The effect of genotype and drying condition on the bioactive compounds of sour cherry pomace

Roberto Ciccoritti[1], Mariano Paliotta[1], Luna Centioni[1,2], Fabio Mencarelli[2], Katya Carbone[1*]

[1]Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria— Research Centre for Olive, Citrus and Tree Fruit; Via di Fioranello, 52-00134 Rome, Italy;

[2] Department for Innovation in Biological Agro-food and Forest systems (DIBAF), Postharvest Laboratory, University of Tuscia, Viterbo, Via San Camillo de Lellis snc, 01100 Viterbo, Italy

*Corresponding author: katya.carbone@crea.gov.it

Abstract

Sour cherry (*Prunus cerasus* L.) contains substantial quantities of phenolic compounds, specifically anthocyanins, and is mainly processed into different products including juice, whose by-product, namely sour cherry pomace (SCP), represents a potential source of high-added bioactive compounds currently underutilized. Oven-drying (OD) and freeze-drying (FD) are simple methods, often used to stabilize food matrices, and can be also successfully employed for the stabilization of sour cherry pomace. In the present study, the influence of genotype (Bianchi d'Offagna and Montmorency cvs), drying method and their interaction on the extractability of phenolic and anthocyanin profiles of sour cherry pomace were evaluated. Both genotype and drying method significantly influenced ($p \leq 0.001$) the overall phytochemical content (total monomeric anthocyanin, total flavan-3-ol, total phenolic, and vitamin C) of analysed pomaces. The interrelationships between the parameters analyzed, the genotype, and the different drying conditions, as well as the relationships among variables, were investigated by principal component analysis (PCA). PCA results pointed out that the phytochemical content of sour cherry pomace was firstly influenced by the cultivar (which accounted for about 70% of the total variance), followed by drying process (about 18% of the total variance), and their interaction (about 12% of the total variance), with the exception of total flavan-3-ols, where a similar contribution (about 50%) of the two factors was observed. Besides, antidiabetic potential of SCPs was also investigated, pointing Bianchi D'Offagna FD pomace as the most active. Obtained results

point out the SCP stabilized by means of freeze-drying process as a valuable second-generation biorefinery for the food supplements, nutraceutical and pharmaceutical markets.

Keyword sour cherry by-products, drying methods, anthocyanins, antioxidant activity, α-glucosidase

Introduction

The Recent European Guidelines on cardiovascular disease prevention in clinical practice recommend eating at least 200 g of fruit (2–3 servings) and 200 g of vegetables (2–3 servings) *per* day [1]. At the same time, several studies regarding the well-being, together with a stronger awareness towards health benefits of bioactive compounds in foods, are influencing the choices of customers, addressing them to consume more fruits, vegetables, functional foods, and vitamin juices [2].

In this scenario, the cultivation and consumption of sweet (*Prunus avium L.*) and sour cherry (*Prunus cerasus L.*) increased about 6% in 2013 [3] due to their quality attributes and health promoting compounds, such as antioxidants, phytochemicals, vitamins, beta carotene, folic acid, lycopene, melatonin [4], and mineral elements like Ca, P, Fe, K, Mg, and Se [5].

Sour cherry belongs to the family of Rosaceae, and it has been reported to be native of Northwest and Central Europe [6]. At present, this species is highly widespread thanks to its strong adaptability. Sour cherry is a resilient species and its cultivation can be afforded also in organic farming. Montmorency is the most cultivated variety in the world [7], for this reason it is often taken as the reference cultivar. The largest areas of sour cherry cultivation in the world are located in Europe (80%), accounting for the 66% of the total world production. Russian Federation, with a production of 198,000 t followed by the Ukraina (182,880 t), Turkey (182,577 t), Poland (176,545)and United States (197,983 t), are the leading crop producers in the world, whereas the Italian production is only 7,541 t [3].

Recent studies suggest that the powerful pool of sour cherry bioactive compounds is linked to a broad spectrum of human health benefits like antioxidant and anti-inflammatory effects [8], anticancer and antineurodegenerative properties [9,10], reduction activity of muscle pain [11], cardiovascular

protective action. Most of these properties are due to their polyphenols and flavonoids and to their ability to scavenge oxygen radicals and other reactive species that are higher in ripe fruits and influenced by the genotype [12,13]. These substances, as well as their sugar derivates, mainly anthocyanins [14], are reported to be also inhibitors of α-glucosidase, contributing to the reduction of type-2 diabetes [15]. One of the therapeutic approaches to treat the diabetes is to decrease the postprandial hyperglycemia by retarding absorption of glucose. Inhibition of carbohydrate-hydrolyzing enzymes, such as α-glucosidase, is considered a possible pathway to achieve this goal as the enzyme plays a key role in digesting carbohydrates [16].

The main phenolic compounds in sour cherries are: anthocyanins, mainly cyanidin 3-glucoside, cyanidin 3-rutinoside, cyanidin 3-sophoroside, cyanidin 3-glucosylrutinoside [17,18], and flavan- 3-ols (37 % and 28 % of total polyphenolic compounds, respectively). They are also present, but to a lesser extent, phenolic acids (23 %) and flavanols (11 %) [19]. Literature data [20, 21] pointed out a high variability in the phytochemical content of fruits related to the genotype, mainly in the case of total anthocyanins that ranged from 27.8 to 80.4 mg/100g [20, 21].

Due to their acidulous taste, sour cherry is mostly processed into juice concentrates, jams, and marmalade [19, 22-24]. Approximately 99% of tart cherry crop was processed as frozen (>50%), canned (>33%), or brined, dried, and for juice production (>10%) [25].

Generally, the juice extraction process produces large amounts of waste (about 20-30% of fresh fruit weight), namely seeds and pomace, which still retained a great amount of underutilized bioactive compounds [26, 27]. This production model is misaligned with the new principles of the circular economy that, among the main targets, suggests minimizing the generation of waste because it is considered an essential contribution to the EU's efforts for the development of a sustainable, low carbon, resource efficient, and competitive economy [28].

About that, Cilek et al. [29] reported a total polyphenolic content (TPC) of sour cherry pomace powder of 91.29 mg GAE/g dry weight, while Djilas et al. [27] highlighted that about 30% of TPC of fresh fruits is still retained in the pomace after juice processing. Moreover, Yilmaz et al.[30]

79 reported that of the phenolic compounds, cyanidin-3-glucosyl-rutinoside, neochlorogenic acid and
80 catechin were the most abundant ones found in the pomace. Besides, no literature data are available
81 on the influence of genotype on the nutraceutical quality of sour cherry pomace.
82 This agro-food waste can therefore represent a precious resource of potentially valuable molecules
83 both for the market of food ingredients and the nutraceutical sector. It is thus clear how the society
84 can derive environmental and economic benefit through better utilization of these resources.
85 However, it is necessary to stabilize pomace, eliminating the residual moisture, mainly to diminish
86 microbiological spoilage and increase its shelf life, but also to reduce the packaging costs, and to
87 lower the shipping weights. One of the oldest methods of food preservation is undoubtedly drying.
88 At present, different drying techniques are available, which affect matrix quality in different ways.
89 Convective drying, conventionally referred also as oven drying (OD), is one of the simplest and
90 cheapest drying methods for the stabilization of food matrices [31] with limited rehydration, and at
91 the same time it is an easy process to scale up. Besides, the technique has some negative aspects such
92 as a relative long duration and high temperature, which in some cases can compromise the quality of
93 the final product [32]. On the other hand, the quality of freeze-dried products is considered as the
94 highest of any dehydration techniques, though the process is longer and more expensive than OD.
95 To the best of our knowledge, there are no literature data about the effect of drying method and the
96 influence of genotype, as well as their interaction, on the yield of polyphenol extraction from sour
97 cherry pomace. Moreover, no data on the influence of these parameters on the anthocyanin profiles
98 and in vitro biological activity were reported.
99 In light of these considerations, the aim of the present study was to describe for the first time the
100 influence of two different drying methods, convective drying *versus* freeze-drying, on the
101 nutraceutical traits, single anthocyanin content and profile (evaluated by means of HPLC-DAD
102 analysis), and antiradical capacity of two single-cultivar sour cherry pomaces. Another goal of the
103 work was to assess the influence and interaction of chosen drying methods and genotype on the alpha-

Glucosidase inhibition capacity of sour cherry pomace in view of their valorization as a natural adjuvant in the treatment of type-2 diabetes.

Moreover, the interrelationships between the parameters analyzed, the genotypes and different drying treatments applied, as well as the relationships among variables, were investigated by means of the principal component analysis (PCA).

Material and methods

Chemicals

All reagents used were of analytical spectrophotometric grade (Carlo Erba, Rome, Italy). Folin-Ciocalteu reagent, gallic acid, catechin, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), vanillin, 2,2'-azinobis-(3-ethylbenzothiazolin-6-sulfonic acid) (ABTS), potassium persulfate, and vanillin were purchased from Sigma-Aldrich (Milan, Italy). Cyanidin 3-O-glucoside, cyanidin-3-rutinoside and peonidin-3-rutinoside standards used for identification and quantification purposes with HPLC were purchased from Extrasynthese (Genay, France) and Sigma-Aldrich (Milan, Italy). Organic solvents used for chromatography were of HPLC ultra gradient grade (Sigma Aldrich, Milan, Italy), whereas distilled water was obtained by the Milli-Q system (Millipore, Milan, Italy). The 45 µm pore size membrane filters were purchased from Pall (Pall Corporation, Ann Arbor, MI, USA) and were used for filtration of samples.

Plant materials

Single-cultivar pomaces (2.5 kg each one), from the juice processing of two different sour cherry varieties (a local "Morello or visciola" sour cherry selection recovered by Bianchi in Offagna AN, Italy, and Montmorency, namely BO and MM, respectively) grown under organic farming, were kindly provided by Italia Selvatica SRL Agricola Offagna, Italy. Pits, stems and other foreign materials were manually removed from pomaces, which were then stored at -20°C in low-density polyethylene bags until use. An aliquot of pomace (100g) was directly analysed and considered as

control samples (CTRs) whereas (400g) of each variety was subjected to oven drying (OD) at 60° C for 24 h (air velocity: 0.6 ms^{-1}, relative humidity < 0.5%, system power: 1.4 kW/h); model 600, Memmert GmbH + Co.KG, Schwabach, Germany), and the remaining part (400g) was freeze-dried (FD) at -54°C and 0.075 mbar for 72h (model Modulyo 4K, Edwards, United Kingdom). Sample dehydration using all the methods mentioned above was continued up to 9% final moisture content was reached. At the end of each drying treatment, pomaces were finely milled (sieve 0.5 mm) and kept protected from light and humidity until analysis. Three replicates for each treatment were carried on.

Phytochemical determinations

Extraction of bioactive compounds

Pomaces were extracted to determine their phytochemical content e.g., total flavan-3-ol content (FLC), total polyphenol content (TPC), total monomeric anthocyanins (TA), anthocyanin profile, antiradical capacity (AC) and α-Glucosidase inhibitory activity (AGA). A 1.0 g of pomace was placed in a 50-mL tube and extracted with 15 mL of a hydroalcoholic solution (methanol/water = 80:20, v/v) acidified with 0.1 % HCl (v/v), for 30 minutes, under continuous mechanical stirring at room temperature, followed by 30 minutes of ultrasound-assisted extraction. The extraction procedure was repeated twice using 15 mL of fresh solvent, and the two extracts were combined and filtered through Whatman no. 1 paper immediately before all the analyses. The obtained solution was analysed for anthocyanin content by HPLC-DAD and for all the following analysis.

Total monomeric anthocyanin content

The determination of total monomeric anthocyanin content of extracts was performed following the method of Lee [33], which is based on the structural change of the anthocyanin chromophore between pH 1.0 and 4.5. Briefly, 100µL of each extract were diluted in 3mL potassium chloride buffer (0.025M) at pH 1.0, whereas other 100µL were diluted in 3mL sodium acetate buffer (0.4M) at pH

4.5 and incubated for 30 minutes in the dark. The absorbance was read at 520nm and 700nm, respectively. The anthocyanin concentration was expressed as mg cyanidin-3-O-glucoside (cyd-3-glu) equivalents/g of dry weight (d.w.). All determinations were performed in triplicate.

Total flavan-3-ol content

FLC was determined following the vanillin assay method as reported by Carbone et al. [31]. FLC was calculated from a calibration curve, using catechin as a standard. Results were expressed as mg of catechin equivalents *per* g of dry weight (mg CTE/g d.w.). All determinations were performed in triplicate.

Total phenolic content

Total soluble polyphenol content (TPC) of extracts were determined according to Carbone et al. (2011) with same modifications. Briefly, sour cherry extract (50 μL) was mixed with 250 μL of Folin-Ciocalteu reagent (previously diluted to 1:1 ratio with double distilled water) and incubated for 3 min at room temperature (RT), then 750 μL of Na_2CO_3 solution (10% w/v) was added. After incubation at RT for 90 min, the absorbance of the solution was read at 765 nm using an UV-Vis spectrophotometer. TPC was calculated using a gallic acid calibration curve. Results were expressed as mg of gallic acid equivalents (GAE) *per* g of dry weight (mg GAE/g d.w.). All determinations were performed in triplicate.

Ascorbic acid content

Ascorbic acid content (AAC) was determined according to Petriccione et al. [32], with some modifications. Briefly, sour cherry pomaces (0.5 g each) were homogenized using 10 mL of 16% (v/v) metaphosphoric acid solution containing 0.18% (w/v) disodium ethylene diamine tetraacetic acid. The homogenate was centrifuged at 5000 *g* for 10 min, filtered and collected. The assay mixture contained 400 μL of extract, 200 μL of 3% metaphosphoric acid and 200 μL of diluted Folin's reagent

(1:5, v/v) in a final volume of 2 mL. After incubation for 10 min, the absorbance was measured at 760 nm using a UV-VIS spectrophotometer (Evolution 300 Thermo Scientific). AAC was calculated using an ascorbic acid calibration curve and results were expressed as milligrams of ascorbic acid (AA) *per* g of dry weight (mg AA/g d.w.). All determinations were performed in triplicate.

HPLC Anthocyanin profile of sour cherry pomaces

Anthocyanins were separated and identified by an analytical High Performance Liquid Chromatography (HPLC) system (Agilent 1100 series, Agilent, Milan, Italy) equipped with a Diode Array Detector (DAD; Agilent Technologies, Milan, Italy). The separation was carried out on a Zorbax SB C18 column (Agilent, 4.6 x 250 mm; 5 μm particle size, set at 30 °C), according to Carbone and Mencarelli, with minor changes [33]. The following mobile phase was used (flow rate 0.7 mL/min): solvent A, water with 2.5 % (v/v) formic acid (FA); solvent B, 30 % acetonitrile, 10 % methanol, 57.50 % water, and 2.5 % FA. The injection volume was 20 μL and samples were membrane-filtered (45 μm filters, Pall Corporation, Ann Arbor, MI, USA) before HPLC analysis. Anthocyanins were identified at 520 nm by their retention times and spectral data as compared to individual standards, when available, literature data [17, 18], and by the method of standard addition to the samples. Besides, UV-Vis spectra were recorded over the range 200–700 nm, at a spectral acquisition rate of 1.25 scans s^{-1} (peak width 0.2 min).

Analytical data were evaluated using a software-management system of chromatographic data (Chemstation 32.1, Agilent Technologies). All detectable anthocyanins identified in each sample were quantified by an external-matrix matched calibration method on the basis of the area ratios respect to the pure chemical standard cyanidin 3-glucoside and reported as its equivalents (mg/100 g dry weight). The standard curve of this compound showed excellent linearity over the concentration range of 3–50 ppm with correlation coefficient better than 0.9996 and nearly passed through the origin (data not shown). Relative standard deviations were less than 2%. The total anthocyanin content was calculated as the sum of all the chromatographic peaks identified. Stock solution of pure cyanidin 3-

glucoside was prepared in the same solvent of samples, namely methanol:water (80:20, v/v) acidified with 0.1 % HCl (v/v). All determinations were performed in triplicate.

In vitro biological assays

Antiradical capacity assays.

The radical scavenging power of the analyzed samples was assessed by measuring their ability to scavenge synthetic radicals (e.g., DPPH· and $ABTS^{\bullet+}$). ABTS radical cation decolourization assay was performed in 2.5 mL plastic cuvettes by pipetting 20 μL of extract (using the following dilution ratios: 1:4, 1:8, 1:12, 1:16, 1:24, 1:32, 1:64, and 1:128) into 980 μL of $ABTS^{\bullet+}$ radical solution and the absorbance was measured after 60 min at 734 nm. Results were expressed as mg of sample on dry weight required to obtain 50% $ABTS^{\bullet+}$ radical scavenging (EC_{50}) according to Carbone et al. [34].

The $DPPH^{\bullet}$ quenching capacity of extracts was determined spectrophotometrically as reported by Carbone et al. [34]. Results are expressed as mg of sample (on dry weight basis) required to obtain 50% DPPH scavenging (EC_{50}). All determinations were performed in triplicate.

α-Glucosidase inhibition assay

The enzymatic α-Glucosidase activity (AGA) of analysed samples was evaluated according to the Sigma-Aldrich enzymatic assay of α-Glucosidase [37], using p-nitrophenyl α-D-glucoside as substrate Briefly α-Glucosidase (0.075 unit) was premixed with the extract (200 μL). In addition, 3 mM p-nitrophenyl α-d-glucopyranoside (substrate) in phosphate buffer was added to the mixture to start the reaction. The reaction solution was incubated at 37°C for 30 min and then stopped by adding 2 mL of 0.1 M Na_2CO_3. α-Glucosidase activity was spectrophotometrically determined by measuring at 400 nm the release of p-nitrophenol from p-nitrophenyl α-d-glucopyranoside. The activity inhibition was expressed as percentage. All determinations were performed in triplicate.

Statistical analysis

234 Each sample was prepared in duplicate and the experiments were repeated at least in triplicate.
235 Results from all the tests were expressed as mean ± SD. Analysis Of Variance (ANOVA) and means
236 testing (Duncan's range test) were performed with level of significance set at $p \leq 0.05$ using MSTATC
237 software (Michigan State University, East Lansing, MI, USA). No parametric correlation of
238 Spearmen was calculated using SPSS 20 software (SPSS, Inc., Chicago, Illinois).
239 In addition, Principal Component Analysis (PCA) was carried out on normalized dataset for all
240 parameters to investigate the within-set data profile and to study the correlations between data.
241 Pearson correlation coefficients were also calculated as a measure of that association. These analyses
242 were performed using MATLAB software (R2010a version, MathWorks Inc., USA).
243

244 ## Result and discussion

245

246 ### Influence of genotype and drying method on the phytochemical content of single-cultivar sour
247 cherry pomace

248 Both genotype and drying method significantly influenced ($p \leq 0.001$) the overall phytochemical
249 content (total monomeric anthocyanin, total flavan-3-ol, total phenolic, and vitamin C) of analysed
250 pomaces, which resulted firstly influenced by the cultivar (about 70% of total variances), followed
251 by drying process (about 18%), and their interaction (about 12%), with the exception of FLC where
252 a similar contribution (about 50%) of the two factors was observed. On average, BO CTR samples
253 showed the highest amount of total monomeric anthocyanins (4.3 ± 0.1 mg cyd-3-O-glu/g) compare
254 to MM CTR ones (0.22 ± 0.1 mg cyd-3-O-glu/g d.w.; Fig. 1 A). Literature data highlight the influence
255 of genotype on TA of sour cherry fruits [12].
256 The influence of drying process on the TA of single-cultivar SCPs is shown in Fig. 1A. As it can see,
257 the overall TA was affected to a different extent by the drying process applied. In general, data pointed
258 out that FD was the most efficient method to preserve the original anthocyanin content of SCP,
259 whereas OD SCP samples showed a strong reduction of TA compared to control ones (-57% and -

32% for BO and MM, respectively). The anthocyanin loss following the oven-drying treatment, caused by oxidation, cleavage of covalent bonds or enhanced oxidation reactions, can be attributed to a degradation process due to the thermal processing of samples, as reported in literature [38, 39]. All BO samples analysed showed a TA content five times higher than the one found by Yilmaz et al. [30], which was similar to the one found in all MM samples.. These differences can be due to various items such as the cultivar, the expression of data (equivalents of cyanidin-3-O-rutinoside *vs* cyanidin-3-O-glucoside), the extraction solvent (ethanol *vs* methanol) and the drying process applied (oven drying samples at 40°C *vs* FD and OD at 60°C).

In the present study, different vitamin C content was observed between the two single-cultivar SCPs (Fig. 1B), being BO CTR samples the richest ones (2.5 ± 0.3 mg AA/g d.w.). To the best of our knowledge, no literature data are available on the vitamin C content of sour cherry pomace. Drying process influenced in a different manner the vitamin C recovery from pomace, in relation to the considered cultivars. A limited but significant reduction with respect to the control samples (-16 and -26% for BO and MM, respectively) was observed between the two cultivars when the FD process was applied. Whilst, a high decrease in ascorbic acid content was evidenced in BO and MM samples following the OD treatment (about -34 and -36%, respectively). This can be explained by a long exposure of the samples to oxygen during long drying operations like OD one as reported by Horuz et al. [41].

From the two single-cultivar SCPs investigated, BO CTR sample extracts showed the highest FLC (23.1 ± 0.3 mg CTE/g d.w.), which was twice than that of MM CTR ones (14.2 ± 0.1 mg CTE/g d.w.; Fig. 1B). Similar results were observed by Capanoglu et al. [42], who reported different composition and quantity of total (+) catechin, free (+) catechin, total (–) epicatechin, and free (–) epicatechin in three sour cherry fruit cultivars. FLC of analysed samples showed a similar trend to that observed for TA as a result of the different drying treatments applied. Although oven-drying represents a cheaper stabilization process compared to freeze-drying, it was not able to preserve completely the compounds under study, leading to a loss of FLC higher than 50% with respect to freeze-drying and

control samples, regardless of the cultivar (Fig. 1C). These results were in agreement with data on sour cherry fruit reported by Wojdyło et al. [19], who reported a drastic reduction of flavan-3-ols after hot drying at 60°C. However, the impact of different drying treatments applied on the FLC of SCPs analysed may be due not only to a degradation process following oven-drying treatment but could be also ascribed on how the applied process may influence the linkages of FLC to different molecules protecting or releasing them. This hypothesis was supported by many studies [40-44] that reported how phenolic compound extractions from food matrix could be promote or not by thermal treatments. As shown in Figure 1D, the highest average amount of total phenolic compounds was found in BO CTR samples (45 ± 1 mg GAE/g d.w.) compared to MM CTR ones (19.0 ± 0.5 mg GAE/g d.w.), highlighting a high variability due to the genotype as also observed in previous studies on sour cherries fruits [45-46]. TPC of BO and MM CTR samples were from 3.5 to 1.4 higher than that found by Yilmaz et al.[30] using a hydro alcoholic solution for polyphenol extraction from sour cherry pomace, where the Authors provided no information about the genotype used in the juice production. As well as observed for anthocyanins and total flavan-3-ols, a significant reduction of TPC was found in oven-dried pomaces compared to FD and CTR samples from both cultivars (-37 and -45%, respectively). Similar results were reported by Wojdyło et al. [19], who observed in sour cherry fruits a high amount of TPC in the freeze-dried samples compared with the hot-dried ones, highlighting a clear dependency between hot air temperature and polyphenols loss. The Authors pointed out a significant variability in the loss of polyphenols (44%, 48%, and 49%) in relation to different drying temperatures applied (50°C, 60°C, and 70°C, respectively). Irreversible oxidative processes that occur during drying process and by a long heat intensity exposure could explain this.

HPLC-DAD anthocyanin profile

The HPLC-DAD analysis carried out on all the filtered hydro alcoholic extracts, obtained from the differently stabilized single cultivar-pomaces as well as their control samples, revealed a specific anthocyanin profile. Five different anthocyanins were simultaneously found, separated and identified

312 in all pomace extracts analysed: cyanidin-3-sophoroside and cyanidin-3-glucosilrutinoside
313 (coeluted), cyanidin-3-O-glucoside, cyanidin-3-rutinoside, and peonidin-3-rutinoside.
314 After identification, the detectable anthocyanins were quantified on the basis of the area ratios of the
315 chromatographic peaks respect to pure cyanidin 3-O-glucoside, used as a standard, and the
316 corresponding results are reported in Table 1. Data about total anthocyanin content in untreated SCP
317 extracts pointed out a strong genotype effect [47]. BO CTR extracts showed a content of total
318 anthocyanins 75 times higher than that of MM CTR samples. Moreover, the two untreated SCP
319 samples exhibited RP-HPLC patterns that clearly differed from each other as MM CTR extracts
320 lacked specific compounds, namely peonidin-3-rutinoside. The anthocyanin patterns also showed
321 major quantitative differences on the single compounds, linked to the genotype. For instance, higher
322 amount of cyanidin-3-O-glucoside (about +26%) and lower levels of cyanidin-3-rutinoside (about
323 2%) were observed in BO samples compared to MM ones. Nevertheless, the major anthocyanin found
324 in all CTR extracts was cyanidin-3-glucosyl-rutinoside, corresponding to more than 50% of the
325 identified anthocyanins, regardless the cultivar considered, as also reported by Kołodziejczyk et al.
326 [48].
327 It is also interesting to note that the total anthocyanin contents obtained for control samples by HPLC
328 (Table 1) are slightly different from those obtained by the differential pH method.
329 From Table 1, it can be noted that the concentration of individual anthocyanins in the extracts varies
330 significantly with the drying process applied. Oven-drying process caused losses of all anthocyanins
331 detected in particular in BO samples, where the loss of each individual anthocyanin was more than
332 65% compared to its relative content in the CTR samples. Besides, limited losses (lower than 20%)
333 of these bioactive compounds were recorded in FD samples with respect to the controls, regardless
334 the cultivars. These results may be explained taking into account the different behaviour of different
335 anthocyanins to thermal treatments due to their different chemical structure. At this regard,
336 Rubinskiene et al. [49] demonstrated that cyanidin-3-O-rutinoside showed the highest stability after
337 a thermal treatment at 95 °C with respect to the other fractions present in blackcurrants.

Influence of genotype and drying method on the in vitro biological activity of sour cherry pomace

In the present study, it was observed that the antiradical capacity (AC) of single-cultivar SCPs changed due to the different drying treatments applied, following the same trend previously reported for the phytochemicals. As well known, TPC of raw materials is largely responsible of their antiradical capacity. Higher EC_{50} values (corresponding to a lower antiradical capacity) were observed in MM samples, which showed the lowest phytochemical content (Table 2). Khoo et al. [12] observed a similar behaviour and variability in the phenol content and antiradical capacity of different sour cherry cultivars. As regards the stabilization treatments, OD samples showed the highest EC_{50} values compared to FD ones, according to their phytochemical contents. This results were similar to that found by Wojdyło et al. [19] for sour cherry fruit but in contrast with those reported by Larrauri et al. [50], who did not noticed any difference in the antioxidant activity between freeze-dried and hot-dried (60°C) grape peel samples.

Type 2 diabetes is on the rise worldwide. Anthocyanins are reported to have a positive impact on factors involved in this common disease. Seymour et al. [51] showed in an obese rat model that the intake of 1% tart cherry powder with the diet reduced the risks associated with the metabolic syndrome. Moreover, many studies evidenced that phenolic compounds such as anthocyanins, and flavan3-ols exert inhibitory effects on AGH [52,53], highlighting a potential interaction of these compounds with the human glucose metabolism for the reduction of the type-2 diabetes onset. In light of these considerations, and in order to explore the hypoglycemic function of SCPs, all the extracts analysed were evaluated also for their α-Glucosidase inhibitory power. As reported in Table 2, BO CTR extracts showed the highest inhibitory activity (83 ± 1%), followed by BO-FD > MM-CTR > MM-FD > BO-OD > MM-OD.

Besides, a positive and significant correlation was observed among the percentage of α-Glucosidase inhibition and all parameters analysed (Table 3) except for the antiradical capacity. The α-

Glucosidase inhibition by anthocyanins was also observed by McDougal et al. [54], who reported a strong relation between the anthocyanin content of extracts from red fruits (strawberry, raspberry, blueberry, blackcurrant, or red cabbage) by-products and their inhibitory activity.

However, we cannot exclude that other compounds present in the extracts but not characterized herein could be also play a role in the α-Glucosidase inhibition by SCPs.

Principal component analysis (PCA)

To better understand the interrelations among all the parameters analysed, genotype, and technological treatments applied the entire dataset was normalized and subjected to principal component analysis (PCA).

The bi-plot of PCA1 vs PC2 reported in Fig. 3 shows the scores of the two single-cultivar pomaces analysed for each thermal treatment applied, related to the different parameters included in the analysis, in which each loading vector represents one of the parameters under study and the proximity of vectors is indicative of the correlation among parameters.

The first component, which accounted for 80.2% of the total variance, was associated with TA, Cya^{X1}, Cya^{X2}, Cya^{3}, Cya^{4}, Peo^{5}, and TPC. PC2 (accounting for 10.1%) was mainly associated with AC_{DPPH} and AC_{ABTS}. AGA and FLC were associated at both components in the same manner. The chemometric analysis permits to group the samples into two major clusters, each showing two subpopulations in agreement with the different stabilization process applied. The main cluster located in the negative side of PC1 grouped all the samples of MM cultivar, whereas BO was grouped in the positive side of same component. Besides, freeze-dried samples were mainly separated from oven-dried ones due to their higher phenol content and antiradical capacity. Interestingly, FD samples clustered with the corresponding CTR samples, regardless the genotype, indicating a preservation of the original nutritional and nutraceutical characteristics following the lyophilisation adopted as a stabilization process.

The bi-plot showed a positive correlation among Cya^{X1}, Cya^{X2}, Cya^{3}, Cya^{4}, Peo^{5}, and TA (always higher then $r = 0.97$, $p < 0.05$); between TPC and TA, and between TPC and FLC ($r = 0.97$; $p < 0.05$ and $r = 0.94$; $p < 0.05$, respectively). Low but positive correlations were found among TPC, FLC, TA, Cya^{X1}, Cya^{X2}, Cya^{3}, Cya^{4}, Peo^{5}, and AAC ($r \geq 0.97$, $p < 0.05$). Very good negative correlations were found between AC_{DPPH} and TPC; AC_{DPPH} and TA, AC_{ABTS} and FLC ($r = 0.91$; $p < 0.05$, $r = 0.91$; $p < 0.05$ and $r = 0.92$; $p < 0.05$, respectively), whereas no significant correlations were found among other parameters (data not shown). These results were in agreement with a previous study of Dragović-Uzelac et al. [46], who reported a direct correlation between AC_{DPPH} and TA content of fruit extracts ($r = 0.89$, $p < 0.05$), whereas using ABTS method to determine the antiradical capacity only a weak correlation was found ($r = 0.5$, $p < 0.05$). Moreover, the Authors reported a remarkable correlation between TPC and ABTS ($r = 0.84$, $p < 0.05$) and FLC and ABTS, whereas AC determined using other methods was not in correlation with TPC and FLC.

Conclusion

Agro-industrial biomass is rich in high added value compounds, mainly antioxidants and fibres, which once extracted and stabilized can serve as green fine chemicals or can be used in food supplements and/or nutraceutical sector.

In the present study, pomace from sour cherry juice processing was investigated as a valuable and low-cost source of bioactive compounds. Influence of genotype and thermal stabilization treatment on the phytochemical content, anthocyanin profile and in vitro biological activities were evaluated. Results showed a strong influence of genotype on the phytochemical content of SCP and how different drying treatments affected the stability and availability of these compounds to a different extent depending on the cultivar. These findings highlight that the choice of the genotype is important not only in order to obtain juices of a higher quality, but also to increase the market potential when a circular economy model for the exploitation of waste is applied to the production process.

Generally, freeze-drying process, commonly applied to preserve the native characteristics of the matrices, did not determine remarkable changes in the antioxidant profiles, whereas a significant decrease was observed when oven- drying was applied. Besides, this study has demonstrated that stabilized FD SCP may be a potential resource for developing not only antioxidant supplements for nutraceutical market but also a low cost coadjuvant in the type-2 diabete treatment. In conclusion, this work gives new insights into the valorization of agroindustrial by-products, which are now more than ever a valuable asset in a bio-economy approach to the management of agro-food pipeline.

Acknowledgments: This research was carried out within the project "Wild Cherry" (prot. N. 40343, 07/03/2015) funded by Italia Selvatica srl Agricola.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

References

1. Germano G, Hoes A, Karadeniz S, Mezzani A, Prescott E, Ryden L, Vrints C (2012) European Guidelines on cardiovascular disease prevention in clinical practice. Eur Heart J 33: 1635-1701.
2. Annunziata A, Vecchio R (2011) Functional foods development in the European market: A consumer perspective. J Funct Foods 3: 223-228.

438 3. Food and Agriculture Organization of the United Nations, 2014; http://faostat.fao.org. Accessed
439 10 Ago 2016
440 4. Burkhardt S, Tan D X, Manchester L C, Hardeland R, Reiter R J (2001). Detection and
441 quantification of the antioxidant melatonin in Montmorency and Balaton tart cherries (Prunus
442 cerasus). J Agric Food Chem 49: 4898-4902.
443 5. Pedišić S, Levaj B, Dragović-Uzelac V, Škevin D, Skendrović Babojelić M. (2009). Color
444 parameters and total anthocyanins of sour cherries (Prunus cerasus L.) during ripening. Agric
445 Conspec Sci 74: 259-262.
446 6. Rodrigues L C, Morales M R, Fernandes A J B, Ortiz J M (2008). Morphological characterization
447 of sweet and sour cherry cultivars in a germplasm bank at Portugal. Genet Resour Crop Evol 55:
448 593-601.
449 7. Brown SK, Way RD, Terry DE (1998). Sweet and tart cherry varieties: description and cultural
450 recommendations. New York Food and life Sci Bull 127: 1-8.
451 8. Sarić A, Sobocanec S, Balog T, Kusić B. Sverko V, Dragović-Uzelac V, Levaj B, Cosić Z, Macak
452 Safranko Z, Marotti T (2009). Improved antioxidant and anti-inflammatory potential in mice
453 consuming sour cherry juice (Prunus Cerasus cv. Maraska). Plant Foods Hum Nutr 64: 231–237.
454 9. Kim JS, Kwon CS, Son KH (2000). Inhibition of alpha-glucosidase and amylase by luteolin, a
455 flavonoid. Biosci Biotechnol Biochem 64: 2458–2461.
456 10. Khoo G M, Clausen M R, Pedersen B H, Larsen E (2011) Bioactivity and total phenolic
457 content of 34 sour cherry cultivars. J Food Compos Anal 24: 772-776.
458 11. Kuehl K S, Perrier E T, Elliot D L, Chesnutt J C (2010). Efficacy of tart cherry juice in
459 reducing muscle pain during running: A randomized controlled trial. JISSN 7: 1-7
460 12. Ferretti G, Bacchetti T, Belleggia A, Neri D. (2010). Cherry antioxidants: from farm to table.
461 Molecules 15: 6993-7005.

13. Ferretti G, Neri D, Bacchetti T (2014). Effect of Italian sour cherry (Prunus cerasus L.) On the formation of advanced glycation end products and lipid peroxidation. Food and Nutrition Sciences 5: 1568.

14. McDougall G J, Kulkarni N N, Stewart D (2008). Current developments on the inhibitory effects of berry polyphenols on digestive enzymes. BioFactors 34: 73–80.

15. Montagut G, Onnockx S, Vaqué M, Bladé C, Blay M, Fernández-Larrea J, Ardévol A (2010). Oligomers of grape-seed procyanidin extract activate the insulin receptor and key targets of the insulin signaling pathway differently from insulin. J Nutr Biochem 21: 476-481.

16. Bhandari M R, Jong-Anurakkun N, Hong G, Kawabata J (2008). α-Glucosidase and α-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (Bergenia ciliata, Haw). Food Chem *106*: 247-252

17. Bonerz D, Würth K, Dietrich H, Will F. (2007). Analytical characterization and the impact of ageing on anthocyanin composition and degradation in juices from five sour cherry cultivars. Eur Food Res Technol 224: 355-364

18. Chandra, A., Rana, J., & Li, Y. (2001). Separation, identification, quantification, and method validation of anthocyanins in botanical supplement raw materials by HPLC and HPLC− MS. J Agr Food Chem 49: 3515-3521.

19. Wojdyło A, Figiel A, Lech K, Nowicka P, Oszmiański J (2014) Effect of convective and vacuum–microwave drying on the bioactive compounds color and antioxidant capacity of sour cherries. Food Bioprocess Technol 7: 829-841

20. Blando F, Gerardi C, Nicoletti I. (2004). Sour cherry (Prunus cerasus L) anthocyanins as ingredients for functional foods. BioMed Res Int 5: 253-258.

21. Picariello G, De Vito V, Ferranti P, Paolucci M, Volpe M G (2016). Species- and cultivar-dependent traits of Prunus avium and Prunus cerasus polyphenols. J Food Compos Anal 45: 50-57.

22. Damar I; Eksi A (2012) Antioxidant capacity and anthocyanin profile of sour cherry (Prunus cerasus L.) juice. Food Chem 135: 2910-2914.

23. Kim D O, Padilla-Zakour O I (2004) Jam processing effect on phenolics and antioxidant capacity in anthocyanin-rich fruits: cherry, plum, and raspberry. J Food Sci 69: 395-400.

24. Sumic Z, Tepic A, Vidovic S, Jokic S, Malbasa R (2013) Optimization of frozen sour cherries vacuum drying process. Food Chem 136: 55-63.

25. Chaovanalikit A, Wrolstad R E (2004). Total anthocyanins and total phenolics of fresh and processed cherries and their antioxidant properties. J. Food Sci., 69:67-72.

26. Barrett D M, Somogyi L, Ramaswamy H S. (2004). Processing fruits: science and technology. CRC Press, New York.

27. Dilas J, Eggers S. (2016). Integrating strategic noise management into the operation and maintenance of national road networks. Final Programme Report, Germany.

28. European Commission (2015) Communication from the commission to the european parliament, the council, the european economic and social committee and the committee of the regions. Closing the loop - An EU action plan for the Circular Economy. http://eur-lex.europa.eu/legal-content/ Accessed 10 Ago 2016

29. Cilek B, Luca A, Hasirci V, Sahin S, Sumnu G (2012). Microencapsulation of phenolic compounds extracted from sour cherry pomace: effect of formulation, ultrasonication time and core to coating ratio. Eur Food Res Technol *235:* 587-596.

30. Yılmaz F M, Karaaslan M, Vardin H (2015). Optimization of extraction parameters on the isolation of phenolic compounds from sour cherry (Prunus cerasus L.) pomace. *J Food Sci Tech Mys, 52:* 2851-2859.

31. Lewicki P P (2006). Design of hot air drying for better foods. Trends Food Sci Tech 17:, 153–163.

511 32. Michalska A, Wojdyło A, Lech K, Łysiak G P, Figiel A (2016). Physicochemical properties of
512 whole fruit plum powders obtained using different drying technologies. Food Chem 207: 223-
513 232.
514 33. Lee J, Durst R W, Wrolstad R E (2005) Determination of total monomeric anthocyanin
515 pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential
516 method: collaborative study. J AOAC int. 88: 1269-1278.
517 34. Carbone K, Giannini B, Picchi V, Lo Scalzo R, Cecchini F (2011) Phenolic composition and
518 free radical scavenging activity of different apple varieties in relation to the cultivar, tissue type
519 and storage. Food Chem 127: 493-500.
520 35. Petriccione M, Mastrobuoni F, Pasquariello M S, Zampella L, Nobis E, Capriolo G,
521 Scortichini M (2015). Effect of chitosan coating on the postharvest quality and antioxidant enzyme
522 system response of strawberry fruit during cold storage. Foods 4: 501-523.
523 36. Carbone K, Mencarelli F (2015). Influence of short-term postharvest ozone treatments in
524 nitrogen or air atmosphere on the metabolic response of white wine grapes. Food Bioprocess
525 Technol 8: 1739-1749.
526 37. Enzymatic Assay of α-GLUCOSIDASE (1996) Sigma-Aldrich technical bulletin
527 https://www.sigmaaldrich.com/content/dam/sigma-
528 aldrich/docs/Sigma/General_Information/alpha_glucosidase_sed.pdf. Accessed 25 Jul 2016
529 38. Patras A, Brunton N P, O'Donnell C, Tiwari B K. (2010). Effect of thermal processing on
530 anthocyanin stability in foods; mechanisms and kinetics of degradation. Trends Food Sci Technol
531 21: 3-11.
532 39. Adams J B (1973). Thermal degradation of anthocyanin with particular reference on 3
533 glucosides of cyanidin. In acidified aqueous solution at 100 C. J Sci Fd Agric 24: 747-762
534 40. Lee S K, Kader A A (2000). Preharvest and postharvest factors influencing vitamin C content
535 of horticultural crops. Postharvest Biol Tec 20: 207-220

536 41. Horuz E, Bozkurt H, Karataş H, Maskan M (2017) Effects of hybrid (microwave-convectional)
537 and convectional drying on drying kinetics, total phenolics, antioxidant capacity, vitamin C, color
538 and rehydration capacity of sour cherries. Food Chem doi.org/10.1016/j.foodchem.2017.03.046
539 42. Capanoglu E, Boyacioglu D, de Vos R C, Hall R D, Beekwilder J (2011) Procyanidins in fruit
540 from Sour cherry (Prunus cerasus) differ strongly in chainlength from those in Laurel cherry
541 (Prunus lauracerasus) and Cornelian cherry (Cornus mas). J Berry Res 1: 137-146.
542 43. Piga A, Romeo F V, Poiana M, Del Caro A, Sanguinetti A M, Piscopo A (2009) Effect of
543 drying temperature on polyphenolic content and antioxidant activity of apricots. Eur Food Res Tec
544 228: 441-448.
545 44. Del Carlo A, Piga A, Corda G (2004) Effect of drying conditions and storage period on
546 polyphenolic content antioxidant capacity and ascorbic acid of prunes. J Agr Food Chem 52: 4780-
547 4784.
548 45. Kim D O, Padilla-Zakour O I (2004) Jam processing effect on phenolics and antioxidant
549 capacity in anthocyanin-rich fruits: cherry, plum, and raspberry. J Food Sci 69: 395-400.
550 46. Dragović-Uzelac V, Levaj B, Bursać D, Pedisić S, Radojčić I, Biško A. (2007) Total phenolics
551 and antioxidant capacity assays of selected fruits. Agric Conspectus Sci 72: 279-284.
552 47. González-Gómez D, Lozano M, Fernández-León M F, Bernalte M J, Ayuso M C, Rodríguez
553 A B. (2010). Sweet cherry phytochemicals: Identification and characterization by HPLC-
554 DAD/ESI-MS in six sweet-cherry cultivars grown in Valle del Jerte (Spain). J Food Comp Anal
555 23: 533-539
556 48. Kołodziejczyk K, Sójka M, Abadias M, Viñas I, Guyot S, Baron, A. (2013). Polyphenol
557 composition, antioxidant capacity, and antimicrobial activity of the extracts obtained from
558 industrial sour cherry pomace. Ind Cro Pro, 51: 279-288.
559 49. Rubinskiene M, Viskelis P, Jasutiene I, Viskeliene R, Bobinas C (2005) Impact of various
560 factors on the composition and stability of black currant anthocyanins Food Res Int 38: 867-871.

561 50. Larrauri J A, Rupérez P, Saura-Calixto F (1997) Effect of drying temperature on the stability
562 of polyphenols and antioxidant activity of red grape pomace peels. J Agr Food Chem 45: 1390-
563 1393.
564 51. Seymour E M, Singer A A, Kirakosyan A, Urcuyo-Llanes D E, Kaufman P B, Bolling S F.
565 (2008). Altered hyperlipidemia, hepatic steatosis, and hepatic peroxisome proliferator-activated
566 receptors in rats with intake of tart cherry. J Med Food 11: 252-259.
567 52. Matsui T, Ueda T, Oki T, Sugita K, Terahara N, Matsumoto K (2001) α-Glucosidase
568 inhibitory action of natural acylated anthocyanins. 2. α-Glucosidase inhibition by isolated acylated
569 anthocyanins. J Agr Food Chem 49: 1952-1956.
570 53. Ma C M, Sato N, Li X Y, Nakamura N, Hattori M (2010). Flavan-3-ol contents, anti-oxidative
571 and α-glucosidase inhibitory activities of Cynomorium songaricum. Food Chem 118: 116-119.
572 54. McDougall G J, Shpiro F, Dobson P, Smith P, Blake A, Stewart D. (2005). Different
573 polyphenolic components of soft fruits inhibit α-amylase and α-glucosidase. J Agr Food Chem 53:
574 2760-2766.
575