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Comparative study of red berries pomaces (blueberry, red raspberry, red currant and blackberry) as source of antioxidants and pigments.

Abbreviated title: Red berry pomaces as antioxidant and pigment sources

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Abstract

Anthocyanins are phenolic compounds with important technological applications due to its bioactive and color properties. In this study, pomaces from four red berries (blueberries, red raspberries, red currants and blackberries) have been analyzed as sources of anthocyanins. Anthocyanins were determined by high performance liquid chromatography/mass spectrometry (HPLC/MS), total phenolic content (TPC) by Folin-Ciocalteu method, antioxidant activity (AA) by ABTS assay, and color by Tristimulus Colorimetry.

A total of fifteen anthocyanins were identified and quantified in the different pomaces from red berries. Pomaces exhibited different qualitative and quantitative anthocyanin profile and antioxidant activity, depending on type of red berry. The highest amounts of anthocyanins were found in blueberries (1188 mg/100 g), however red currant pomaces exhibited the highest TPC (3447 mg/100 g) and AA (61 mmol/100 g). Color of extracts was different depends on individual and total content of anthocyanins.

Results indicate that these berries pomaces are a natural source of antioxidants and pigments, and they may be useful for industrial purposes. Therefore, the exploitation of these pomaces, like possible byproducts for their reuse in the food, cosmetics, and drug industries, could be of great interest, considering either the whole pomaces or its individual components.

Keywords: Red berries; pomaces; anthocyanins; antioxidant activity; pigments; color

Abbreviations: AA: antioxidant activity, ABTS: 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid), BB: blueberry, RR: red raspberry, RC: red currants, BK: blackberry, TA: Total anthocyanins, TPC: total phenolic content, DM: dry matter, TE: Trolox equivalent, SLDA: stepwise linear discriminant analysis

Introduction

Phenolic compounds are secondary plant metabolites, which constitute one of the most numerous and widely distributed groups of natural products in the plant kingdom, and can in general be classified into two main groups: flavonoids (flavanols, flavonols and anthocyanins) and non-flavonoids (phenolic acids and stilbenes) [1]. These compounds are important determinants in the sensory and nutritional properties of fruits and vegetables, and they have received attention because of their antioxidant activity. Specifically, anthocyanins are a subgroup of flavonoids that are found in nature as conjugated anthocyanidins. There are six anthocyanidins distributed throughout the plant kingdom: cyanidin, malvidin, delphinidin, peonidin, petunidin and pelargonidin. These compounds are one the most common pigments in nature and they are widely distributed in fruits and vegetables, being responsible for their color [2]. Anthocyanins display a great diversity of colors, from orange and red through purple and blue hues. In addition, anthocyanins have been associated with positive health effects, such as protection against oxidative stress and coronary heart disease, antimicrobial, anti-inflammatory and anticarcinogenic activities, control of obesity and diabetes, or improvement of vision [3, 4].

Red berries such as blueberries, blackberries, raspberries, strawberries, elderberries, blackcurrants, red currants and cranberries have been postulated to be a good source of phenolic compounds, chiefly anthocyanins [5-11].

In Spain the cultivation of these fruits has increased in recent years and approximately 80% is used for fresh consumption. Also, these fruits are used in food industry for the elaboration of some products such as juices [12]. In this processing, berries are crushed and pressed and solid parts are discarded after extracting juice, which generates an intensive accumulation of byproducts consisting of seeds, peels and pulp rests. Through common processing, only a part of the phenolic compounds is transferred to juice and then the generated byproducts are rich in anthocyanins and other compounds [7]. In this sense, the recovery of anthocyanins from solid wastes (pomaces) represents an attractive, sustainable and cost-effective source of these compounds, which could be incorporated into foods to improve their biological value, and also could be used as natural colorants [6, 13].

Actually, consumers demand functional foods and healthy products, therefore food industry has been more conscious of the importance to use natural additives which provide the final product with a healthy added value. Due to the increasing demand for nutraceutical, antioxidant compounds and natural colorants, the study of pomaces like byproducts from fruits may be useful for industrial purposes [14].

Although red berries have been extensively investigated, as far as we know, no many studies regarding red berries byproducts have been published. Therefore, the aim of this work was to carry out the determination of anthocyanin profile, total phenolic content, and antioxidant activity, and the colorimetric characterization of the extracts of pomaces from blueberries, red raspberries, red currants and blackberries.

Materials and methods

Chemicals and reagents

Sodium carbonate, potassium persulfate and phosphate-buffered saline (PBS) were purchased from Sigma-Aldrich (Madrid, Spain). 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Fluka (Madrid, Spain). Acetonitrile, Folin reagent, methanol and formic acid were obtained from Panreac (Barcelona, Spain).

Samples

Samples used in this study were berries from four species: blueberries (*Vaccinium myrtillus*) (BB), red raspberries (*Rubus idaeus*) (RR), red currants (*Ribes rubrum)* (RC) and blackberries *(Rubus fructicosus*) (BK). They were collected during a period of suitable ripening from the 2015 harvest in Almonte, Huelva (southwestern Spain). Immediately after collecting, berries were pressed to mix the peel, the seeds and the pulp. The obtained juice was discarded, and the pomace samples were obtained. The pomaces were weighed, stored at -20 °C and lyophilized for 48 h (lyophilizer Cryodos-80, Telstar Varian DS 102); finally, the samples were ground and stored until phenolic extraction. After lyophilizing 100 g of each fresh pomace, 15.5, 26.1, 28.8 and 20.9 g of dry pomace from blueberries, red raspberries, red currants and blackberries were obtained, respectively.

Preparation of extracts

The extraction was carried out with 75% methanol (1% 1N HCl) according to the methodology described as follows: the dry pomace (1 g) was homogenized in 5 mL of the solvent kept under shaking for 12 h in an incubating minishaker at 25 ºC, and further centrifuged at 4190*g* for 10 min at 10 ºC; the supernatant was collected, and the residue submitted to the same process until loss of color. Finally the supernatants were combined and were adjusted to 25 mL with 75% methanol.

Total phenolic content

The total phenolic content (TPC) was determined using the Folin-Ciocalteu assay with some modifications [15]. Gallic acid was employed as a calibration and results were expressed as gallic acid equivalents (mg GAE/100 g of dry matter (DM)).

Determination of anthocyanins by HPLC/MS analysis

The anthocyanins were determined by HPLC/MS following the method described by Fernández-Lara et al. [16]. Analyses were carried out in a Hewlett-Packard 1200 series liquid chromatograph (Agilent Technologies, PaloAlto, CA, USA) equipped with a diode-array detector, and a Zorbax SB C18 column (4.6 mm x 250 mm, 4.6 μm particle size) using an injection volume of 50 μL. Detection was carried out at 525 nm and was also performed in a micrOTOF-QII High Resolution Time-of-Flight mass spectrometer (UHR-TOF) with Q-TOF geometry (Bruker Daltonics, Bremen, 194 Germany) equipped with an electrospray ionization (ESI) interface. The instrument was operated in positive ion mode (ESI+) using a scan range from m/z 50-1200. Nitrogen was used as the dry gas at a flow rate of 8 mL/min with nebulizing (1.2 bar), and nebulized temperature was set at 200 ºC.

Anthocyanins were identified by their retention time, UV-vis spectra and mass spectra, as well as by comparison with our data library. The quantification of the compounds was carried out by external calibration from the areas of the chromatographic peaks obtained by UV detection at 525 nm using a calibration curve of cyaniding-3-*O*-glucoside. All analyses were performed in triplicate and the results were expressed as milligrams per 100 g of DM (mg/100 g DM). Total anthocyanins (TA) were also estimated by summing the content of all of individual anthocyanins quantified.

Antioxidant activity by ABTS assay

The antioxidant activity (AA) was determined by the ABTS assay according to the methodology described previously [17]. The results (ABTS values) were expressed as millimol of Trolox equivalents (TE) per 100 grams of DM (mmol TE/100 g DM). This result indicates the Trolox-equivalent antioxidant capacity (TEAC): mmol of Trolox with the same antioxidant activity as 100 g of the pomace).

Colorimetric measurements

A Hewlett-Packard UV-vis HP8452 spectrophotometer (Palo Alto, CA) was used to carry out color measurements. Spectrophotometric measurement of the visible absorption spectrum (380-770 nm) at constant intervals ($\Delta\lambda = 2$ nm) was made, using 2 mm path length glass cells and distilled water as reference. The CIELAB parameters (L*, a*, b*, C^*_{ab}, and h_{ab}) were determined by using the original software CromaLab© [18] following the Commission Internationale de L'Eclariage's recommendations [19]: the CIE 1976 10° Standard Observer and the Standard Illuminant D65.

Statistical analysis

These statistical analyses of the data were performed using the Statsoft Statistica® V 8.0 software [20]. One way analysis of variance (ANOVA) was applied to determine whether significant differences ($p < 0.05$) exist among the different pomaces. A stepwise linear discriminant analysis (SLDA) was applied on experimental standardized data in order to classify red berries pomaces.

Results and discussion

Anthocyanin profile and total phenolic content

Table 1 shows retention times, and mass spectrometric data of each anthocyanin identified in the HPLC-DAD-MS analysis. A total of fifteen anthocyanins were identified and quantified in the different pomaces, according to their mass and chromatographic data and also by comparison with literature [21-24]. Figure 1 shows the HPLC chromatogram with the anthocyanin profile of blueberry (a), red raspberry (b), red currant (c) and blackberry (d) pomaces.

Peak 1, 2 and 5 were corresponded to delphinidin-3-*O*-galactoside (m/z = 465, MS^2 = 303), delphinidin-3-*O*-glucoside (m/z = 465, MS^2 = 303) and delphinidin-3-*O*-arabinoside (m/z = 435, MS^2 = 303), respectively. Peaks 3, 4, 6, 7, 8 and 10 with main MS fragmentation ions at m/z 287 were attributed to cyanidin derivatives. These peaks were identified as cyanidin-3-*O*-sophoroside (Peak 3: m/z = 611, MS^2 = 287, loss of 324 amu, corresponding to a sophorosyl unit), cyanidin-3-*O*-glucosyl-rutinoside (Peak 4: m/z 757, MS^2 = 287, loss of 146 amu corresponding to rhamnosyl group, and 324 amu corresponding to two glucosyl moieties), cyanidin-3-*O*-sambubioside (Peak 6: m/z 581 and MS^2 = 287, loss of xylose or arabinose (132 amu) and glucose (162 amu)), cyanidin-3-*O*-glucoside (Peak 7: m/z 449, MS^2 = 287), cyanidin-3-*O*-rutinoside (Peak 8: m/z 595, MS^2 = 287, loss of a rutinosyl unit (308 amu)) and cyaniding-3-*O*-arabinoside (Peak 10: m/z 419, MS^2 = 287). Peaks 9 and 11 were identified as petunidin-3-*O*-galactoside (m/z 479 and MS^2 = 317) and petunidin-3-*O*-arabinoside (m/z 449 and MS^2 = 317), respectively. Peaks 12, 13 and 15 were identified as malvidin-3-*O*-galactoside (m/z 493 and MS^2 = 331), malvidin-3-*O*-glucoside (m/z 493 and MS^2 = 331), and malvidin-3-*O*-arabinoside (m/z 463 and MS^2 = 331), respectively. Finally, peak 14 was corresponded to peonidin-3-*O*-arabinoside (m/z 433 and MS^2 = 301). Glycosides were assigned based on the characteristics losses of fragments, i.e., 162 amu (glucosides and galactosides), and 132 amu (arabinosides).

. Different qualitative and quantitative anthocyanin profiles were found in the pomaces (Table 1). As can be seen in this table, eleven different anthocyanins were identified in blueberry pomaces (delphinidin-3-*O*-galactoside, delphinidin-3-*O*-glucoside, delphinidin-3-*O*-arabinoside, cyanidin-3-*O*-glucoside, petunidin-3-*O*-galactoside, cyanidin-3-*O*-arabinoside, petunidin-3-O-arabinoside, malvidin-3-*O*-galactoside, malvidin-3-*O*-glucoside, peonidin-3-*O*-arabinoside, malvidin-3-*O*-arabinoside) whereas in red raspberry (cyanidin-3-*O*-sophoroside, cyanidin-3-*O*-(2"-O-glucosyl)rutinoside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside) , red currant (cyanidin-3-*O*-sambusoside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside) and blackcurrant pomaces (cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside) were identified four, three and two anthocyanins, respectively. In blueberry pomaces were identified delphinidin-, cyanidin-, petunidin-, peonidin- and malvidin-, based anthocyanins, while than in the rest of pomaces only cyanidin derivates.

The most abundant anthocyanins in blueberry pomaces were malvidin-3-*O*-galactoside and malvidin-3-*O*-arbinoside (30 and 29 % of the anthocyanin content, respectively). The percentages of these two compounds are consistent with previous reports: in a work about ripe samples of blueberries (*Vaccinium corymbosum*) represented by four varieties, these anthocyanins represented between 28 and 36 % and between 11 and 16 % of the anthocyanin content, respectively [22]. In other study [21], a total of fifteen anthocyanins were identified and quantified in blueberries (*Vaccinium corymbosum*), and malvidin-3-*O*-galactoside and malvidin-3-*O*-arabinoside were also the most abundant anthocyanins (21 and 18 % of total content, respectively). According literature [25, 26], malvidin glycosides are the main anthocyanins in blueberries and they constitute between 30 and 50% of the total anthocyanin content in blueberries (*Vaccinium myrtillus*), being malvidin-3-*O*-galactoside the predominant anthocyanin (15%).

Other compounds as delphinidin-3-*O*-galactoside, petunidin-3-*O*-galactoside, delphinidin-3-*O*-arabinoside and petunidin-3-*O*-arabinoside also showed relevant contribution to the levels of total anthocyanins in blueberry pomaces (10, 9, 8 and 7 %, respectively). However, the concentration of cyanidin-3-*O*-arabinoside and cyanidin-3-*O*-glucoside in blueberry was low, representing the 1 and 0.4 % of total anthocyanins, respectively. These anthocyanins have also been identified by other authors in blueberries [21, 22, 25, 26].

The main anthocyanins in the red raspberry pomaces were cyanidin-3-*O*-sophoroside and cyanidin-3-*O*-glucoside (32 and 31 %, respectively). Cyanidin-3-*O*-sophoroside, has been previously described as the

major anthocyanin (42 % of total anthocyanins) in red raspberries of the same specie as the one analyzed in this study [21]. Therefore, this compound has been described as an important and distinctive anthocyanin in red raspberries [21, 27]. However, in a study on raspberries grown in USA, this compound was the less abundant among the four anthocyanins identified [28]. Cyanidin-3-*O*-sophoroside was not identified in blueberry, red currant and blackberry pomaces.. Cyanidin-3-*O*-(2"-*O*-glucosyl)-rutinoside and cyanidin-3-*O*-rutinoside were also quantified in high concentrations (around 20 and 17 % of total anthocyanin content, respectively), the last one has been identified in black raspberry seeds as principal anthocyanin [29].

The predominant compound in red currant extracts was cyanidin-3-*O*-sambubioside (82 % of the anthocyanin content), which only was identified in pomace of this fruit. This compound has been also identified in red currants of the same studied specie (*Ribes rubrum*), although in lower proportion (around 12 %) [22]. In blackberry pomaces, cyanidin-3-*O*-glucoside was the main anthocyanin (166 mg/100 g DM), which represented 86 % of anthocyanin content.

Cyanidin-3-*O*-glucoside was the only compound identified in all pomaces, with highest concentration in blackberry pomaces followed by red raspberry, blueberry and red currant pomaces (59, 5 and 4 mg/100 g DM, respectively), and significant differences ($p < 0.05$) were found (Table 1). Significant differences were found in the content of cyanidin-3-*O*-rutinoside in red raspberry, red currant and blackberry pomaces (32, 22 and 26 mg/100 g DM, respectively).

The different pomaces (blueberry, red raspberry, red currant and blackberry) showed significant differences ($p < 0.05$) in their contents of TA (Table 2). The highest amounts were found in blueberries (1188 mg/100 g DM), that was around 6-fold the concentration of blackberries (192 mg/100 g DM) and red raspberries (190 mg/100 g DM) and 8-fold the content of red currants, which presented the lowest one (147 mg/100 g DM).

The TPC values for pomaces from blueberries, red raspberries, red currants and blackberries are shown in Table 2. The comparison among the four studied type of pomaces revealed that red currant extracts exhibited higher TPC (3447 mg GAE/100 g; around 2-fold the concentration of the other samples) followed by red raspberry (2015 mg GAE/100 g), blueberry (1955 mg GAE/100 g) and blackberry (1699 mg GAE/100 g) pomaces. The ANOVA test applied to the set of data indicated that significant differences were assigned between red currants and rest of the pomaces.

Other authors analyzed the whole berries from blueberries (*Vaccinium corymbosum*) and red raspberries (*Rubus idaeus*) and they indicated that have higher phenolic content that red currants (*Ribes rubrum*) [21,

22]. However, the results cannot be compared in quantitative terms with ours because they are expressed in different units, and moreover the samples of our study are pomaces and not the whole fruit.

In a study on blueberry residues obtained in southern Brazil [30], the TPC values were up to 80% higher than those for our blueberry pomace. This difference could be explained by the variety used in each study, and by using supercritical and pressurized liquids in phenolic extraction, that were not used in this study. Contents between 600 and 4900 mg/100 g reported in residues obtained from blackberry *(Rubus sp.*) grown in southeastern Brazil [31]. These contents, which depended on the extraction process, framed our TPC value for blackberry pomace (1699 mg/100 g)."

In general terms, these differences are logical because the phenolic content is different in these fruits and their byproducts, and also is influenced by factors such as specie, variety, cultivar, climatic and growing conditions, and phenolic extraction process [29, 32-34].

Antioxidant activity

It is well-known that phenolic compounds, and among them anthocyanins, are antioxidants [11, 35]; therefore, the AA of pomaces was measured by the ABTS assay (Table 2). The ABTS assay measures the *in vitro* antioxidant activity on the basis of the ability of samples to scavenge the $ABTS^{\bullet+}$ radical. In this study, the ability of phenolic extracts from pomaces to scavenge the radical is measured using Trolox as reference antioxidant compound.

Red currant pomaces showed the highest AA (61 mmol TE/100 g DM), followed by red raspberry, blueberry and blackberry pomaces (30, 27 y 23 mmol TE/100 g DM, respectively). The antioxidant activity values of blueberry residues reported in a previous study were lower than the values determined in this study (2.4-10.3 vs. 26.9 mmol TE/100 g) [30]. Also, our data were higher than values found in other previous study on blackberry residues (22.5 vs. 5.3-15.4 mmol TE/100 g) [31].

. These results of AA agreed with the TPC, which was significantly correlated with ABTS values, as showed by regression analysis ($p < 0.05$; r = 0.97). However, correlation between ABTS values and TA was lower (r = 0.75), which indicates that antioxidant activity of pomaces depend on all phenolic compounds, and anthocyanins only are responsible partially of this activity. Similar results have been reported by other authors [33], who indicated that antioxidant activity in blueberries was highly correlated with phenol content (r = 0.981) and a less linear correlation with anthocyanin content (r = 0.817). It has been suggested that phenolic compounds other than the anthocyanins contributed positively to the total antioxidant activity [32], which is in accordance with our study.

In a previous work, the contribution of anthocyanin composition to the total antioxidant capacity of berries (blackberry, black currant and blueberry) having different anthocyanin composition was studied, and results indicated that anthocyanins contribute to the total antioxidant activity, but this contribution depends on type and content of each individual anthocyanin identified in the berries [36].

All types of pomaces had antioxidant activity by the ABTS assay, indicating that their phenolic compounds can combat the free radicals. These radicals generate important cell damage and subsequent diseases and phenolic compounds from red berries pomaces are antioxidants that prevent the attack of radicals and therefore cell damage.

Color characteristics

There is considerable demand for food colorants from natural sources that can serve as alternatives to the use of synthetic dyes [37]. Anthocyanin-rich byproducts are recognized as natural pigments sources and therefore they are interesting to be used as natural colorants. In this sense, the measurement of the color of the pomace extracts, obtained from freshly processed berries, is very useful to establish the optimum chromatic characteristics that define the extract color. Thus, the extracts color could be used as a quality parameter in the food coloring production.

In order to study the practical application of the extracts as a source of natural pigments, the color of them was calculated by Tristimulus Colorimetry. The values of L*, C^*_{ab} and h_{ab} of each type of extract are shown in Table 3. Figure 2 shows the color points represented in the (a*b*) diagram of the sample extracts. It can be noticed that all samples were located in the first quadrant of the (a*b*)-plane (positive values of a* and b*) corresponding to reddish region. As can be seen, two different groups are formed: on the one hand, red raspberry, red currant and blackberry pomaces are located in a red hue area of the (a*b*)-plane, having he angles around 10-20°, and on the other hand blueberry pomaces are above 30° (red-orange region). Chroma (C^*_{ab}) showed higher values for blueberry pomaces (65 CIELAB units versus 51-58 CIELAB units in the red currant, red raspberry and blackberry pomace extracts) indicating the 'higher' colorfulness of the blueberry pomace extracts (Figure 2). This can be due to the higher concentration of TA in blueberry pomace extracts (1188.34 mg/100g versus 192.41, 188.05 and 149.91 mg/100g in blackberry, red raspberry and red currant pomace extracts respectively) (Table 2).Regarding the CIELAB color space variables, lightness (L*) showed values ranging between 24 and 65 CIELAB u for blueberry and red currant extracts, respectively (Table 3). Considering hue (h_{ab}) values, blueberry extracts had a very intense red-orange (h_{ab}

= 32°) color, while blackcurrant, red currant and red raspberry extracts showed a red color (h_{ab} = 21º, 19º and 17º, respectively). The chroma (C^*_{ab}) of the samples revealed that they showed a deep color. The highest values corresponded to blueberry extracts (65 CIELAB u), followed by blackberry, red raspberry and red currant extracts (58, 54 and 51 CIELAB u, respectively). Significant differences (Table 3) among the extracts were found in terms of chroma, lightness and hue, which indicate that the differences observed among the color characteristics of the four type of extracts were quantitative and qualitative. As described previously, the color characteristics of foods are affected by their phenolic composition [38, 39] and thus color-composition relationships were explored. Correlations between TPC and the color parameters (L*, C^*_{ab}, and h_{ab}) were performed and no significant correlations were found. Also, univariate linear regression analysis was applied to these data to explore relationships between the TA and the individual anthocyanins with the color parameters. Regarding chroma (C^*_{ab}) and hue (h_{ab}), a high and significant correlation was found with TA ($p < 0.05$; r > 0.9), and all individual anthocyanins ($p < 0.05$; r > 0.9), except cyanidin derivatives. The positive correlation with C^*_{ab} indicates that higher values are related to higher concentration of anthocyanins. Also, a negative and significant correlation ($p < 0.05$; r = -0.96) was found with lightness (L*), indicating that higher L* values are related to lower TA and individual anthocyanin content.

In general, the L* and C^*_{ab} values of the byproduct extracts from studied red fruits are higher than the results obtained by other authors in the study of the red fruits color [34, 40, 41]. These results are logical since during the fruit processing a quite important amount of pigments, and therefore of color, stay in the juice, hence the pomace had to have less pigments concentrations than the complete fruit.

Finally, to ascertain whether it was possible to discriminate between blueberry, red raspberry, red currants and blackberry pomaces as a function of TPC, TA, AA and color parameters (L*, C^*_{ab}, and h_{ab}) a SLDA was carried out. Four variables were found statistically significant ($p < 0.05$): TA, AA, L* and C_{ab} Two classification functions were obtained, which yielded a good separation among samples (Figure 3). The discriminant function 1 provides a good separation among pomaces. Respect to positive and negative zone, this function clearly discriminates the BB (which had highest TA) and the RC samples (highest AA), respectively. On the other hand, discriminant function 2 was mainly linked to colorimetric parameters. This discriminant function mostly discriminates between BK (positive sign, highest values of C^*_{ab}) and RC samples (negative sign, highest values of L*).

Conclusions

In summary, it might be concluded that these pomaces could be considered as an important source of natural antioxidants and pigments. Pomaces from blueberries, red raspberries, red currants and blackberries exhibited a different qualitative and quantitative phenolic profile respect to anthocyanins, and different antioxidant activities and color characteristics. Although further studies could be required in relation to other bioactive compounds present in the pomaces from red berries, this study provides relevant information that may be useful for industrial purposes. Therefore, the exploitation of these pomaces, like possible byproducts for their reuse in the food, cosmetics, and drug industries, could be of great interest, considering either the whole pomaces or its individual components.

In addition, this study is important for food research and technology because identify bioactive compounds present in different byproducts that can be obtained from production of red berries juice. The extraction of these bioactive compounds from byproducts is the first step for them to be used in functional food development, and besides these compounds could have another technological use due to their color properties.

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Compliance with ethical standards

Conflict of interest Authors declare no conflict of interest

Compliance with ethics requirements All authors declare that this article does not contain any studies with human or animal subjects

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Figure captions

Fig. 1 HPLC chromatograms recorded at 525 nm of blueberry (a), red raspberry (b), red currant (c) and blackberry (d) pomace extracts. Peaks: 1, delphinidin-3-*O*-galactoside; 2, delphinidin-3-*O*-glucoside; 3, cyanidin-3-*O*-sophoroside; 4, cyanidin-3-*O*-(2”-O-glucosyl)rutinoside; 5, delphinidin-3-*O*-arabinoside; 6, cyanidin-3-*O*-sambusoside; 7, cyanidin-3-*O*-glucoside; 8, Cyanidin-3-*O*-rutinoside; 9, petunidin-3-*O*-galactoside; 10, cyanidin-3-*O*-arabinoside; 11, petunidin-3-O-arabinoside; 12, malvidin-3-*O*-galactoside; 13, malvidin-3-*O*-glucoside; 14, peonidin-3-*O*-arabinoside; 15, malvidin-3-*O*-arabinoside.

Fig. 2 Representation of blueberry, red raspberry, red currant and blackberry pomace extracts in the (a*b*) plane. Two different groups: red raspberry, red currant and blackberry pomaces in a red hue area, and blueberry pomaces in a red-orange region.

Fig. 3 Scatterplot of the four types of samples (blueberry, red raspberry, red currant and blackberry pomaces) in the plane defined by the canonical function when phenolic composition, antioxidant activity and color parameters are considered for discrimination by applying a stepwise linear discriminant analysis (SLDA)**.**

Figure 1.

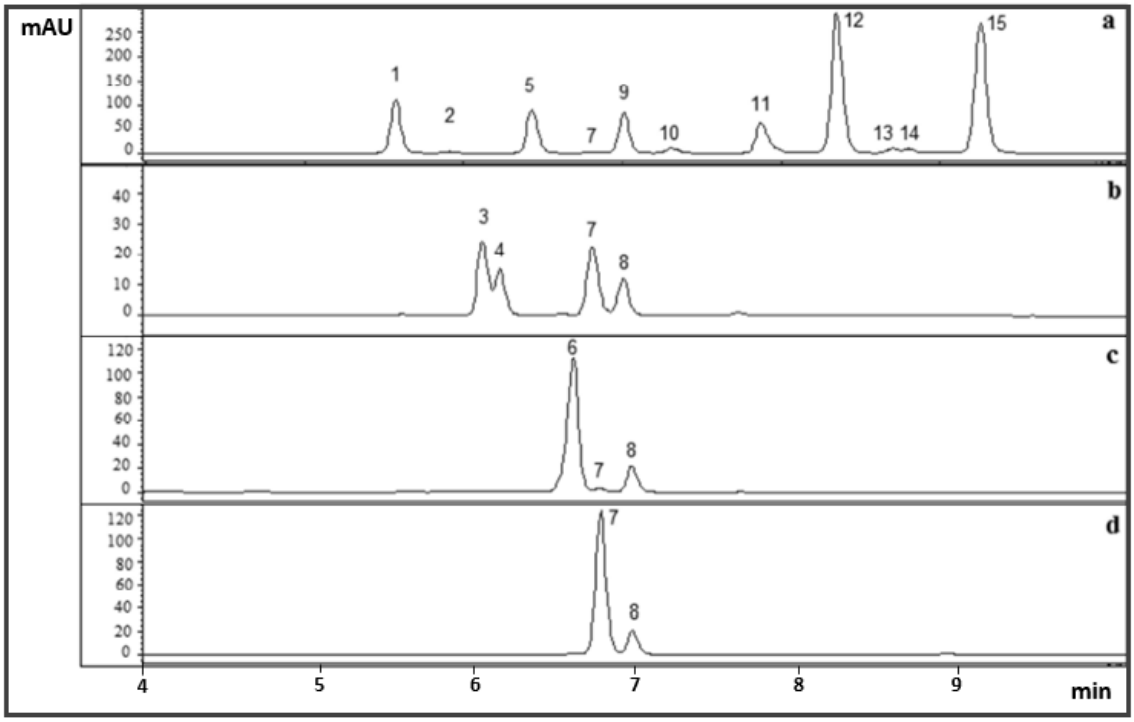

Figure 2.

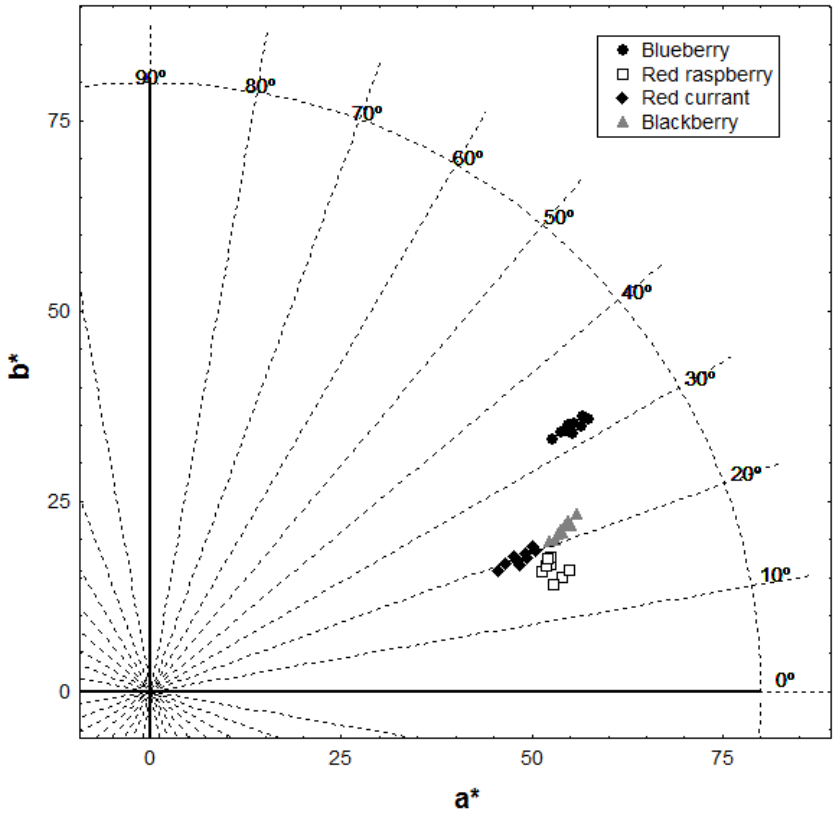
Blueberry
Red raspberry
Red currant
Blackberry
90º
80º
70º
60º
50º
40º
30º
20º
10º
0º
75
50
25
0
b*
0
25
50
75
a*

Figure 3.

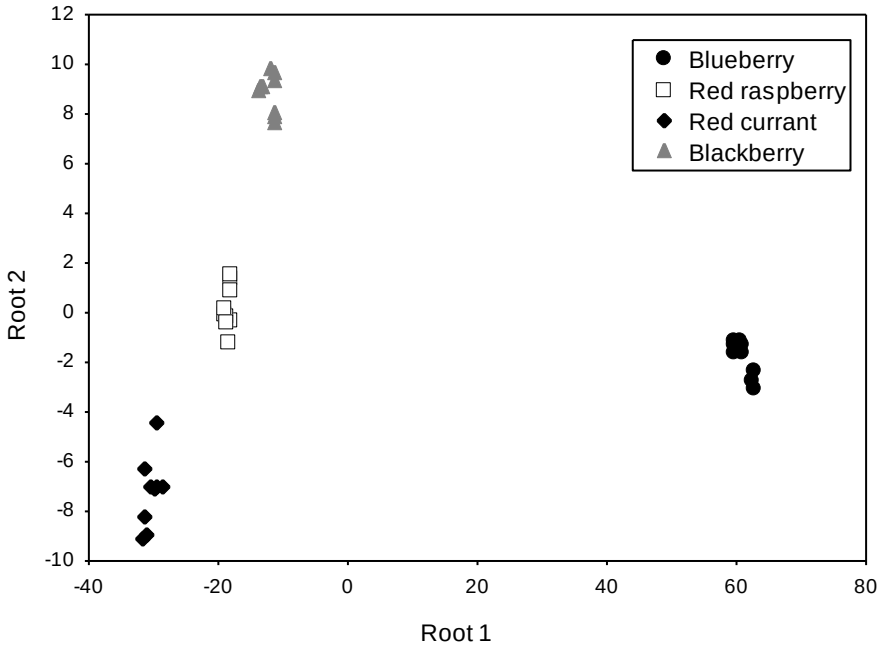
Blueberry
Red raspberry
Red currant
Blackberry
Root 2
Root 1
12
10
8
6
4
2
0
-2
-4
-6
-8
-10
-40
-20
0
20
40
60
80

Table 1. HPLC retention times (TR), mass spectrometry data of anthocyanins detected and quantitative anthocyanin profile (mg/100g DM) in blueberry (BB), red raspberry (RR), red currants (RC) and blackberry (BK) pomaces.

Peak	TR (min)	MS^+ (m/z)	MS^2	Compound	BB	RR	RC	BK
1	5.55	465	303	Delphinidin-3-*O*-galactoside	130.08 ± 2.16	nd	nd	nd
2	5.89	465	303	Delphinidin-3-*O*-glucoside	4.40 ± 0.16	nd	nd	nd
3	6.12	611	287	Cyanidin-3-*O*-sophoroside	nd	60.55 ± 4.25	nd	nd
4	6.23	757	287	Cyanidin-3-*O*-(2”-O-glucosyl)rutinoside	nd	37.32 ± 2.73	nd	nd
5	6.41	435	303	Delphinidin-3-*O*-arabinoside	97.20 ± 1.33	nd	nd	nd
6	6.62	581	287	Cyanidin-3-*O*-sambusoside	nd	nd	124.33 ± 16.71	nd
7	6.79	449	287	Cyanidin-3-*O*-glucoside	4.89 ± 1.32[a]	59.47 ± 3.59[b]	3.58 ± 0.48[a]	166.39 ± 19.11[c]
8	6.93	595	287	Cyanidin-3-*O*-rutinoside	nd	31.71 ± 2.27[a]	22.00 ± 2.05[b]	26.01 ± 1.76[c]
9	6.99	479	317	Petunidin-3-*O*-galactoside	106.76 ± 2.28	nd	nd	nd
10	7.29	419	287	Cyanidin-3-*O*-arabinoside	14.25 ± 0.76	nd	nd	nd
11	7.85	449	317	Petunidin-3-O-arabinoside	89.17 ± 1.72	nd	nd	nd
12	8.33	493	331	Malvidin-3-*O*-galactoside	363.14 ± 6.18	nd	nd	nd
13	8.69	493	331	Malvidin-3-*O*-glucoside	11.25 ± 0.23	nd	nd	nd
14	8.78	433	301	Peonidin-3-*O*-arabinoside	11.39 ± 0.25	nd	nd	nd
15	9.23	463	331	Malvidin-3-*O*-arabinoside	355.79 ± 7.72	nd	nd	nd

nd: not detected

Values in the same row followed by different letters are significantly different ($p < 0.05$).

Table 2. Total phenolic content, total anthocyanins and antioxidant activity of blueberry (BB), red raspberry (RR), red currants (RC) and blackberry (BK) pomaces.

	BB	RR	RC	BK
TPC (mg GAE/100 g DM)	$1954.54 \pm 177.82^{a,c}$	2014.66 ± 100.91^{a}	3446.59 ± 805.17^{b}	1699.62 ± 174.50^{c}
TA (mg/100 g DM)	1188.34 ± 19.35^{a}	188.05 ± 12.55^{b}	149.91 ± 19.90^{c}	192.41 ± 20.83^{b}
AA (mmol TE/100 g DM)	26.98 ± 0.35^{a}	29.75 ± 0.73^{a}	60.83 ± 5.29^{b}	22.54 ± 4.45^{c}

Each value represents mean (n=9) ± SD.
Values in the same row followed by different letters are significantly different by ANOVA test ($p < 0.05$).

Table 3. Color parameters of extracts from blueberry (BB), red raspberry (RR), red currants (RC) and blackberry (BK) pomaces.

	L^*	C^*_{ab}	h_{ab}
BB	24.08 ± 1.25 [a]	65.24 ± 1.51 [a]	32.11 ± 0.92 [a]
RR	62.05 ± 1.96 [b]	54.18 ± 1.75 [b]	17.96 ± 2.08 [b]
RC	65.03 ± 1.44 [c]	51.46 ± 1.68 [c]	19.77 ± 0.91 [c]
BK	52.44 ± 0.92 [d]	58.14 ± 1.21 [d]	21.41 ± 0.99 [c]

Each value represents mean (n=9) ± SD.
Values in the same column followed by different letters are significantly different by ANOVA test ($p < 0.05$).