Hydroxycinnamic acids	33.7 ± 4.31 a	33.2 ± 3.47 a	25.5 ± 5.77 ab	35.4 ± 4.90 a	21.5 ± 2.99 b	43.8 ± 0.11 a	27.2 ± 0.19 b	29.0 ± 2.64 b	26.7 ± 0.86 b
Total Hydroxybenzoic acids	546 ± 23.7 a	451 ± 0.40 b	445 ± 27.0 b	450 ± 27.0 b	476 ± 5.48 b	462 ± 13.5 c	619 ± 16.1 a	529 ± 26.2 b	435 ± 15.4 c
Total Phenolic acids	580 ± 28.0 A	484 ± 3.87 B	471 ± 32.7 B	486 ± 31.9 B	498 ± 8.47 B	506 ± 13.6 BC	646 ± 15.9 A	558 ± 28.8 B	462 ± 16.3 C
Total Phenyl alcohols	0.97 ± 0.24 A	1.11 ± 0.01 A	0.97 ± 0.37 A	1.02 ± 0.20 A	1.37 ± 0.15 A	1.13 ± 0.02 AB	0.84 ± 0.13 B	1.18 ± 0.15 A	1.02 ± 0.12 AB
Total Isorhamnetin	1.98 ± 0.17 ab	1.58 ± 0.18 bc	2.13 ± 0.36 a	1.66 ± 0.15 abc	1.41 ± 0.06 c	3.11 ± 0.12 a	1.56 ± 0.03 c	2.25 ± 0.22 b	2.84 ± 0.19 a
Total Kaempferol	2.01 ± 0.06 b	2.69 ± 0.33 a	2.91 ± 0.22 a	2.75 ± 0.06 a	2.01 ± 0.06 b	4.27 ± 0.03 a	1.60 ± 0.02 b	2.80 ± 1.31 ab	3.63 ± 0.13 a
Total Myricetin	3.11 ± 0.05 c	5.10 ± 0.22 a	4.57 ± 0.71 abc	3.41 ± 0.27 bc	4.87 ± 0.64 a	9.48 ± 1.12 a	3.50 ± 0.03 b	3.18 ± 0.22 b	4.04 ± 0.29 b
Total Quercetin	51.5 ± 2.83 a	37.4 ± 0.41 b	36.3 ± 3.81 b	38.8 ± 1.28 b	22.1 ± 1.73 c	116 ± 7.11 a	23.4 ± 1.27 b	33.8 ± 2.82 b	35.2 ± 4.88 b
Total Laricitrin	0.85 ± 0.03 b	0.91 ± 0.11 b	1.32 ± 0.10 a	1.31 ± 0.14 a	1.07 ± 0.18 a	1.78 ± 0.14 a	0.87 ± 0.02 c	1.33 ± 0.08 b	1.76 ± 0.20 a
Total Syringetin	1.19 ± 0.05 c	1.18 ± 0.15 c	2.33 ± 0.01 a	2.02 ± 0.05 b	2.23 ± 0.05 a	2.09 ± 0.17 b	1.21 ± 0.00 c	1.96 ± 0.15 bc	3.50 ± 0.57 a
Total Astilbins	1.67 ± 0.36 a	1.33 ± 0.23 a	1.70 ± 0.17 a	1.36 ± 0.15 a	0.66 ± 0.07 b	2.91 ± 0.32 a	1.52 ± 0.00 b	1.60 ± 0.22 b	1.75 ± 0.40 b
Total Flavonols	62.3 ± 2.99 A	50.1 ± 1.62 B	51.2 ± 5.17 B	51.3 ± 1.69 B	34.4 ± 2.56 C	140 ± 8.96 A	33.7 ± 1.28 C	47.0 ± 3.97 BC	52.8 ± 6.66 B
Total Catechin derivates	1040 ± 115 a	1033 ± 109 a	938 ± 175 ab	726 ± 5.20 bc	561 ± 19.9 c	1727 ± 0.01 a	1751 ± 68.5 a	1271 ± 125 b	889 ± 61.3 c
Total Procyanidins	848 ± 77.4 a	623 ± 24.6 b	833 ± 85.1 a	793 ± 80.6 a	333 ± 4.70 c	886 ± 104 a	1006 ± 140 a	909 ± 30.7 a	846 ± 205 a
Total Proanthocynidins	1888 ± 192 A	1656 ± 84.3 A	1770 ± 260 A	1520 ± 75.4 A	893 ± 15.2 B	2613 ± 104 AB	2757 ± 209 A	2180 ± 156 B	1734 ± 144 C
Total Stilbens	6.27 ± 0.52 A	5.62 ± 0.65 A	5.70 ± 0.91 A	3.62 ± 0.34 B	6.06 ± 0.80 A	35.6 ± 6.20 A	9.93 ± 0.49 B	4.84 ± 0.34 B	4.83 ± 0.80 B
Total Lignans	150 ± 7.96 ABC	127 ± 24.5 C	182 ± 16.0 A	172 ± 4.91 AB	144 ± 1.57 BC	109 ± 3.88 C	149 ± 14.4 AB	186 ± 14.8 A	142 ± 19.5 BC
Total non-coloured phenols	**2687 ± 232 A**	**2324 ± 115 A**	**2480 ± 315 A**	**2233 ± 101 A**	**1577 ± 18.6 B**	**3405 ± 129 AB**	**3596 ± 177 A**	**2977 ± 196 B**	**2397 ± 187 C**

For each row in CF and CM independently, values not displaying the same letter are significantly different (one-way ANOVA, Fisher's test between all means, $p < 0.05$).
[a] The average concentrations of the individual compounds for each phenolic group can be consulted in Supplementary Table 4S.

2.3. Dehydration process of winemaking by-products

The following dehydration processes were studied: lyophilisation, air-drying chamber at 40 °C and 60 °C, a microwave system and Spiral Flash air drying. Prior to the drying process, the by-product samples were thawed in a cold chamber (5–8 °C). This process was performed for all processes except for freeze-drying, in which the samples were placed directly in the freeze-dryer. In the case of CM by-products, a step was carried out to separate the stems from the grape pomace (Fig. S1 of Supplementary Material).

2.3.1. Lyophilisation

Due to the possible presence of ethanol in the pomace grapes, the process was conducted in a Telstar LyoQuest-85 lyophiliser (Terrassa, Spain). For the stem samples, which do not contain ethanol, the Scanvac-CoolSafe-95-16-Pro freeze-dryer control (Bjarkesvej, Denmark) was used. The freeze-drying was performed at 0.1 bar with a temperature ramp of −20 to 0 °C over 48 h.

2.3.2. Air-drying

Studies of drying kinetics were carried out to optimise the time and temperature parameters in the air oven (POL-EKO, SLW400-STD, Wodzisław Śląski, Poland). The optimised drying time (hours)/temperature, for each type of by-product, was 27 and 10 h at 40 °C for pomace, and 17 and 5 h at 60 °C for stems.

2.3.3. Spiral Flash air dryer

The dehydration process by Spiral Flash dryer was carried out in the pilot plant of the INGETECSA company (Barcelona, Spain). This dryer system is made up of a vertical chamber with a static blade ring and

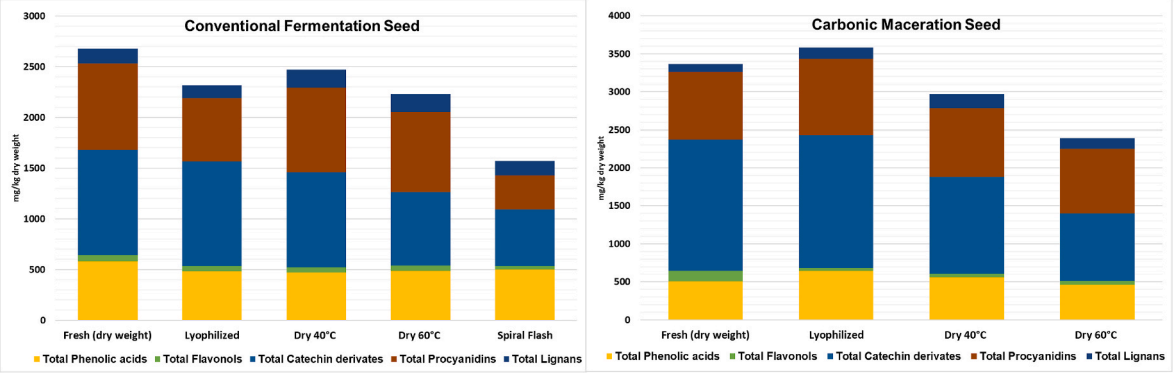

Fig. 2. Impact of dehydration technology on major polyphenols present in the seeds from conventional (CF) and maceration carbonic (MC) winemaking.

upper extraction, which can simultaneously dry and disperse organic materials without any risk of explosion due to mechanical friction (Fig. S2 of Supplementary Material). In this system, the filtered hot air is pushed by a fan into the drying chamber, flowing through a static blade ring. The blades have a fixed orientation, which generates a highly turbulent air flow. The product to be dehydrated is introduced above the blade ring, and as the product falls it is mixed with the hot air flow and the drying process is carried out quickly. Summarising the process, the moist material is dosed into the drying chamber by a feeder, and dried by stirring and vortex flow. In the present work, this dehydration technology was only applied to grape pomace from the conventional winemaking process (CF) (Fig. S1 of Supplementary Material), as a first proof of concept that will be extended to the other by-products in the future.

2.3.4. Microwave

The dehydration process was done in a prototype continuous-flow microwave oven (Model SI MAQ0101; Sairem Iberica S.L., Barcelona, Spain). This microwave system contains 4 magnetrons, each with a power of 1000 W. In the present work, this type of drying was only applied to the skin and stem samples from the CM process (Fig. S1 of Supplementary Material). For each experiment, the sample was placed in an aluminium tray in the oven and dried at 50% of the output power using two magnetrons. In addition, cold air was introduced to avoid an excessive rise in temperature inside the product, and the conveyor belt system moved back and forth to ensure homogeneous treatment.

2.3.5. Conditioning of dehydrated by-products

The dehydrated grape pomace was sieved to give two fractions: skin and seeds (Fig. S1 of Supplementary Material). All dehydrated samples (skin, seeds and stem) were crushed directly in the IKA Instruments grinder (Staufen, Germany) with a power 420 W and sieved (ø 0.5 mm). The samples were transferred to falcon tubes and then stored at −80 °C until their chromatographic analysis.

2.4. Determination of individual phenolic compounds by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-QqQ-MS/MS)

Sample pre-treatment. Immediately before the chromatographic analysis, the phenolic compounds were extracted from the samples by a solid-liquid extraction (SLE) based on the method of Royo et al. (2021) with modifications. Briefly, dehydrated (200 mg) and fresh (600 mg) samples were weighed in a falcon tube (15 mL). Then, 4 mL of methanol/Milli-Q water/formic acid (79:20:1, v/v/v) were added. The mixture was vortexed and macerated overnight at 4 °C in the dark. Later, samples were sonicated (5 min, 40 Hz frequency) using an ultrasonic bath (Ultrasons P. Selecta, Barcelona, Spain) and were centrifuged at 9000 rpm (10 min, 20 °C) in a Sorvall LYNX 4000 Superspeed Centrifuge (Thermo ScientificTM, Madison, WI, USA) to collect the supernatants. The extraction procedure was repeated twice, adding 3 mL of extraction solution to the solid residue, sonicating and centrifuging. The supernatants from each extraction cycle were collected, adjusted to 10 mL with the extraction solvent and filtered with a 0.22 μm PTFE filter (Scharlab Chemie, Catalonia, Spain) prior to chromatographic analysis.

Chromatographic analysis. The phenol extracts were analyzed by UHPLC/QqQ-MS/MS based on the method described by (Royo et al., 2021). LC analyses were carried out in a liquid chromatograph (Shimadzu Nexera, Shimadzu Corporation, Japan), coupled to an 3200QTRAP triple quadrupole mass spectrometer (AB Sciex, USA) equipped with an electrospray ionisation source (ESI Turbo V™ Source). Two chromatographic methods were used for the analysis of 1) anthocyanins, and 2) the non-coloured phenolic compounds. The polyphenol separation was performed on a Waters AcQuity BEH C18 column (100 mm × 2.1 mm, 1.7 μm) equipped with a VanGuardTM AcQuity BEH C18 Pre-Column (5 × 2.1 mm, 1.7 μm) supplied by Waters (Milford, MA, USA).

The electrospray (ESI) interface was used in the positive mode $[M-H]^+$ for the analysis of anthocyanins, and in the negative mode $[M-H]^-$ for the rest of the phenolic compounds. The ionisation source parameters and the data acquisition through multiple reaction monitoring (MRM) are described in Royo et al. (2021). The retention time and MRM transitions for quantification and identification for each phenolic compound are presented in Table S1 of Supplementary Material. Data acquisition was carried out with the Analyst® 1.6.2 software (AB Sciex, USA).

Phenolic compounds were identified by comparing their spectra and retention times with those of externally injected standards. Compounds for which standards were not available were identified using MRM transitions with the mass of the parent ion (M−H) and typical MS fragmentation pattern described in the literature. Some of the phenolic compounds were quantified using the calibration curves of their corresponding pure commercial standards. The other compounds were tentatively quantified using the calibration curves of standards with similar chemical structures (Table S2 of Supplementary Material). The results were expressed as mg compound/kg grape skin, seed or stem samples.

2.5. Statistical analysis

Concentration values of the phenolic compounds studied were reported as means ± standard deviation (SD). One-way analysis of variance (ANOVA) and Fisher's test at a level of 0.05 were used to determine the significance of differences among the dehydration processes. All data were analyzed with the Minitab Statistical Software, version 17.2.1 (Minitab Inc., State College, Pennsylvania, United States).

Table 3
Impact of drying processes on the anthocyanin and non-coloured phenols contents of the grape stem from conventional fermentation (CF) and carbonic maceration (CM) winemaking, respectively. Results are expressed as mean (mg/kg) ± standard deviation ($n = 10$).

Compound (mg/kg)[a]	Stem from conventional fermentation (CF) winemaking				Stem from carbonic maceration (CM) winemaking				
	Fresh (dry weight)	Lyophilisation	Air-drying 40 °C	Air-drying 60 °C	Fresh (dry weight)	Lyophilisation	Air-drying 40 °C	Air-drying 60 °C	Microwave
Total Malvidins	706 ± 107 a	534 ± 0.11 b	376 ± 5.31 c	272 ± 5.14 c	1134 ± 31.2 a	397 ± 2.52 d	453 ± 35.6 c	518 ± 7.12 b	403 ± 0.07 cd
Total Petunidins	55.5 ± 3.82 b	67.8 ± 1.01 a	31.9 ± 0.88 c	19.3 ± 1.36 d	107 ± 0.27 a	42.6 ± 1.24 c	24.1 ± 1.35 e	30.0 ± 0.87 d	49.6 ± 2.08 b
Total Delphinidins	52.6 ± 0.88 b	96.3 ± 1.94 a	33.1 ± 0.98 c	20.5 ± 0.41 d	121 ± 1.12 a	52.5 ± 1.09 c	25.1 ± 1.75 d	32.1 ± 5.93 d	77.7 ± 3.87 b
Total Peonidins	178 ± 30.0 a	145 ± 9.78 a	94.0 ± 5.24 b	71.4 ± 3.19 b	95.0 ± 2.32 a	37.0 ± 0.64 d	62.5 ± 4.63 c	83.8 ± 2.49 b	68.6 ± 2.92 c
Total Cyanidins	39.7 ± 0.17 a	42.8 ± 2.45 a	21.9 ± 0.12 b	16.7 ± 2.07 c	13.3 ± 0.03 a	7.74 ± 0.01 c	2.72 ± 0.21 e	4.48 ± 0.52 d	10.2 ± 0.46 b
Pelarg-3-G-6-Gluc	0.66 ± 0.06 a	0.56 ± 0.02 b	0.36 ± 0.02 c	0.31 ± 0.01 c	0.25 ± 0.01 a	0.12 ± 0.00 c	0.14 ± 0.02 bc	0.16 ± 0.00 b	0.27 ± 0.00 a
Vitisins A	n.d.	n.d.	n.d.	n.d.	0.57 ± 0.05 a	0.14 ± 0.00 c	0.21 ± 0.02 b	0.19 ± 0.01 bc	0.22 ± 0.01 b
Vitisins B	0.08 ± 0.03 ab	0.07 ± 0.00 ab	0.05 ± 0.01 b	0.11 ± 0.01 a	8.56 ± 0.20 a	2.73 ± 0.10 c	3.88 ± 0.27 b	3.85 ± 0.26 b	3.89 ± 0.14 b
Total Anthocyanins	**1033 ± 140 A**	**887 ± 9.19 A**	**557 ± 12.6 B**	**401 ± 1.67 B**	**1479 ± 34.5 A**	**539 ± 0.37 D**	**571 ± 43.9 CD**	**672 ± 11.7 B**	**613 ± 8.47 BC**
Total Hydroxycinnamic acids	587 ± 1.12 d	2487 ± 17.5 a	1014 ± 6.32 b	900 ± 3.35 c	194 ± 3.95 b	222 ± 10.7 a	87.9 ± 4.60 d	102 ± 10.5 d	129 ± 3.69 c
Total Hydroxybenzoic acids	708 ± 15.1 a	692 ± 4.50 a	489 ± 38.6 b	461 ± 7.28 b	304 ± 7.07 a	163 ± 9.55 c	146 ± 2.38 d	164 ± 0.28 c	226 ± 5.14 b
Total Phenolic acids	1295 ± 14.0 D	3179 ± 22.0 A	1503 ± 32.3 B	1360 ± 10.6 C	498 ± 11.0 A	385 ± 1.17 B	233 ± 2.23 E	266 ± 10.2 D	355 ± 8.83 C
Total Phenyl alcohols	11.5 ± 0.37 A	7.94 ± 0.26 C	9.30 ± 0.17 B	7.40 ± 0.17 C	6.44 ± 0.50 A	3.00 ± 0.04 C	5.26 ± 0.32 AB	4.70 ± 0.96 B	3.02 ± 0.01 C
Total Flavanones	10.0 ± 0.85 A	5.90 ± 0.13 B	6.67 ± 0.33 B	7.13 ± 0.15 B	13.4 ± 0.01 A	4.85 ± 0.09 BC	5.52 ± 0.63 B	5.47 ± 0.20 B	4.08 ± 0.16 C
Total Isorhamnetin	14.9 ± 0.30 a	12.5 ± 0.24 b	9.12 ± 0.15 c	7.54 ± 0.32 d	19.2 ± 0.19 b	6.56 ± 0.05 e	14.3 ± 0.73 c	20.5 ± 0.04 a	10.8 ± 0.34 d
Total Kaempferol	137 ± 4.36 c	165 ± 1.77 b	182 ± 1.11 a	123 ± 5.92 d	91.3 ± 3.72 a	57.0 ± 1.77 b	21.4 ± 0.30 d	33.8 ± 0.07 c	38.0 ± 1.50 c
Total Miricetin	36.2 ± 0.25 b	48.7 ± 1.27 a	26.6 ± 1.94 c	21.8 ± 1.60 d	148 ± 3.12 a	47.4 ± 5.28 c	22.2 ± 2.51 d	26.1 ± 1.30 d	58.6 ± 1.44 b
Total Quercetin	3098 ± 27.3 a	2922 ± 48.5 ab	2755 ± 102 b	1826 ± 71.6 c	1248 ± 49.5 a	763 ± 4.82 b	564 ± 20.9 c	768 ± 23.1 b	443 ± 11.0 d
Total Laricitrin	2.70 ± 0.23 a	2.14 ± 0.23 ab	1.77 ± 0.30 b	2.11 ± 0.08 ab	24.7 ± 0.16 a	8.05 ± 0.03 cd	7.84 ± 0.43 d	8.74 ± 0.43 c	11.8 ± 0.10 b
Total Syringetin	3.93 ± 0.23 a	3.26 ± 0.07 b	2.79 ± 0.05 c	3.81 ± 0.11 a	31.2 ± 1.33 a	12.2 ± 0.27 d	19.4 ± 0.50 c	20.7 ± 0.12 c	22.6 ± 0.38 b
Total Astilbins	127 ± 8.37 a	99.4 ± 1.17 b	76.4 ± 2.46 c	66.0 ± 4.80 c	24.7 ± 1.12 c	16.1 ± 0.33 d	34.4 ± 0.74 b	39.5 ± 1.49 a	8.62 ± 0.48 e
Total Flavonols	3420 ± 15.1 A	3253 ± 47.3 AB	3054 ± 106 B	2050 ± 84.2 C	1588 ± 58.8 A	911 ± 2.26 B	684 ± 24.0 C	918 ± 26.4 B	594 ± 11.4 D
Total Catechin derivatives	1393 ± 254 b	2770 ± 1.67 a	1193 ± 11.8 b	1112 ± 32.0 b	236 ± 11.7 b	355 ± 5.71 a	176 ± 2.04 c	333 ± 22.0 a	326 ± 13.1 a
Total Procyanidins	4845 ± 171 a	4958 ± 2.70 a	3359 ± 28.4 b	2739 ± 55.6 c	447 ± 33.8 b	545 ± 17.3 a	272 ± 5.77 c	306 ± 15.3 c	307 ± 6.88 c
Total Proanthocyanidins	6238 ± 425 B	7728 ± 1.03 A	4552 ± 16.6 C	3851 ± 23.6 D	684 ± 45.4 B	899 ± 23.0 A	448 ± 7.81 C	638 ± 37.3 B	633 ± 20.0 B
Total Stilbenes	404 ± 31.1 A	261 ± 0.19 B	190 ± 4.30 C	186 ± 2.28 C	112 ± 8.28 A	113 ± 20.3 A	49.5 ± 4.78 B	58.1 ± 1.26 B	63.6 ± 0.33 B
Total Lignans	515 ± 73.8 A	289 ± 4.93 B	298 ± 22.2 B	258 ± 7.85 B	104 ± 8.08 A	84.6 ± 4.10 B	65.6 ± 7.17 C	49.1 ± 1.88 D	58.0 ± 2.94 CD
Total non-coloured phenols	**11884 ± 381 B**	**14718 ± 65.0 A**	**9607 ± 72.0 C**	**7712 ± 129 D**	**2992 ± 23.7 A**	**2396 ± 7.89 B**	**1486 ± 25.6 E**	**1934 ± 21.4 C**	**1706 ± 43.5 D**

For each row in CF and CM independently, values not displaying the same letter are significantly different (one-way ANOVA, Fisher's test between all means, $p < 0.05$). n.d.: not detected.

[a] The average concentrations of the individual compounds for each phenolic group can be consulted in Supplementary Table 5S.

3. Results and discussion

3.1. Impact of drying processes on the phenolic composition of grape pomace from conventional fermentation (CF) and carbonic maceration (CM) winemaking processes

In order to estimate the impact of the different dehydration processes on the phenolic composition of the grape pomace (skin and seeds) and stems, targeted UHPLC-QqQ-MS/MS chromatography was used. Using the fresh product before dehydration process as a reference, a wide range of anthocyanins and non-coloured phenols (hydroxybenzoic and hydroxycinnamic acids, phenyl alcohols, flavonols, flavan-3-ols, proanthocyanidins, stilbenes and lignans) were identified and quantified in the skin, seed and stem samples.

3.1.1. Impact of drying processes on skin grape phenolic composition

Results show a similar phenolic composition of the skin before the dehydration (fresh sample expressed as dry weight), regardless of the

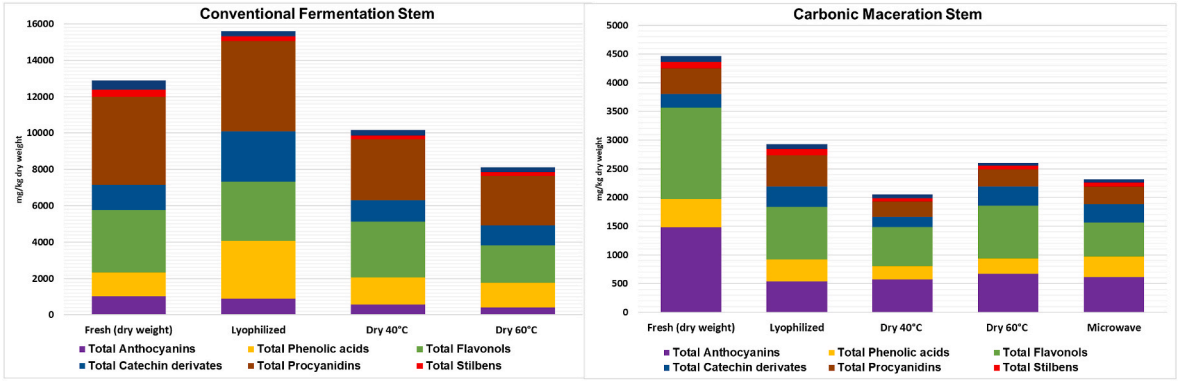

Fig. 3. Impact of dehydration technology on major polyphenols present in stems from conventional (CF) and maceration carbonic (MC) winemaking.

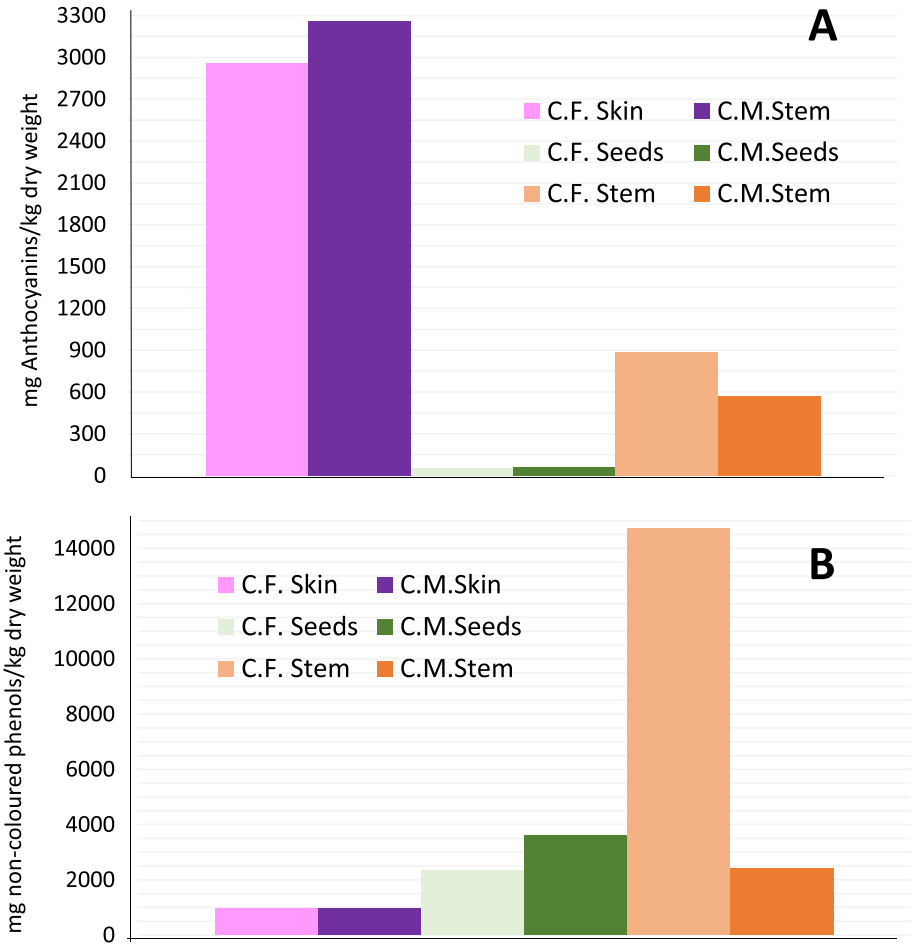

Fig. 4. Content of total anthocyanins (A) and non-coloured phenols (B) by each type of by-product and comparison between the different by-products and winemaking processes: conventional fermentation (CF) and maceration carbonic (MC).

winemaking process (Table 1). Anthocyanins are the major class of phenols in the skin samples, being the malvidin derivatives the most abundant, especially malvidin-3-glucoside following by delphinidin-type anthocyanins (Table S3 of Supplementary Material). Concerning the non-coloured phenols, flavonols were the most abundant (Table 1), showing quercetin derivatives as the predominant flavonols (Table S3 of Supplementary Material). These results are in agreement with those found by Carmona-Jiménez et al. (2018) evaluating the use of a climatic chamber to dry grape pomace from five grape pomace varieties, including Tempranillo variety. In addition, important amounts of hydroxybenzoic and hydroxycinnamic acids were also detected in the skin samples (Table 1 and Table S3 of Supplementary Material).

Regarding the dehydration process, in general, in studies evaluating the phenolic composition of foods, freeze-drying (lyophilisation) is used as a reference, since the low temperature (from −2 to −10 °C) and pressure that favour the absence of oxygen during the drying process. This leads to less degradation of the most labile compounds as well as facilitating the extraction of the bound phenolic compounds from the sample for subsequent analysis (Alonso et al., 2002; Barcia et al., 2014). The results of the present study showed that lyophilisation preserved the anthocyanins in the grape skin samples. No significant differences were observed in anthocyanin concentration in relation to fresh skin

(Table 1). By contrast, the hot air dehydration processes (air-drying at 40 and 60 °C and Spiral Flash) resulted in a significant increase ($p<0.05$) in the concentration of non-coloured phenols, these being mainly proanthocyanidins (catechin derivatives and procyanidins) (Table 1 and Table S3 of Supplementary Material). Unlike previous studies that have observed an improvement in the extractability of bound phenolic compounds by lyophilisation, in our study the highest extraction of hydroxycinnamic acids and proanthocyanidins was obtained with the hot-air treatments (air-drying at 40 °C and 60 °C and Spiral Flash dryer).

However, at an industrial level, lyophilisation is costly due to a need for refrigeration and vacuum systems, multiplying the energy costs by around 4–8 times compared to those of hot-air or convective drying, restricted its application to high-value aggregated products such coffee and medicinal herbs (Ratti, 2001). In addition, the freeze-dried product needs major care in packaging and storage, due to increased porosity and hygroscopy, which can reduce their shelf-life (Karam et al., 2016).

Compared to lyophilisation, air-drying at 40 °C was shown to be a good drying alternative because it retained the anthocyanins and produced a slight increase in the concentration of the other phenolic groups (Table 1 and Fig. 1). This increase in the concentration of non-coloured phenols was also observed after the air-drying at 60 °C, probably as consequence of the impact of thermal processing on the release of the bound phenolics. In fact, phenolic acids, such as hydroxycinnamic and hydroxybenzoic acids, form ether linkages with lignin through their hydroxyl groups in the aromatic ring and ester linkages with structural carbohydrates and proteins through their carboxylic group (Acosta-Estrada et al., 2014).

Interestingly, the concentration of anthocyanins in the skin samples from conventional fermentation winemaking (CD) dried by Spiral Flash technology did not show significant differences in relation to fresh and lyophilised skin samples (Table 1). In parallel, and similar to that observed in air-dried samples, a significant increase ($p<0.05$) in the concentration of non-coloured phenols was observed. The flash drying technology has been used in different industries, including the agro-food and chemical sectors. However, there is no information regarding the impact of this air-drying technology on the preservation of phenolic compounds. The results obtained in our study reveal a great potential for applying this technology for the production of grape skin derived products with retention of bioactive phenolic compounds on an industrial-scale.

The comparative analysis of the different drying processes (Fig. 1) showed that air-drying at 60 °C caused a significant loss of anthocyanins, in the samples from both the CF and CM winemaking processes. This may be due to the exposure of skin fragments to oxygen and the impact of time and temperature (60 °C). These results agree with a previous study by Barcia et al. (2014) that reported a loss of anthocyanins (40%) in the grape skin of *Cabernet Sauvignon* and *Cabernet Franc* after air-drying compared to lyophilisation process.

When analysing the impact of the microwave oven drying technology, the results showed a significant ($p<0.05$) loss of anthocyanins (around 75%) (Table 1 and Fig. 1). In addition, from a sensory viewpoint (data not shown), the CM skins dried by microwave had a brown/black coloration, and a very strong and unpleasant toasted aroma, which hinders the application of this by-product for food purposes.

3.1.2. Impact of the drying processes on seed phenolic composition

The concentration of individual phenolic compounds from the fresh seeds in grape pomace expressed as dry weight is shown in Table 2 and Table S4 of Supplementary Material. Anthocyanins do not usually accumulate in seeds of commercial grape varieties (Barros et al., 2014; Cerda-Carrasco et al., 2015; Royo et al., 2021). Surprisingly, anthocyanins have been detected in seed samples in this study, mostly malvidin derivatives. The presence of anthocyanins on the surface of the seed samples could be a consequence of their contact with the grape skin during the maceration phase of the alcoholic fermentation (CD) processes. In the case of the carbonic maceration (CM), the grapes remain intact during 6 days in the tank. This could favour the direct contact of the seeds with anthocyanins extracted from the skin to fermenting wine explaining the higher content of anthocyanins in CM seeds (Table 2).

Regarding non-coloured phenols, a wide range of compounds was detected (Table 2 and Table S4 of Supplementary Material). With the exception of lyophilisation, the dehydration processes using hot-air led to a slight loss of proanthocyanidins (catechin derivatives and procyanidins) in the seed samples (Table 2 and Fig. 2). By contrast, Kim et al. (2006) showed that the fast thermal treatment (100–150 °C/10–50 min) of *Campbell* grape seeds increased the content of gallocatechin gallate and the antioxidant activity of grape seed extracts. In our study, the higher temperatures applied with Spiral Flash drying produced significant losses ($p<0.05$) of non-coloured phenols in grape seeds, mainly proanthocyanidins (catechin derivatives and procyanidins) (Table 2).

Regarding phenolic acids, hydroxybenzoic acids constituted ~95% of these in the seed samples (Table 2 and Fig. 2), the most abundant being gallic acid in its glucoside and free forms, together with vanillic acid hexose (Table S4 of Supplementary Material). These results agree with those observed by other authors (Fanzone et al., 2011; Maier et al., 2009; Obreque-Slier et al., 2012) in red grape seeds. The concentration of lignans detected in all the seed samples is remarkable (Table 2). These were made up of the glycosylated forms of secoisolariciresinol and iso-lariciresinol (Table S4 of Supplementary Material). In recent years, research into lignans has been drawing attention given their antioxidant activity and potential anti-inflammatory and anticancer activities (Saleem et al., 2005; Zálešák et al., 2019). Secoisolariciresinol and matairesinol have been identified in a large number of plant foods, of which flaxseed is the richest known source of these lignans. Such other foods as cereals, vegetables and fruits have lower concentrations (Touré & Xueming, 2010). However, studies of lignans in winemaking by-products are scarce. Some researchers have focused on the study of the trace amount of lignans naturally present in wine (Nurmi et al., 2003), or assessed the impact of wine/must fortification with lignan extracts, obtained from other plants, on antioxidant and anti-mutagenicity activity (Balík et al., 2017). In our study, an average of 50 mg/kg of secoisolariciresinol-glucoside was found in the seed samples, independently of the type of winemaking (CF and CM) or dehydration processes (Table S4 of Supplementary Material), while higher concentrations of iso-lariciresinol-glucoside were quantified (79.8–124.4 mg/kg).

Unlike in the skin, the different drying technologies did not significantly affect the concentrations of the seed phenolics. Nevertheless, Spiral Flash drying decreased the concentration of non-coloured phenols, mainly proanthocyanidins (catechin derivatives and procyanidins) (Table 2). This fact could be explained by the direct contact of seeds with the static blade ring where there is a cone with an opening that can be used to discharge heavy or off-spec particles (Fig. S2 of Supplementary Material), such as seeds from the grape-pomace sample.

3.1.3. Impact of drying processes on stem phenolic composition

Little attention has been devoted to grape stems, which comprise the woody part of grape clusters and constitute a waste product in winemaking. Grape stems are removed before the vinification process, since their presence during fermentation increases astringency, negatively affecting the organoleptic properties of the finished wine. Currently, the commercial value of grape stems is low, reflecting their use mostly as animal feed or soil fertilizer (Anastasiadi et al., 2012). In the present study, the targeted UHPLC-MS/MS analysis of stem samples from conventional winemaking (CF) and carbonic maceration (CM) enabled 25 anthocyanins and 63 non-coloured phenols to be identified. It is important to note that regardless of the dehydration process, the stems showed important amounts of non-coloured phenols (mainly hydroxycinnamic acids), quercetin derivatives (mainly quercetin glucuronide) and proanthocyanidins (catechin derivatives and anthocyanins) and an interesting concentration of anthocyanins (mainly malvidin derivatives) (Table 3 and Table S5 of Supplementary Material). The concentration of

proanthocyanidins detected in the stems is notable being the dimer B1 the predominant in both CF and CM stem samples, followed by dimers B3 and B2 (Table S5 of Supplementary Material). The phenolic composition of stem samples also revealed the presence of stilbenes, being the *trans*-resveratrol and its glucoside derivatives the main compounds (Table 3 and Table S5 of Supplementary Material). Recent studies have also detected stilbenes in grape stems (Jiménez-Moreno et al., 2019; Leal et al., 2020). In contrast to the other parts of the grape-pomace (skin and seeds), the winemaking procedure (CF and CM) had an important impact on the phenol concentration in the fresh stems (dry weight) (Fig. 3), mainly non-coloured phenols.

Similar to what was observed in skin and seeds, the lyophilisation preserved the concentration of anthocyanins and the main non-coloured phenolic fractions (quercetin derivatives and proanthocyanidins) in the stem samples (Table 3). When compared to the concentration of phenolic compounds of fresh stem samples (expressed as dry weight), air drying (at 40 and 60 °C) and microwave dehydration processes resulted in a significant decrease ($p<0.05$) of the main groups of the non-coloured phenols (phenolic acids, quercetin derivatives and proanthocyanidins) in stem dehydrated samples (Table 3 and Table S5 of Supplementary Material). Unlike what was observed in the skin and grape seed samples, the concentration of stilbenes and lignans in stem samples was reduced after air drying (40 and 60 °C) and microwave dehydration processes.

3.2. Comparative analysis of the phenolic composition of different fractions of winemaking by-products

With the aim of exploring the potential of each of the by-products studied as functional additives, we compared the composition of anthocyanins and non-coloured phenols between lyophilised samples of skin, seeds and stems. A common phenolic profile but important quantitative differences were observed (Tables 1–3 and Tables S3–S5 of Supplementary Material).

Anthocyanins constituted up to 70% of the total phenolic contents in the skin fraction of winemaking (CF and CM) by-products (Fig. 4A), whereas the non-coloured phenols represented around 20% (Fig. 4B). The seeds showed a distinct phenolic composition from the skin, and non-coloured phenols constituted up to 97% of the total phenolic content (Fig. 4B), whereas the anthocyanins represented about 2–3% (Fig. 4A).

It should be highlighted that the high content and diversity of phenolic compounds in the stem samples displayed a similar phenolic profile to the seeds but with important quantitative differences. The comparative analysis of the CF and CM samples revealed that the non-coloured phenol concentration of CF stems was about 3–4 times higher than that of the CM stems (Fig. 4B). These differences could be due to the presence of the stems during the carbonic maceration (CM) of grape clusters under CO_2 atmosphere to favour the intracellular fermentation/maceration and subsequent pressing of the mash, favouring the transfer of phenolic compounds from the stems to the fermenting must (Blackford et al., 2021; Busse-Valverde et al., 2011; Favre et al., 2014). Related to this, wines obtained by carbonic maceration have higher contents of both catechins and oligomeric and polymeric proanthocyanidins compared with wine made conventionally where the stems are removed prior to alcoholic fermentation (Spranger et al., 2004; Sun et al., 2001). Recently, the phenolic extracts obtained from grape stems are gaining increasing attention as antioxidants (Anastasiadi et al., 2012; Esparza et al., 2021; Ewald et al., 2017; Jiménez-Moreno et al., 2019; Teixeira et al., 2018) and as SO_2 substitutes (Esparza et al., 2020).

4. Conclusion

The results of the present study showed that lyophilisation preserved the anthocyanins and the non-coloured phenols in the different winemaking by-products studied, grape pomace (skin and seeds) and stems. However, at an industrial level, lyophilisation is costly due to a need for refrigeration and vacuum systems, multiplying the energy costs by around 4–8 times compared to those of hot-air or convective drying, restricted its application to high-value aggregated products. As alternative to lyophilisation, the Spiral Flash is a technology for high-speed drying that combines the advantages of flash drying and fluidized bed. The results obtained in our study reveal a great potential of application of this technology to dehydration of grape pomace at industrial-scale, retaining the heat-sensitive anthocyanins and non-coloured phenolic compounds. In the present work, this dehydration technology was only applied to grape pomace as a first proof of concept that will be extended to the other by-products in the future. In contrast, air-drying at 60 °C resulted in significant loss of anthocyanins mainly in skin samples. However, air-drying resulted in an increase of phenolic acids probably related with a better extractability of bound phenolic compounds. Based on the present study, the winemaking process provides an excellent source of raw materials for the recovery of ingredients rich in valuable phenolic compounds, thus contributing to the circular bioeconomy in the production of high added-value products.

CRediT authorship contribution statement

Bianca Souza da Costa: Investigation, Methodology, Validation, Formal analysis, Writing – original draft, All the authors have read and approved the final manuscript. **Germán Soldevilla Muro:** Project administration, Supervision, Funding acquisition, Writing – review & editing, All the authors have read and approved the final manuscript. **Marta Oliván García:** Investigation, Formal analysis, Writing – review & editing, All the authors have read and approved the final manuscript. **Maria-Jose Motilva:** Conceptualization, Methodology, Writing – original draft, Supervision, Project administration, All the authors have read and approved the final manuscript.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2022.113774.

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