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Abstract

There has been a growing interest in the recovery and valorization of chemical products from biomass wastes. In the present study pomace from sour cherry liquor was analyzed in order to evaluate its potential for valorization. Two different samples of liquor pomace and two different extraction methods were screened through determination of their phenolic content (HPLC/PDA) and antioxidant activity (FRAP and DPPH assay). Results obtained showed that skins (pomace without kernel) presented a higher extraction yield, polyphenolic content and antioxidant activity than pomace with kernel (skin+kernel). Decoction at 100 °C allowed a higher recovery of phenolic compounds, but, maceration with water at 25 °C was considered a more sustainable process. HPLC analyses allowed the identification and quantification of phenolic compounds such as cyanidin-3-*O*-glucoside, (+)catechin and (−)epicatechin and some phenolic acids. The analyzed by-products might be a promising source of natural polyphenolic compounds, which can act as a new eco-friendly antioxidant ingredient, with potential to be incorporated in nutraceutical formulations or applied in food or cosmetic industries. The residues remaining after extraction have a high calorific value and fat content, suggesting its valorization as a source of energy or through the extraction of value-added oil.

Keywords (separated by '-')	Agro-industrial waste - Antioxidant activity - Biorefinery - Liquor industries - Phenolic compounds - Sour cherry liquor
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ORIGINAL PAPER

Evaluation of Industrial Sour Cherry Liquor Wastes as an Ecofriendly Source of Added Value Chemical Compounds and Energy

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Abstract

There has been a growing interest in the recovery and valorization of chemical products from biomass wastes. In the present study pomace from sour cherry liquor was analyzed in order to evaluate its potential for valorization. Two different samples of liquor pomace and two different extraction methods were screened through determination of their phenolic content (HPLC/PDA) and antioxidant activity (FRAP and DPPH assay). Results obtained showed that skins (pomace without kernel) presented a higher extraction yield, polyphenolic content and antioxidant activity than pomace with kernel (skin+kernel). Decoction at 100 °C allowed a higher recovery of phenolic compounds, but, maceration with water at 25 °C was considered a more sustainable process. HPLC analyses allowed the identification and quantification of phenolic compounds such as cyanidin-3-*O*-glucoside, (+)catechin and (−)epicatechin and some phenolic acids. The analyzed by-products might be a promising source of natural polyphenolic compounds, which can act as a new eco-friendly antioxidant ingredient, with potential to be incorporated in nutraceutical formulations or applied in food or cosmetic industries. The residues remaining after extraction have a high calorific value and fat content, suggesting its valorization as a source of energy or through the extraction of value-added oil.

Keywords Agro-industrial waste · Antioxidant activity · Biorefinery · Liquor industries · Phenolic compounds · Sour cherry liquor

Statement of Novelty

The aim of this work was to evaluate industrial sour cherry liquor wastes as an ecofriendly source of added value chemical compounds and energy. The study focused on the analyses made to two different samples of liquor pomace (skins and skins+kernel) and on the obtained extracts from two different methods (decoction at 100 °C and maceration with water at 25 °C). The residual fraction obtained after extraction was also analyzed towards its valorization for energy. As far as we know, information about the recovery of sour cherry liquor by-products and their chemical and antioxidant evaluation has not been thoroughly described by other authors. Moreover, information concerning the chemical composition (ash, fat, sugar and nitrogen content) of the remaining residues in order to evaluate their potential to be valorized for energy production was not yet described before. Additionally, the study shows the prospects of using an ecofriendly and more sustainable process (maceration with water at 25 °C) applied to recover added value-products, compared to a process that demands more energy (decoction at 100 °C) and that causes degradation of some compounds due to the effect of high temperature. The authors believe that this work will add up to the (scarce) information about the recovery of pomace by-products of

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sour cherry liquor industry, their transformation into eco-friendly extracts, and the potential of bio-active products from those extracts to promote health.

Introduction

In the European Union the production of large amounts of waste and by-products is a concern. According to the European Environment Agency [1], it has been estimated that around 89 million tons of food waste was generated in the EU in 2006, of which 39% were food industry waste. These by-products are wasted without any valorization and deposited in soil and water, giving rise to environmental issues. Thus, reducing the environmental impact of industrial by-products, maximizing their recovery and recycling has been a challenge in recent years [2].

According to the recent circular economy development model it is possible to build a more harmonious society that efficiently manage all natural resources in a sustainable way by increasing the share of renewable and recyclable resources while reducing the consumption of raw materials and energy as well as the emissions and losses of material. In this perspective, the recovery and recycling of by-products and materials and their conversion into new and low cost interesting products, will play a significant role in maintaining the utility of products, components and materials and retaining their value, reducing the environmental pollution generated at each step and in saving resources [3–5].

There are many scientific works concerning the recovery of phenolic compounds from food industry by-products. In fact, in most cases those by-products can be regarded as potential sources of antioxidants that might be interesting for different applications in pharmaceutical, food or cosmetic industries [6–8]. Some authors have reported lycopene extraction from tomato waste [9] or some applications of by-products derived from grape marc [10], pomegranate leaves and peels [11] or olive oil production [12, 13], including its wastewater [14, 15], and olive leaves [16], from which antioxidant compounds such as hydroxytyrosol, tyrosol and oleuropein may be extracted [17]. Even the production of fruit juices and the transformation of fruit and vegetables have shown to be also a potential source of polyphenols, which are still mainly located in the skins of those by-products [18–21]. Other examples studied are the citrus, apples and tomato industries that produce, as by-products, a large amount of skins which could have an even higher phenolic content than that of the edible part of the fruit [22–25]. Thus, the recovery of these pomaces seems to be a natural way to obtain active food-value ingredients, rich in bioactive compounds such anthocyanins and other phenolics, and that can substitute synthetic food colorants and antioxidant ingredients [2, 26].

Sour cherry (*Prunus cerasus* L.) is an acid fruit widely used for liquors production that can be found in many countries of Europe and Southwestern Asia. These liquors are produced in several industrial units and have a typical taste that makes them one of the most appreciated beverages all over the world. In the manufacturing process, fruits are first infused in an alcoholic solution for about 12 months, to extract all the aromatic and color compounds, and afterwards separated from the liquid using a press (crush). At the end of the process the juice of the fruit is mixed with sugar and alcohol to make the final product [27]. However, this manufacturing process generates many by-products, mainly skins and kernels (20% of the cherry), arising from industrial pressing and that are normally discarded without any valorization or environmental concerns. Only a small percentage of those wastes have other final destination. Some authors have reported their transformation into organic fertilizer or their distillation for ethanol production, but without any recovery of the bioactive compounds [18, 27–29].

In recent years, many studies on sour cherries have revealed that they are rich sources of bioactive compounds, due to their polyphenolic phytochemicals, mainly phenolic acids and flavonoids, and nutritional value [30–32]. The most common flavonoids identified by several authors on cherries and sour cherries are essentially anthocyanins, such as cyanidin, flavonols, such as quercetin, kaempferol and rutin, and flavanols, such as (+)catechin and (−)epicatechin. These compounds have showed a high antioxidant capacity and other pharmacological properties [25, 33–36]. Those compounds are mainly located in the fruit skin and contribute to its organoleptic and sensory properties, such as taste or astringency [37]. Besides providing fruit color, anthocyanins from cherries may be behind several of the important health benefits attributed to those fruits. They are reported to exhibit antioxidant [38–42], anti-inflammatory [11, 38, 43, 44], anticancer [17, 45], and anti-diabetic activities [46], and protective properties against cardiovascular and neurodegenerative diseases [47, 48].

However, information about the recovery of pomace by-products of sour cherry liquor industry, their transformation into ecofriendly extracts, and the potential of those extracts to promote health, has not been thoroughly described in literature and no previous studies were performed concerning their composition and antioxidant activity. Moreover, information concerning the potential of the remaining residue to be valorized for energy production was not yet describe. Therefore, this work aimed to investigate the transformation of these by-products in extracts with biological properties that can act as functional ingredients when added to food, nutraceuticals and cosmetics. For this, the phenolic profile and antioxidant activity of two different samples of pomace from sour cherry liquor industry, namely skin and a mix of skin and kernels, were determined in order to evaluate

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their bioactive potential. Additionally, the chemical composition (organic matter, fat, sugar and nitrogen content) of the remaining residue was also analyzed in order evaluate its potential as an energy source with the perspective of total recycling of by-products reducing its environmental impact.

Materials and Methods

Sample Preparation

Plant and fruits of sour cherry *P. cer*asus L. were collected in Sobral, Óbidos and voucher specimens were deposited in *herbarium* "João de Carvalho e Vasconcellos", of the "Instituto Superior de Agronomia", Lisbon, Portugal, with identification LISI-345/2013, 2013. At the production unit fruits were squeezed and crushed to collect the liquid for the manufacture of the liquor. After crushing many by-products are generated mainly composed by a mixture of skins and kernels (pomace). Skin represents about 24% of the total pomace (skin+kernel) weight. Studied pomace was provided by Frutóbidos liquor industry, from Óbidos, Portugal. The pomace was transported in cold conditions to the laboratory where it was divided in two halves: pomace (skin+kernel) and skin. The skin fraction was obtained from the pomace by manual separation. Skin was also characterized because it is known that phenolic compounds are mainly located in the skin of the fruits. Samples were than stored at − 80 °C for further analysis.

Reagents and Reference Compounds

Folin–Ciocalteu (FC) reagent, gallic acid and sodium carbonate were purchased from VWR (Leuven, Belgium). 2,4,6-Tri(2-pyridyl)-s-triazine) (TPTZ) were purchased from Fluka (Steinheim, Alemanha) and iron chloride(III) hexa-hydrated 97%, potassium chloride and sodium acetate tri-hydratated 99,9% from Panreac, (Barcelona, Spain). 2,2-Diphenyl-1-picrylhydrazyl (DPPH); methanol, chlorogenic and *p*-coumaric acids, kaempherol and cyanidin-3-glucoside were purchased from Sigma-Aldrich (Steinheim, Germany). Procatechuic, ferulic, *p*-hydroxybenzoic and vanillic acids, as well as, catechin, epicatechin, quercetin-3-*O*-glucoside and kaempherol-3-*O*-rhamno-glucoside were isolated and provided by Doctor Ana M. Díaz-Lanza, from the University of Alcalá de Henares; Madrid, Spain.

Extract Preparation

Two kinds of samples were used for extract preparation: pomace (skin+kernel) and skins after manual removal of the kernels (skin). Samples were first ground in a coffee mill for 3 min, in order to reduce the particle size and increase the surface area, and then homogeneously mixed and riddled in a Retsch®-Test Sieve up to a particle diameter of 0.5 mm. The extraction was performed by maceration with water at 25 °C during 24 h and by decoction in boiling water for 15 min. All the extracts were prepared with the proportion 1/10 plant/water, under constant stirring (900 rpm) and reduced-light conditions. Finally, samples were filtered through quantitative filter paper (P5; Fisher Scientific, Pittsburgh, PA, USA) and freeze-dried (Freezone 2.5 L, Freeze-dryer Labconco, Kansas City, MO, USA). The dry weight of each extract was determined in percentage of yield. All the extracts were stored at − 20 °C for further analysis. All the analytical procedures were performed in triplicate.

Total Phenolic Content

The total phenolic content (TPC) was determined according to the modified FC colorimetric method using gallic acid as a standard [49]. Briefly, 100 µL of each extract (or its dilutions) were mixed with 500 µL of FC reagent and 1.5 mL of sodium carbonate 20% (w/v), and water up to 10 mL. The absorbance at 765 nm of the blue coloration formed was measured after 2 h of incubation in the dark at 25 °C against the blank standard. TPC was calculated with respect to gallic acid standard curve (concentration range 50–500 mg/L). Results are expressed in mg equivalent of gallic acid (GAE)/100 g of fresh biomass.

Total Anthocyanin Content

Total anthocyanins content were estimated by the pH differential method [31, 36]. Absorbance was measured at 510 and 700 nm in buffers at pH 1.0 (potassium chloride, 0.025 M) and pH 4.5 (sodium acetate 0.4 M). Results were expressed in mg of cyanidin 3-glucoside equivalents (EC3G)/100 g of fresh biomass, using a molar extinction coefficient of 29,600 L/mol/cm.

Antioxidant Capacity

DPPH Assay

Radical scavenging capacity was determined by the DPPH assay. Briefly diluted samples were added to the DPPH methanolic solution (0.1 mM) and the absorbance was measured at 517 nm immediately and after 30 min. IC_{50} was defined as the dry matter content of the sample in µg/mL, required for decreasing the initial DPPH concentration by 50% [50].

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FRAP Assay

The FRAP assay was carried according to the procedure described by Ramful et al. [51]. The principle of this method is based on the ability of substances to reduce Fe(III)-2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ). The absorbance was read at 593 nm after 4 min incubation at 37 °C. A calibration curve of ferrous sulphate (0–1.2 mM) was used and results were expressed as mmol Fe^{2+}/100 g fresh biomass.

High-Performance Liquid Chromatography (HPLC) Analysis

Phenolic profile of the extracts was evaluated by HPLC, using a HPLC system (DIONEX ICS3000) equipped with a photodiode array detector (PDA) (ICS series DIONEX), and 3.9 × 150 mm Novapak C18 column from WATERS (Massachusetts, USA). Extracts were prepared and diluted in methanol:water (70:30, v/v) and analyzed by HPLC, injecting 20 µL at 30 °C and using a two-solvent gradient elution. The solvent compositions used were A: water–methanol–acetic acid (88:10:2, v/v/v) and B: water–methanol–acetic acid (8:90:2, v/v/v).

The solvent composition changed according to the following gradient: 0–10 min 100% A; 25 min 85% A + 15% B; 35 min 50% A + 50% B; 35–44 min 50% A + 50% B; 50 min 30% A + 70% B. The flow rate was set at 0.5 mL/min. Detection was carried out between 200 and 550 nm with a PDA detector. Retention times and UV–Vis absorbance spectra were used to identify the different phenolic compounds. Quantification of polyphenols was performed using calibration curves (peak area vs. concentration) obtained with individual standards prepared and diluted in methanol: water (70:30, v/v) and analyzed in the same conditions. Results were expressed as µg/100 g fresh biomass. All analyses were performed in triplicate.

Chemical Analyses of the Remaining Residue

The chemical composition of the residue obtained after pomace/skin extract preparation was determined in order to evaluate its potential as an energy source. Thus the residues were analyzed for moisture, protein, fat, carbohydrates and ash. Moisture was determined by the loss of weight after drying at 105 ± 2 °C (2 h), repeated until constant weight, expressed in relation to a wet sample [52]. Ash content was determined by incineration in a muffle at 550 ± 50 °C [52]. The crude protein content was estimated from the total nitrogen Kjeldahl content (N × 6.25) determined after mineralization with H_2SO_4, distillation and titration with H_2SO_4 0.02N [52]. The crude fat was determined gravimetrically after extraction with petroleum ether in a Soxhlet apparatus [52]. Total carbohydrates were calculated by difference: Total carbohydrates = 100 − (g moisture + g protein + g fat + g ash) [53]. Total energy was calculated according to the following equation: Energy (kcal) = 4 × (g protein + g carbohydrate) + 9 × (g lipid) [53].

Statistical Analysis

Data are expressed as mean ± standard deviation. Statistical analysis was performed by Kruskal–Wallis-test using SPSS Statistics® software, version 22.0, Chicago, USA. Results with $p < 0.05$ were considered significantly different.

Results and Discussion

Results from extraction yields, TPC, anthocyanin content, and antioxidant activity of aqueous pomace (skin with and without kernels) extracts prepared by decoction at 100 °C (W100) or maceration at 25 °C (W25) are presented in Table 1. Results showed that in general, extracts from pomace without kernel (skins) showed a higher extraction yield, phenolic and anthocyanin contents and antioxidant activity than those from pomace with kernel (skin+kernel), showing that the sour cherry skin contributes more to the total

Table 1 Characterization of aqueous extracts prepared by decoction at 100 °C (W100) or by maceration at 25 °C (W25)

Sample	Water extracts	Yield (%)	Phenolic content (mg GAE/100 g)	Anthocyanin content (mg EC3G/100 g)	Antioxidant activity	
					FRAP (mmol Fe^{2+}/100 g)	DPPH (IC_{50}) (µg dry extract/mL)
Skin	W100	18 ± 2^{a}	289 ± 5^{a}	1.6 ± 0.9^{a}	7.5 ± 0.3^{a}	380 ± 3^{c}
	W25	6.8 ± 0.4^{b}	31 ± 1^{c}	1.8 ± 0.3^{a}	3.0 ± 0.4^{b}	880 ± 21^{b}
Skin+kernel	W100	14 ± 2^{a}	173 ± 8^{b}	0.5 ± 0.3^{b}	1.9 ± 0.1^{c}	407 ± 14^{c}
	W25	1.7 ± 0.6^{c}	40 ± 4^{c}	0.9 ± 0.4^{b}	1.9 ± 0.2^{c}	946 ± 21^{a}

Within the same column different letters indicate significant differences ($p < 0.05$)

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phenolic and anthocyanin contents of pomace extracts as well as for its antioxidant capacity. Those results are in agreement with the results from other authors that refer that most of skin phenolic compounds can remain after fruit pressing because they are associated with the skin pectin matrix [23–25, 54].

In what concerns the extraction yields (Table 1), the best results were obtained by the decoction method. Skin extracts prepared at 25 °C presented an extraction yield significantly higher ($p < 0.05$) than skin+kernel extracts prepared by the same method. In contrast, the extraction yield obtained with decoction at 100 °C showed no significant differences between the skin and skin+kernel ($p > 0.05$).

For both samples (skin and skin+kernel), extracts prepared at 100 °C presented a significantly higher phenolic content ($p < 0.05$) than extracts prepared at 25 °C. Thus, according to our results the decoction method seems to be more efficient in extracting phenolic compounds than the maceration method. Other authors refer that temperature can potentially increase polyphenol extraction, which may be due to increased diffusion of phenolic compounds [55, 56].

Decoction skin extract presented a significantly higher ($p < 0.05$) phenolic content than decoction skin+kernel extract. This result may be due to the presence of phenolic compounds in the fruit skin. As previously stated, skin represents only about 24% of the total weight of skin+kernel sample. Thus, this small amount of skin may explain the observed differences in the phenolic content of skin and skin+kernel extracts. When analyzing the pulp and skin of *P. cerasus* fruits, Chaovanalikit and Wrolstad [25], reported a TPC higher in skin (5.58 mg/g fresh weight) than in pulp (3.01 mg/g fresh weight). These authors found that phenolic compounds are mainly located in the skin of those fruits, which can make the by-products resulting from the processing of sour cherry an interesting source of polyphenolic compounds. In comparison with other fruit by-products, the values obtained for total phenols were higher than those found by Bonilla et al. [6] for grape pomace extracts carried out by maceration for 6 h with ethyl acetate/water 50:50 (v:v). For those extracts the TPC was about 100 mg GAE/100 g of marc by-product. Additionally, Rødtjer et al. [57], extracted cherry liquor pomace with different solvents (methanol, ethanol, acetone and 2-propanol), and obtained phenolic contents (78–74 mg GAE/100 g) lower than those obtained in the present study using water as extraction solvent.

The anthocyanin content was significantly higher ($p < 0.05$) for skin extracts than for skin+kernel extracts (Table 1). Once again, these results may be due to the presence and permanence of these compounds in fruit skin, even after industrial pressing, and to the smaller amount of skin used to prepare the skin+kernel extracts when compared to the amount of skin used to prepare the skin extracts. On the other hand, for both samples (skin and skin+kernel), the anthocyanin content of extracts obtained by decoction at 100 °C, was not significantly higher ($p > 0.05$) than anthocyanin content of extracts obtained by maceration at 25 °C. These results could be related to the vulnerability of anthocyanins to high temperatures, which may have led to their destruction in the samples extracted at 100 °C. It has been reported that increases in temperature in aqueous extract of red fruit can increase the content of other phenolic compounds that are not thermo labile, such as phenolic acids and flavones. Nevertheless, increases in temperature almost always result in a marked decrease in anthocyanin content of aqueous extracts [56, 58], which is in agreement with the results obtained in the present work.

Also, according to FRAP and DPPH assays (Table 1), the extract that presented the best antioxidant activity was the skin extract obtained by decoction at 100 °C. This result is in agreement with the previously discussed phenolic content and emphasizes the involvement of phenolic compounds on the antioxidant activity of the extracts. Conversely, the values obtained with skin+kernel extracts obtained by decoction or maceration were quite different in FRAP and DPPH assays. Thus, in FRAP assay both skin+kernel extracts showed similar antioxidant activity, but in DPPH assay the antioxidant activity of skin+kernel extract was significantly higher ($p < 0.05$) when the extract was obtained by decoction than when the extract was obtained by maceration, similarly to what was observed with the skin extracts.

Additionally aqueous extracts were analyzed by HPLC in order to identify and quantify the phenolic compounds that can contribute to the antioxidant activity previously demonstrated. Table 2 shows the quantification of the main phenolic compounds identified in the different by-products extracts. The major polyphenols identified were phenolic acids (protocatechuic, chlorogenic, *p*-hydroxybenzoic, coumaric, vanillic and ferulic acids), and flavonoids, mainly flavanols [(+)catechin and (−)epicatechin]; flavonols (kaempferol and quercetin-3-*O*-glucoside) and anthocyanins (cyanidin-3-*O*-glucoside).

Table 2 shows that extracts from pomace without kernel (skins) presented a higher concentration in polyphenols, with a total of 370 µg/100 g for W25 and 1767 µg/100 g for W100, than extracts from pomace with kernel (skin+kernel), with a total phenolic compounds of 157 µg/100 g for W25 and 1529 µg/100 g for W100 extracts. It is also possible to observe that values obtained for total phenol contents by HPLC were much lower than those observed for the FC method, maybe due to the fact that not all the phenolic compounds in the sample have been identified by the HPLC analysis. However a significantly positive correlation was observed between the TPC quantified by those two methods ($r = 0.9529$).

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Table 2 Quantification of phenolic compounds by HPLC (μg/100 g fresh biomass) in the aqueous extracts prepared by decoction at 100 °C (W100) and by maceration at 25 °C (W25)

Samples	Skin		Skin+kernel	
Compounds	W25	W100	W25	W100
Protocatechuic acid	260 ± 10^{c}	1261 ± 189^{a}	110 ± 8^{d}	980 ± 58^{b}
(+)Catechin	15.1 ± 0.9^{c}	63 ± 8^{a}	6.5 ± 0.0^{d}	58 ± 1^{b}
p-Hydroxybenzoic acid	3.2 ± 0.2^{c}	19 ± 1^{b}	3.6 ± 0.1^{c}	48 ± 3^{ca}
Chlorogenic acid	58 ± 1^{b}	305 ± 42^{a}	27 ± 2^{c}	288 ± 17^{a}
Vanillic acid	4.4 ± 0.5^{c}	20 ± 2^{b}	3.9 ± 0.1^{c}	47 ± 2^{a}
(−)Epicatechin	3.8 ± 0.2^{c}	46 ± 6^{a}	n.d	17.1 ± 0.1^{b}
Cyanidin-3-*O*-glucoside	14.3 ± 0.0^{b}	23 ± 5^{a}	3.3 ± 0.1^{c}	18.0 ± 0.0^{a}
Coumaric acid	1.0 ± 0.0^{b}	7.5 ± 0.3^{a}	n.d	8.9 ± 0.4^{a}
Ferulic acid	3.2 ± 0.2^{c}	17 ± 1^{a}	1.9 ± 0.0^{d}	11.2 ± 0.4^{b}
Quercetin-3-*O*-glucoside	4.4 ± 1.0^{b}	n.d	n.d	16 ± 1^{a}
Kaempferol	n.d	22.6 ± 1.7^{a}	n.d	25 ± 1^{a}
Kaempferol-3-*O*-rhamno-glucoside	2.1 ± 1.6	n.d	n.d	n.d
Total	370	1767	157	1529

Within the same line different letters indicate significant differences ($p < 0.05$)

n.d Not detected

It can also be observed that most of the identified compounds presented significantly higher ($p < 0.05$) concentrations in extracts obtained by decoction (W100) than in extracts obtained by maceration (W25) for both samples (skin and skin+kernel). It is also possible to observe that in relation to skin+kernel extracts, skin extracts presented a higher phenolic content for the majority of the compounds identified, except for the vanillic and *p*-hydroxybenzoic acids and quercetin 3-*O*-glucoside that presented higher values for skin+kernel decoction extract than for skin decoction extract. Perhaps these compounds are present in the skin and in the kernel of the fruit. These results are in agreement with those obtained for polyphenols and antioxidant activity.

Rødtjer et al. [57] also identified vanillic acid, (+)catechin, and (−)epicatechin in extracts from *P. cerasus* (cv. Stevns cherry) pomace, obtained in distillers from Denmark. Moreover, chlorogenic and protocatechuic acids [25, 37] as well as flavonoid compounds such as catechins and epicatechin [36, 59–61] were identified in cherry and sour cherry juice and fruit extracts. All of these compounds have also been identified in our pomace extracts, suggesting that after industrial extraction they remain in the pomace, possibly linked to the matrix of the skin. The presence of these compounds can justify the antioxidant activity observed.

Several compounds found in *P. cerasus* pomace extracts, namely phenolic acids such as protocatechuic, chlorogenic, vanillin and *p*-hydroxybenzoic acids [6, 59] and flavanols such as catechins and epicatechins [62], demonstrated in vitro antioxidant activity, acting as efficient scavengers of free radicals. Also Picollela et al. [34] examined the antioxidant capacity of pure compounds isolated from methanolic extracts and found that the more active compounds were catechins, epicatechins, and chlorogenic acid. Thus, it is likely that the presence of these compounds in the extracts under study can contribute to the observed antioxidant activity. However, it must be take into account that these compounds may have a different activity when they are isolated or when they are present in a complex sample, like pomace extracts, where synergy or antagonistic relationships may occur [63].

The cyanidin-3-*O*-glucoside content ranged from 3.3 to 23 μg/100 g in which the higher value was observed in the W100 skin extract and the lower in the W25 skin+kernel extract. Anthocyanins remain in skin even after the industrial pressing [54]. However in decoction extracts (W100) there were no significant differences ($p > 0.05$) between cyanidin-3-*O*-glucoside content in skin and skin+kernel samples, perhaps due to the thermal degradation of the anthocyanins. In fact, degradation of anthocyanins has been reported to occur at temperatures above 40 °C [58, 64]. These results are in agreement with that observed in this study for the quantification of total anthocyanins content by the pH differential method.

The observed presence of anthocyanins in the liquor pomace by-products extracts shows that those compounds can be recovered and used as natural antioxidants in cosmetics, drinks and nutraceuticals, providing many benefits for human health. Similar studies have revealed that anthocyanins from sour cherry exhibit in vitro antioxidant activities that can be comparable to synthetic commercial products, such as butylated hydroxyanisole and butylated hydroxytoluene, and even superior to vitamin E at 2 mM concentration [40, 65]. Thus anthocyanins can be considered as natural antioxidants that can be added to functional products.

The chemical composition (ash, fat, sugar and nitrogen content) of the remaining residues obtained after pomace extractions were also analyzed in order evaluate its potential

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as an energy source (Fig. 1). The fat content of the residue obtained after extraction at 100 °C (RW100) was lower than the fat content of the residue obtained after extraction at 25 °C (RW25). However, results showed that both the remaining residues still have significant oil content. This oil can be extracted and valorized in a biorefinery context. The remaining high sugar content suggests that the residues have potential for the production of ethanol or they can be used directly for combustion purposes. In fact, the remaining residues presented a high heat of combustion (19.9 MJ/kg dry matter for RW25 and 18.9 MJ/kg dry matter for RW100) and a low ash and nitrogen contents. Ash and nitrogen contents are important criteria for thermal conversion technologies. High ash content leads to fouling problems, especially if the ash is high in metal halides (e.g., potassium), thus these content should be lower than 5 g/100 g of dried matter in order to avoid the occurrence of slagging processes [66]. Moreover, ash does not contribute to energy production and it generally cost money to discard [67]. On the other hand, lower nitrogen content should lead to lower NO_x emissions. Thus, this value should be lower than 1 g/100 g dry matter as the lower the nitrogen absorbed by the biomass, the lower the N emissions that will contribute to the greenhouse gases emissions [66–68].

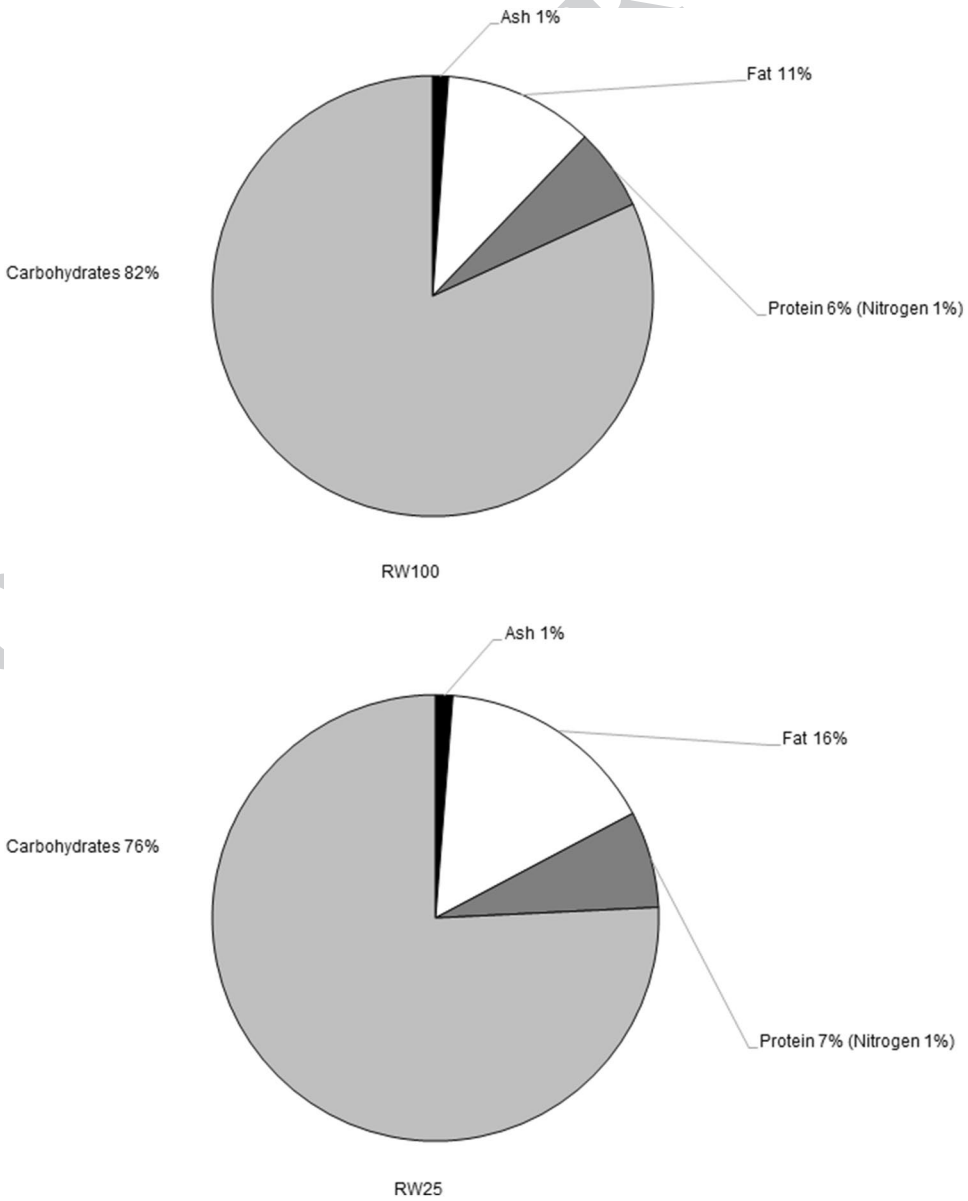

Fig. 1 Chemical composition of the remaining residue after water extraction at 100 °C (RW100) and at 25 °C (RW25)

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Conclusions

Results obtained in this study showed that the sample without kernel (skins) showed a higher extraction yield, polyphenolic content and antioxidant activity than the sample from pomace with kernel (skin+kernel). Results also showed that decoction at 100 °C was the best extraction method, except for the anthocyanins, that probably suffered some degradation with temperature. However, despite allowing a higher recovery of phenolic compounds, extraction at 100 °C requires also higher energy consumption, and thus extraction at 25 °C seems to be a more sustainable process. In conclusion, results obtained allow us to conclude that the cherry pomace could be regarded as a promising eco-friendly agro-industrial by-product. It can be considered a potential low-cost source of polyphenols, with potential to be used as functional ingredients when added to food, nutraceuticals and cosmetics. Additionality the transformation of these by-products in an energy source can be regarded in the perspective of total recycling of those by-products.

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