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Research article

Monitoring effects on anthocyanins, non-anthocyanin phenolics and $ORAC_{FL}$ values of Colombian bilberry (*V. meridionale Swartz)* during pulping and thermal operations

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ABSTRACT

Processing of berries usually degrades anthocyanin and non-anthocyanin phenolics and diminishes antioxidant activity. In Colombia, jelly produced from the fruit of *Vaccinium meridionale* Swartz is a popular product among consumers. The aim of this study was to determine the effect of jelly processing steps on bioactive components. Analysis of anthocyanins (ACNs) and non-anthocyanin phenolics was performed via HPLC-PDA. Antioxidant activity was assessed by the $ORAC_{FL}$ method. The pulping step had the highest impact on ACNs, whose total content was significantly higher in the pomace (747.6 ± 59.2 mg cyanidin 3-glucoside (cyn 3-glu)/100 g) than in the pulp (102.7 ± 8.3 mg cyn 3-glu/100 g). Similarly, pulping caused a significant decrease in flavonols, procyanidins (PACs) and $ORAC_{FL}$ values.

Despite the effects of processing, Colombian bilberry jelly can be considered a good source of phenolic compounds with high antioxidant activity. The final concentration of ACNs, hydroxycinnamic acids (HCAs) and flavonols, as well as the $ORAC_{FL}$ values in this product were comparable to those of fresh cranberry (*Vaccinium oxycoccos*) and black currant (*Ribes nigrum*). The results also suggest that the pomace of *V. meridionale* can be recovered as an excellent source of bioactive compounds.

1. Introduction

The fruit of Colombian agraz (*Vaccinium meridionale* Swartz) is a wild bilberry endemic to mountain slopes between 2000 and 3500 m above sea level. Since this fruit is not exposed to pesticides, it is highly valued among a selected circle of consumers, who demand natural sources of beneficial compounds, especially polyphenolics.

Due to the tartness and acidity of agraz, this product is currently underutilized; people prefer to eat it in the processed form. At present, jelly is the product with the highest demand among consumers.

Previous reports show that agraz berries contain a variety of polyphenolic compounds with bioactive characteristics including high antioxidant capacity, antiproliferative and cytotoxic effects against cancerous cells [1], cardioprotective properties in trials with ischemia-induced rats [2], anti-inflammatory effects in overweight individuals [3], and protective effects against colorectal cancer at in vitro and in vivo levels [4–7]. In addition, It has been shown that agraz pomace extracts prevent lipid oxidation in Greek yogurt in

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Nomenclature

ACNs	Anthocyanins
GAE	Gallic acid equivalents
HCAs	Hydroxycinnamic acids
PACs	Procyanidins
TE	Trolox equivalents
TPC	Total phenolic contents
RE	Rutin equivalents
CAE	Chlorogenic acid equivalents

pork patties during storage [8,9] also have antimicrobial activity against Gram positive and Gram negative bacteria [10].

The bioactive properties cited above are attributed to the variety of polyphenolic compounds present in this bilberry. Seventy seven percent of the total anthocyanins (ACNs) in this fruit (329.0 ± 28.0 mg cyanidin 3-glucoside equivalents (cyn 3-glu)/100 g FW) is represented by cyanidin glycosides [9], which are reported to confer high antioxidant and radical scavenging activities, comparable to that of α-tocopherol and Trolox [11].

Berries of *V. meridionale* also provide high levels of non-anthocyanin phenolics including hydroxycinnamic acids (HCAs) and flavonols. The most representative HCAs are caffeoylquinic acid, caffeoyl methyl quinate and caffeic acid derivatives, which account for 81.8 % (99.2 ± 6.7 mg/100 g FW) of the non-anthocyanin-phenolics. Among flavonols quercetin glycosides constitute the remaining percentage (41.9 ± 4.9 mg/100 g FW) [11]. Moreover, the pomace of the fruit is rich in A and B-type procyanidins (PACs) from monomers to decamers (140.9 ± 33.3 mg cocoa procyanidin equivalents/100 g FW) [10].

The detrimental effect on polyphenolic compounds during processing is well documented. Interactions with other food components along with reactions and structural changes under the influence of light, pH and temperature occur [12]. For instance, ACN degradation and decrease of their bioactivity is critical during jam production due to level and length of heating [13,14]. Other losses also occur as a result of physical removal of the pomace during the pulping operation since the concentration of bioactive compounds is higher in the peel and seeds [10].

Since berries of *V. meridionale* are rich in phenolics, understanding their stability during processing operations is important. There is no quantitative information on the changes of polyphenolics of this fruit during jelly processing. Accordingly, the aim of this study was to assess the stability of polyphenolics, total phenolics and antioxidant capacity of the fruit and *V. meridionale* during conventional jelly production.

2. Materials and methods

2.1. Reagents

HPLC reagents were acquired from Fisher Scientific. (FairLawn, NJ,USA). A standard mixture of anthocyanin glucosides was from Polyphenols Laboratories AS (Sandnes, Norway). Chlorogenic acid (3-caffeoylquinic acid), rutin, gallic acid, fluorescein sodium salt, 2,2-azobis-(2-amidinopropane) dihydrochloride (AAPH), (−)-epicatechin, and (+)-catechin were purchased from Sigma-Aldrich (St. Louis, MO). Isolated procyanidin standards were from Mars Inc. (Hackettstown, NJ, USA). Sodium carbonate, potassium persulphate, methanol, and Folin–Ciocalteau agent were purchased from Merck (Darmstadt, Germany).

2.2. Fruit processing

The bilberry fruits (*Vaccinium meridionale* S.) used in the production of jelly were obtained from the local market. The berries were selected according to their maturity index and color, sanitized with a 100 mg/L sodium hypochlorite solution, and processed in a stainless steel cylindrical pulper. The pulp was recovered for thermal processing while the recovered pomace was spread on a tray, frozen at −25 °C for 24 h and lyophilized. The conditions of the freeze drier (Labconco Freezone 6L; Kansas City, MO, USA) were: plate temperature of 20 °C, vacuum pressure of 0.130 mBar and condenser temperature of −50 °C. The collected material was pulverized to a fine powder and stored at −4 C for further analysis.

2.3. Jelly processing

The obtained pulp had a pH of 2.96 ± 0.064, a total soluble solids content of 11.83 ± 0.94 °Brix and a titratable acidity of 4.64 ± 1.24. Based on this composition, citric acid was excluded from the formulation. The fruit pulp was heated to a full boil in an open kettle (101–103 °C) and sugar was added and stirred continuously. The mixture was subjected to a second boil (103–105 °C) until obtaining a final total solids concentration of 65 °Brix. The final product was transferred to sterilized glass jars and capped. Glasses were inverted for 5 min to sterile the lids, turned upright and immersed in cooling water.

2.4. Extraction of anthocyanins and non-anthocyanin phenolics

The protocol described by Kim [15] with modifications was applied to extract phenolic compounds. For the pulp and jam, 10 g of material were mixed with 100 mL of methanol and sonicated for 20 min at room temperature in a water bath (B-2200R-1; Branson, Shelton, CT, USA), whereas 80 % aqueous methanol was added to the lyophilized pomace before sonication. After centrifugation at 4000×*g* for 20 min, the supernatant was recovered, and the solid was reextracted until obtaining a colorless solution. Methanol was evaporated from the pooled extracts using a Buchi Rotavapor at 40 °C. The extract was taken to a known volume, lyophilized and stored at 4 °C for further analysis.

2.5. HPLC-PDA analysis

Reverse phase separation of ACNs, HCAs and flavonols was carried out according to Mi et al. [16] with modifications. A Waters HPLC system equipped a model 996 photodiode array detector and a 4.6 mm × 250 mm Symmetry® C-18 column (Waters Corp, Milford, MA, USA) was used. The eluents were: A: Water/formic acid (95:5, v/v) and B: acetonitrile/formic acid (95:5, v/v). The elution conditions were as follows: linear gradient from 5 % to 45 % solvent B over 50 min at 1 ml min^{-1}, from 45 % to 5 % solvent B over 1 min; PDA data were recorded between 200 and 700 nm with parallel detection at 520 nm for ACNs, 320 nm for HCAs and 360 nm for flavonols. Anthocyanins were quantified as mg cyn 3-glu/100 g, using calibration curves of a mixture of anthocyanin glucosides. External standards of rutin were used to quantify flavonols mg rutin equivalents (RE)/100 g and chlorogenic acid solutions at different concentrations were used to calculate HCAs as mg chlorogenic acid equivalents (CAE)/100 g.

Normal phase HPLC was used for procyanidin separation, according to Kelm et al. [17] with modifications. A Develosil diol 100A (5 μm, 250 by 4.6 mm) column (Phenomenex, Torrance, CA) was connected to a Waters Alliance 2690 HPLC (Waters Corporation, Milford, MA) coupled to a scanning fluorescence detector, with excitation at 276 nm and emission at 316 nm, set at 21 °C. The mobile phase consisted of CH_3CN:HOAc (98:2, v/v) (A) and CH_3OH:H_2O:HOAc (95:3:2, v/v/v) (B). Run conditions were flow rate 0.8 mL min^{-1}, 30 °C, linear gradient 0–40 % B over 35 min; isocratic 40 % B over 5-min, followed by re-equilibration over 5 min.

Quantification of PACs with degrees of polymerization from DP1 (monomer) through DP10 (decamer) was achieved with an external calibration curve containing mixed procyanidin standards. Total PACs were determined by adding individual PACs. Results are expressed as mg cocoa procyanidin equivalents/100 g.

Identification of ACNs and non-anthocyanin phenolics in all fractions of the berry and the jelly was based on comparison with results obtained in previous research on the same fruit material [10,11].

2.6. Determination of total phenolics

Total phenolics content (TPC) was assessed in terms of mg of gallic acid equivalents (GAE)/100 g by the Folin-Ciocalteu method [18]. A mixture of 20 μl of extract or gallic acid standard (50–500 mg/L), 1.58 mL water and 100 μl of Folin–Ciocalteau's reagent was obtained and maintained at ambient temperature for 8 min. Then, 300 μl of a 20 % (w/v) aqueous sodium carbonate solution was combined with the previous solution. After 2 h at room temperature, absorbance of the samples was determined at 765 nm on a Shimadzu UV–visible spectrophotometer; model UV 160 U (Kyoto, Japan).

2.7. $ORAC_{FL}$ analysis

The antioxidant activity was determined applying the hydrophilic oxygen radical absorbing capacity (ORACFL) according to Prior et al. [19]. This is probably the most widely recognized and approved method as it has several advantages over other in vitro assays. First, it generates peroxyl free radicals, which are radicals frequently found in the living body. Thus, the measured antioxidant capacity resembles the physiological response [20]. Second, the generation of free radicals during the assay is continuous and thus, it resembles the reactions in vivo. Also, this assay is carried out in a phosphate buffer, with a pH similar to the one of the human body. Finally, the method is automated and highly reproducible [21], which reduces human error.

Forty μL of phosphate buffer (blank solution), extract, or Trolox standards (6.25, 12.5, 25, 50 μM) were dispensed in 48-well microplates. Thereafter, 400 μL of a 0.108 μM fluorescein solution, followed by AAPH (150 μL 31.6 mM) were added to each well. Detection of fluorescence from (AAPH) was recorded at 485 nm (excitation) and 520 nm emission every 192 s for 112 min (95 % loss of fluorescence). $ORAC_{FL}$ values were assessed from the regression equation by interpolating the differences between the blank and the samples in a Standard Trolox curves are expressed as μmol Trolox equivalents (TE)/g.

2.8. Statistical analyses

All experimental results are mean values with their corresponding standard errors. Experiments were replicated four times, and each measurement was repeated three times. Data analysis was performed using the Statgraphics 7 package (Statistical Graphics Corp. Manugistics Inc., Cambridge, MA). The effects of processing on ACNs, HCAs, flavonols, total PACs, TPC, and $ORAC_{FL}$ values were determined by one-way analysis of variance (ANOVA). The least significant difference procedure (LSD) was applied to assess significant differences ($p \leq 0.05$) among means.

Pearson correlation coefficients (R) were calculated to determine the contribution of TPC, total ACNs, HCA, and flavonols to the antioxidant capacity. Correlation coefficients were considered very strong if R = 0.90–0.99, strong when R = 0.70–0.89 and moderate

if R = 0.40–0.69 [22].

3. Results and discussion

3.1. Identification and quantification of ACNs and non-anthocyanin polyphenolics

Fig. 1 is the HPLC chromatogram of the ACNs found in the jelly. The profile was coincident with those from the different fruit fractions. Delphinidin 3-hesoxide, cyanidin 3-galactoside, delphinidin 3-pentoside, cyanidin 3-glucoside, and cyanidin 3-arabinoside were identified in each fraction of the berry and jelly. All HPLC chromatograms of the ACNs were in line with the ones previously identified in the whole fruit and pomace [10,11]. In accordance with Table 1, the total ACN content in the fresh bilberry and in the pomace was 232.1 ± 35.1 mg cyn 3-glu/100 g and 747.6 ± 59.2 mg cyn-3-glu/100 g, respectively. These values are also in agreement with the ones previously reported by the same authors (329 ± 28 mg cyn 3-glu/100 g FW for the berry and 747.6 ± 167.5 mg cyn-3-glu/100 g FW for the pomace).

Similarly, the HPLC profiles of HCAs and flavonols were consistent with fractions of the fruit and jelly. Hydroxycinnamic acids (Fig. 2A) to comprised of caffeoylquinic acid, caffeoyl methyl quinate, and caffeic acid derivatives; flavonols (Fig. 2B) included quercetin hexoside, quercetin pentoside, quercetin rhamnoside, and quercetin hydroxylmethylglutaryl-alpha-rhamnoside. These HCAs and flavonols were previously identified in Colombian bilberry and its pomace [10,11].

The content of individual and total HCAs and flavonols is reported in Table 2. The total amount of HCAs in the fresh bilberries was 258.0 ± 25.3 mg CAE/100 g, which is much higher than value (99.2 ± 6.7 CAE/100 g FW) previously reported [11]. Likewise, the amount of flavonols in the berries (78.2 ± 6.2 mg RE/100 g FW) was about twice as much the concentration found by Garzón et al. [11] (41.9 ± 4.9 mg RE/100 g FW). Multiple environmental factors such as altitude change, precipitation amount, growing site, temperature, wind speed, soil, radiation intensity along with genotype influence secondary metabolite biosynthesis and concentration [23].

The HPLC profile of PACs showed procyanidins of DP1-DP10 in the berry, and the pulp, which matched the pattern in the pomace as previously characterized [10]. The total content of PACs in the pomace was significantly higher ($p \leq 0.05$) than the content in the pulp and the berry. Procyanidins were not detected in the jelly (Table 3).

3.2. Processing effect on anthocyanins and non-anthocyanin polyphenols

Table 1 depicts the concentration of individual and total concentration of each phenolic compound obtained in each fraction of the fruit after the pulping heat treatment operations. In general, the content of each individual ACN and the total ACNs in the fresh pulp (102.7 ± 8.3 cyn 3-glu/100 g) was statistically comparable to the content in the whole berry and jelly, but significantly lower than the content in the pomace ($p \leq 0.05$).

A significant reduction ($p \leq 0.05$) in the content of ACNs in the jelly (27.5. ± 4.9 mg cyn 3-glu/100 g) as compared to the fresh fruit (232.1 ± 35.1 mg cyanidin-3-glucoside/100 g FW) was observed. This reduction confirms that concentration of ACNs is much higher in the exterior layers of fruits, which were removed during the pulping operation. In fact, the pomace of *V. meridionale*, which represents around 20 % of the fresh fruit comprises nearly twice the ACN content in the fruit [10].

During production of jelly the pulp is subjected to a long residence time in the kettle to evaporate most of the water and reach the final sugar concentration. During this step of the process, the pigment is susceptible to degradation by interaction with other components present in the pulp including vitamin C, sugars, and minerals, which are present in considerable concentration in red fruits [24].

The influence of light, oxygen and temperature is also detrimental. Granted that temperature is one of the most critical factors, the decrease in monomeric ACN can be attributed to the shift of the molecule towards the tautomeric open-form and the colorless chalcone because of thermal treatment. Such shift is more pronounced in monoglycosidic anthocyanins [13], which is consistent with presence

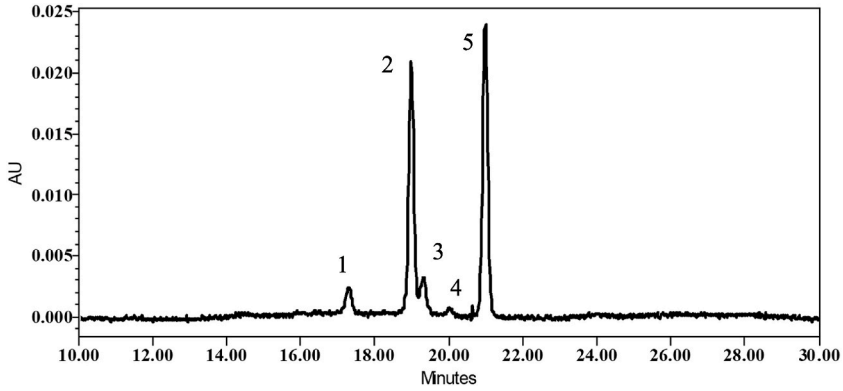

Fig. 1. Representative HPLC-PDA chromatogram of anthocyanins detected at 512 nm in wild bilberry (*V. meridionale* S.) jelly. (1) delphinidin 3-hexoside; (2) cyanidin 3-galactoside; (3) delphinidin 3-pentoside; (4) cyanidin 3-glucoside; (5) cyanidin 3-arabinoside.

Table 1
HPLC-DAD analysis of anthocyanins in each fraction of the fruit and in the jelly of Colombian bilberry (*V. meridionale* S.).

Anthocyanins (mg cyanidin 3-glucoside/100 g)						
Product	Delphinidin 3-hesoxide	Cyanidin 3-galactoside	Delphinidin 3-pentoside	Cyanidin 3-glucoside	Cyanidin 3-arabinoside	Total
Fresh bilberry	11.5 ± 2.5[a]	106.5 ± 16.5[a]	11.1 ± 2.4[a]	4.1 ± 0.8[a]	98.8 ± 13.6[a]	232.1 ± 35.1[a]
Pomace	38.5 ± 6.1[b]	357.7 ± 26.7[b]	34.7 ± 4.9[b]	10.1 ± 0.7[b]	306.6 ± 21.4[b]	747.6 ± 59.2[b]
Pulp	4.8 ± 0.4[a]	42.8 ± 3.4[ac]	6.3 ± 2.1[a]	1.3 ± 0.2[c]	47.0 ± 4.3[ac]	102.7 ± 8.3[ac]
Jelly	1.6 ± 0.3[a]	11.1 ± 2.2[c]	2.9 ± 0.5[a]	0.5 ± 0.1[c]	11.5 ± 1.8[c]	27.5 ± 4.9[c]

Values represent means ± standard error (n = 4).
Values in columns with different letters are significantly different at $p \leq 0.05$.

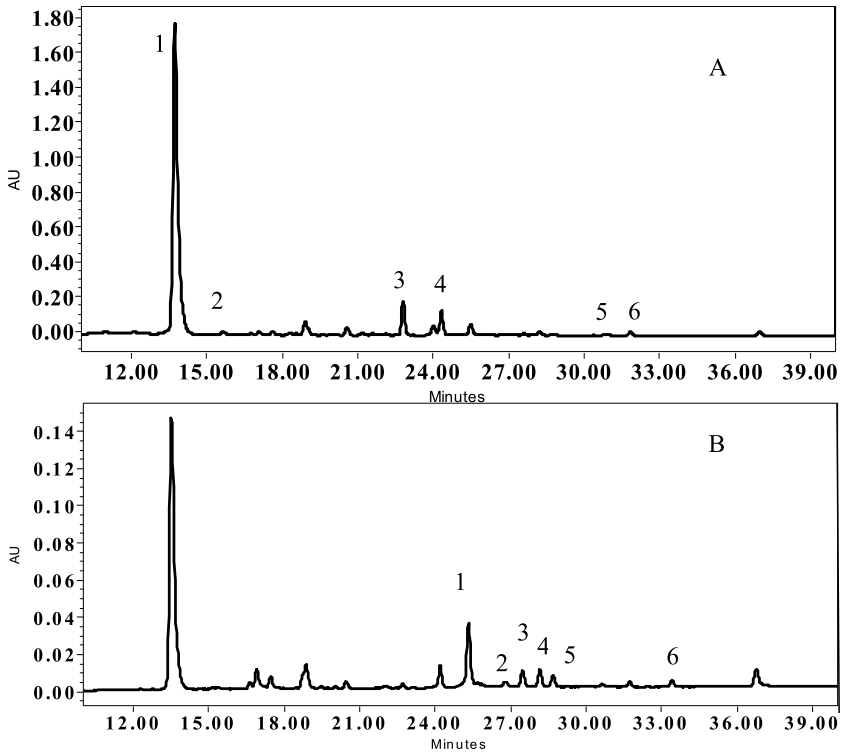

Fig. 2. Representative HPLC-PDA chromatograms of non-anthocyanin phenolics present in wild bilberry (*V. meridionale* S.) jelly. A: Hydroxycinnamic acids detected at 320 nm. (1) caffeoylquinic acid isomer 1; (2) caffeoylquinic acid isomer 2; (3) caffeoyl methyl quinate; (4) caffeic acid derivative; (5) caffeic acid derivative; (6) caffeic acid derivative. B: Flavonols detected at 360 nm. (1) quercetin hexoside; (2) quercetin pentoside; (3) quercetin pentoside; (4) quercetin pentoside; (5) quercetin rhamnoside; (6) quercetin hydroxylmethylglutarylα-rhamnoside. Unlabelled peaks are anthocyanins.

of mg cyanidin-3-galactoside and cyanidin-3-galactoside arabinoside as the major ACNs in *V. meridionale*.

Numerous studies confirm the susceptibility of berry ACNs to temperature. Pigment losses between 28 and 80 % have been observed depending on length of heating and interactions with other molecules present in the food matrix [13].

Despite of the reduction in monomeric pigment, the total content of ACNs in the jelly is within the range of other fresh berries from the *Vaccinium* family such as *Vaccinium vitis-idaea,* (30–50 mg cyn-3-glu/100 g FW), and *Vaccinium macrocarpon* (31–102 mg cyn 3-glu/100 g FW) [25]. The loss of pigment was not significant when comparing the ACN content in the pulp (102.7 ± 8.3 mg cyn 3-glu/100 g) with the content in the jelly (27.5 ± 4.9 mg cyn 3-glu/100 g), which might be related to the high sugar concentration. Consistent with these results, other studies [26,27] have reported excellent retention of individual and total ACNs in jam and marmalade made from highbush blueberry (*Vaccinium corymbosum* L.) and fresh black carrots (*Daucus carota* L. ssp. sativus var. atrorubens Alef.), respectively. Likewise, others [28] found that high additions of sucrose to black currant pigments improved their thermostability, while Watanabe et al. [29] noted a decrease in *k* (rate constant of ACN degradation) when strawberries were impregnated with sucrose

Table 2
HPLC-DAD analysis of hydroxycinnamic acids and flavonols present in each component of the fruit and in the jelly of Colombian bilberry (*V. meridionale* S.).

Hydroxycinnamic acids (mg chlorogenic acid equivalents/100 g)							
Product	Caffeoylquinic acid isomer 1	Caffeoylquinic acid isomer 2	Caffeoyl methyl quinate	Caffeic acid derivative	Caffeic acid derivative isomer 1	Caffeic acid derivative isomer 2	Total
Fresh bilberry	234.0 ± 23.4^{a}	2.7 ± 0.6^{a}	2.8 ± 0.7^{a}	4.1 ± 0.9^{a}	11.9 ± 0.7^{a}	3.1 ± 0.1^{a}	258.0 ± 25.3^{a}
Pomace	246.9 ± 42.6^{a}	5.9 ± 1.4^{b}	2.3 ± 0.6^{a}	2.5 ± 0.6^{ac}	40.1 ± 8.8^{b}	11.4 ± 2.8^{b}	309.1 ± 56.2^{a}
Pulp	231.0 ± 22.7^{a}	2.2 ± 0.3^{a}	2.7 ± 0.5^{a}	4.1 ± 0.7^{a}	4.8 ± 1.6^{a}	6.3 ± 0.9^{ab}	251.1 ± 24.2^{a}
Jam	122.6 ± 24.8^{b}	1.1 ± 0.2^{a}	2.6 ± 0.6^{a}	0.7 ± 0.2^{bc}	3.0 ± 0.1^{a}	1.1 ± 0.2^{a}	129.5 ± 24.2^{b}
Flavonols (mg rutin equivalents/100 g)							
Product	Quercetin hexoside	Quercetin pentoside	Quercetin pentoside	Quercetin pentoside	Quercetin rhamnoside	Quercetin hydroxylmethylglutaryl-alpha-rhamnoside	Total
Fresh bilberry	40.9 ± 4.7^{a}	3.8 ± 0.3^{a}	11.5 ± 1.7^{a}	12.4 ± 1.2^{a}	4.6 ± 1.2^{a}	5.1 ± 0.9^{a}	78.2 ± 6.2^{a}
Pomace	161.2 ± 33.9^{b}	14.2 ± 2.4^{b}	39.0 ± 8.8^{b}	58.8 ± 10.4^{b}	36.9 ± 5.6^{b}	24.9 ± 6.1^{b}	335.0 ± 56.9^{b}
Pulp	15.4 ± 1.2^{a}	2.2 ± 0.1^{a}	3.2 ± 0.3^{a}	4.3 ± 0.3^{a}	2.7 ± 0.7^{a}	2.5 ± 0.3^{a}	30.3 ± 1.3^{a}
Jam	3.7 ± 0.3^{a}	0.6 ± 0.0^{a}	1.0 ± 0.1^{a}	1.0 ± 0.2^{a}	1.2 ± 0.4^{a}	1.3 ± 0.2^{a}	7.6 ± 0.1^{a}

Values represent means ± standard error (n = 4).
Values in columns with different letters are significantly different at $p \leq 0.05$.

Table 3
Normal phase HPLC analysis of procyanidins in each component of the fruit and in the jelly of Colombian bilberry (*V. meridionale* S.).

Degree of polymerization	Fruit fraction			
	Fresh bilberry	Pomace	Pulp	Jam
DP1	11.9 ± 1.6^{a}	20.8 ± 2.8^{b}	6.2 ± 1.5^{a}	ND
DP2	5.2 ± 0.5^{a}	3.6 ± 1.3^{b}	2.9 ± 0.3^{a}	ND
DP3	10.9 ± 2.0^{a}	23.7 ± 2.2^{b}	7.8 ± 0.8^{a}	ND
DP4	7.2 ± 0.7^{a}	15.2 ± 1.4^{b}	5.8 ± 0.7^{a}	ND
DP5	5.1 ± 0.7^{a}	12.0 ± 1.2^{b}	4.6 ± 0.6^{a}	ND
DP6	5.6 ± 0.9^{a}	13.0 ± 1.1^{b}	4.8 ± 0.5^{a}	ND
DP7	3.0 ± 0.4^{a}	8.5 ± 0.8^{b}	2.9 ± 0.9^{a}	ND
DP8	3.3 ± 0.5^{a}	8.5 ± 0.9^{b}	2.9 ± 0.4^{a}	ND
DP9	5.6 ± 0.9^{a}	17.6 ± 1.3^{b}	5.3 ± 0.7^{a}	ND
DP10	2.2 ± 0.7^{a}	8.5 ± 0.8^{b}	3.1 ± 0.4^{a}	ND
Total	59.9 ± 8.8^{a}	140.9 ± 11.8^{b}	46.8 ± 6.0^{a}	ND

Values represent means ± standard error (n = 4).
Values in rows with different letters are significantly different at $p \leq 0.05$.
ND = nondetectable.

before preparation of jam. According to these authors, addition of sucrose in high concentration improves ACN retention in the final products through various mechanisms such as reducing water activity, inhibiting pigment degradative enzymes (polyphenol oxidase and β-glucosidase), providing a partial oxygen barrier, and sterically interfering with condensation between ACNs, other phenolics and ascorbate.

The total content of HCAs was statistically comparable among the fresh berry (258.0 ± 25.3 mg CAE/100 g), pomace (309.1 ± 56.2 mg CAE/100 g) and pulp (251.1 ± 24.2 mg CAE/100 g), indicating that contrary to ACNs, HCAs were not lost during pulping and that the concentration of these compounds was not higher in the exterior layers of fruit. In contrast, there were significant decreases ($p \leq 0.05$) in the content of the predominant HCA (caffeoylquinic acid-isomer 1) and in the total HCAs in the jelly as compared to all the fruit fractions. Caffeoylquinic acid-isomer 1 changed from 234.0 ± 23.4 to 122.6 ± 24.8 mg CAE/100 g while the total HCAs decreased from 258.0 ± 25.3 to 129.5 ± 24.2 mg CAE/100 g. These findings suggests that oxidation reactions of caffeic acid derivatives leading to caffeic acid *ortho*-quinones during heating under aerobic conditions might be the main reason for the HCAs degradation.

In agreement with these result Salazar-orbea [30], reported 44 % decrease in chlorogenic acid in apple puree heated at 90 °C for 30 min in the presence of oxygen, but no change was observed under anerobic conditions.

On the other hand, Szwajgier et al. [31] indicated that thermal processing applied to fruit preserves may cause a differential effect on the concentration of phenolic acids depending on the specific fruit composition. Therefore, conclusions could not be subject to a single explanation.

Despite the decrease in HCAs, the total content of chlorogenic acid in the final jelly is equivalent to the quantity delivered by cultivated half-highbush blueberries (*V. angustifolium* × *corymbosum*) cultivar North blue, which contains 130.4 mg CAE/100 g FW [32]. The amount of chlorogenic acid in the jelly is also consistent with the one delivered by 200-ml serving of brewed coffee, which is known for its high level of chlorogenic acid and potential positive health effects [33].

The total flavonol content in the pulp (30.3 ± 1.3 RE/100 g), fruit (78.2 ± 6.2 RE/100 g) and jelly (7.6 ± 1.1 mg RE/100 g) was statistically comparable, but significantly lower ($p \leq 0.05$) than the content in the pomace (335.0 ± 56.9 mg RE/100 g). These numbers show that a considerable amount of the flavonols remained in the pomace after pulping.

All flavonols present in *V. meridionale* are quercetin derivatives, whose stability is affected during food processing. Recent reports on UPLC-Q-TOF-MS-MS analysis revealed that quercetin is unstable in boiling water. Under this condition, this flavonol degrades readily to produce phenolic acids and other compounds as a result of oxidation, hydroxylation and nucleophilic attack cleavages [34]. In agreement with this hypothesis, Aaby & Amundsen [35] observed increase of free quercetin due to the degradation of quercetin glycosides during thermal treatment of lingonberries (*Vaccinium vitis-idaea* L.) for juice production. Also, Tuárez-García et al. [36] noticed a significant reduction in quercetin hexosides content after applying heat treatments to banana pulp.

Accordingly, heat treatment to produce the jelly might be responsible for the reduction in flavonol content in the final product. Nevertheless, the total flavonol concentration in the jelly was higher than the rutin content in fresh fruits such as gooseberry (*Ribesuva-crispa*) (3.03 ± 0.09 mg RE/100g FW) and blueberries (*Vaccinium corymbosum* L.) (3.1 ± 0.1 mg RE/100g FW) as reported by Multescu & Susman and Može et al., respectively [37,38].

The content of total PACs in the fruit was 59.9 ± 8.8 mg cocoa equivalents/100 g, which is close to the amount (41.0 mg cocoa equivalents/100 g) reported before [11]. This concentration along with the concentration of individual PACs was significantly lower ($p \leq 0.05$) than the concentration in the pomace (140.9 ± 11.8 mg cocoa equivalents/100 g), which confirms that 25–50 % of the PACs of fresh berries remain in this byproduct after juice extraction as found by Struck et al. [39].

Procyanidins were not detectable in the jelly. There are several factors that might explain this observation. These polymers can either be hydrolyzed to cyanidins under thermal treatments [36], or they can interact with polysaccharides specially pectin to form non-covalent interactions [40]. Le Bourvellec et al. [41] hypothesized that *ortho*-quinone produced from caffeoylquinic acid oxidation may react with catechin to yield an oxidation/reduction reaction, which reduces proanthocyanidins concentration. On the other hand,

Howard et al. [42] hypothesized that PACs form larger oligomers in high-calorie jams as a result of the reduced water activity. Such large oligomers cannot be detected by HPLC.

3.3. Processing effect on total phenolics content and antioxidant capacity

Fig. 3 shows TPC values of the berry fractions and the jelly. There was a considerable reduction in TPC due to pulping operations. The TPC in the pomace (15.14 mg GAE/g) was nearly threefold higher than the content in the berry (4.72 mg GAE/g) and about four times higher than the pulp (3.90 mg GAE/g). The TPC value of the jelly (1.98 mg GAE/g) was statistically comparable to that one of the whole berries and to the value in other fresh fruits including raspberry (*Rubus ideaus* L.) (1.14–18.2 mg GAE/g) and blackberries (*Rubus fructicosus* L.) (0.49–6.90 mg/g) [43]. Both berries are classified as fruits with high level of TPC [43].

The antioxidant activity values of the berry fractions and the jelly are shown in Fig. 4. The pomace showed the highest $ORAC_{FL}$ values, with an average value of 286.3 ± 13.1 μmol TE/g, which can attributed to the high amount of ACNs and non-anthocyanin phenolics that remain in this fraction during pulping operations. The $ORAC_{FL}$ mean value for the whole berry (130.0 ± 7.7 μmol TE/g) was comparable to the mean value of the pulp (136.7 ± 9.3 μmol TE/g), but significantly higher ($p < 0.05$) than the mean value of the jelly (89.6 ± 7.2 μmol TE/g). The lowest $ORAC_{FL}$ value of the jelly extracts confirms that thermal treatment applied during processing caused degradation of polyphenolics responsible for antioxidant properties of *V. meridionale*. In other words, degraded polyphenolics loss their ability to transfer hydrogen atoms to AAPH-derived peroxyl radicals.

In spite of the decrease in antioxidant activity, and due to the high phenolic concentration, the jelly is still a product with high $ORAC_{FL}$ value, comparable to that one of cranberry (*Vaccinium oxycoccos*) (18.5–96.8 μmol TE/g FW), and black currant (*Ribes nigrum*) (36.9–93.1 μmol TE/g FW) [24]. Although $ORAC_{FL}$ is an in vitro assay, these findings are relevant. It has been hypothesized that food products with antioxidant capacity can help to reduce the concentration of free radicals in the body, which participate in chronic diseases [24]. In fact, data obtained by the ORAC assay during a recent study on the antioxidant activity of proanthocyanidins was comparable to the results observed in a biological model (the reduction of the activation of NF-κB) verifying that this assay yields a more significative response to foresee the biological effect of the sample [20].

The $ORAC_{FL}$ value found in the jelly could be explained partly by its concentration of cyn 3-galactoside and quercetin-based flavonols and their ortho-hydroxy groups, which allow these phenolics to donate the hydrogen and stabilize phenoxyl radicals. Cyanidin 3-galactoside is known as a compound with a crucial antioxidant role in plants. In vitro assays have demonstrated its high radical scavenging activity as well as its hydrogen peroxide and radical oxygen scavenging capacities. Previous studies have also found that this ACN can perform its physiological functions in the absence of other compounds [25].

In as much the same way, quercetin shows high antioxidant activity and high ability to scavenge free radicals due to its *ortho*-hydroxy group. Clinical studies have proved that quercetin is able to pass through the blood brain barrier and quench free radicals, which results in antiinflammatory, cardioprotective and neuroprotective effects in patients suffering from Alzheimer's disease [44].

3.4. Correlation between antioxidant capacity and phenolic composition

The highest correlations between each group of phenolic compounds and the antioxidant activity were found in in the pomace. Correlation coefficients (R) were 0.97 for TPC and $ORAC_{FL}$, 1 for HCAs and $ORAC_{FL}$, 0.98 for PACs and $ORAC_{FL}$, 0.94 for ACNs and

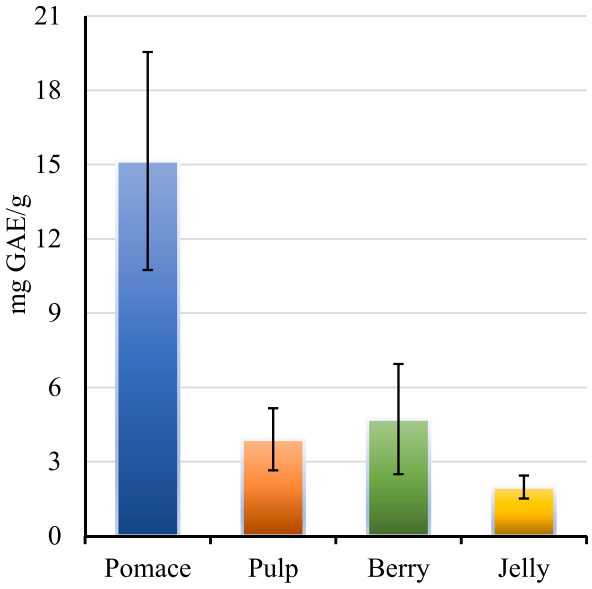

Fig. 3. Total phenolics content of *V.meridionale* S. fruit components and jelly. Bars represent the mean ± SEM (n = 4).

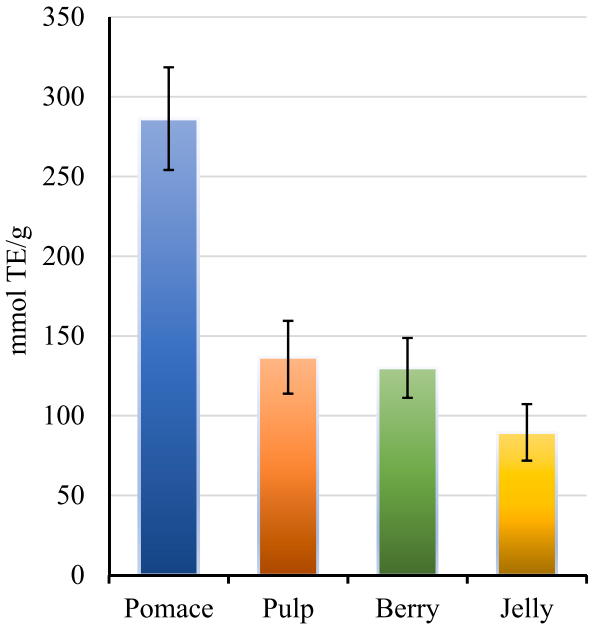

Fig. 4. $ORAC_{FL}$ of *V.meridionale* S. fruit components and jelly. Bars represent the mean ± SEM (n = 4).

$ORAC_{FL}$, and 0.94 for flavonols and $ORAC_{FL}$.

For the whole berry, very strong correlations were found between TPC and $ORAC_{FL}$ (R = 0.99), and PACs and $ORAC_{FL}$ (R = 0.98). Anthocyanins, flavonols and HCAs showed strong correlation with $ORAC_{FL}$ (R = 0.83, 0.78 and 0.75, respectively).

Total phenolics content and $ORAC_{FL}$ presented a very strong correlation (R = 0.98) in the pulp while ACNS, flavonols and HCAs showed strong correlations (R = 0.89, 0.83 and 0.81, individually). A weak correlation of R = 0.23 was found between PACs and $ORAC_{FL}$.

Regarding the relation between TPC and $ORAC_{FL}$ in the jelly, a linear correlation coefficient with the value of 0.73 was detected. A moderate linear correlation between the total HCAs and $ORAC_{FL}$ was analyzed (R = 0.45); however, stronger correlations were established between flavonols and $ORAC_{FL}$ (R = 0.98) and ACNs and $ORAC_{FL}$ (R = 0.89).

The observed degree of correlation found among the different group of polyphenolics and $ORAC_{FL}$ is explained by the concentration of these compounds in each fruit fraction and in the jelly, and by the chemical structure of phenolics. Chemical configuration of the molecules is closely related to their antioxidant properties; cyanidin based-ACNs, caffeoylquinic acid, quercetin derivatives and PACs exhibit *ortho*-dihydroxy structure phenolic, which show a higher ability to donate a hydrogen atom to an unpaired electron than monohydroxy phenolics [21]. Therefore, they are excellent radical scavengers.

Accordingly, the high correlations among polyphenolics and antioxidant activity in *V. meridionale* are supported by the *ortho*-dihydroxy configuration of these phenolics. Cyanidin based-ACNs, caffeoylquinic acid, quercetin derivatives and PACs exhibit *ortho*-dihydroxy structure.

Correlation coefficients showed that PACs made a high contribution to the antioxidant power of the fruit and the pomace of *V. meridionale,* but not to the one of pulp and jelly. The explanation for these numbers is that the highest amount of A and B-type PACs remains in the peel of the fruit, which is separated during pulping. It has been demonstrated that because PACs have more than 1 ortho hydroxyl substituent per molecule, they are better hydrogen donors than smaller molecules with such configuration. The higher the degree of polymerization, the higher the number of *ortho hydroxyl* substituent per molecule; thus, oligomeric and polymeric PACs present in agraz must account for the observed correlation coefficients.

These observations are significant as it has been reported that procyanidin-rich extracts specially A-type show physiological responses against oxidative stress that cause metabolic diseases [20,45,46].

4. Conclusions

In summary, the characterization of phenolic compounds present in the different fractions of the fruit of *V. meridionale* during jelly processing was attained. It was also determined that obtention of agraz jelly resulted in large decrease of HCAs, total ACNs, flavonols, PACs, and $ORAC_{FL}$ values. Nevertheless, this product can be considered a good source of bioactive compounds as levels of ACNs were close to those of *Vaccinium vitis-idaea* and *Vaccinium macrocarpon,* while the concentration of HCAs is comparable to the one of cultivated half-highbush blueberries (*V. angustifolium* × *corymbosum*) cultivar North blue. Similarly the amount of flavonols compares with the one found in gooseberry (*Ribesuva-crispa*) and blueberries (*Vaccinium corymbosum* L.)

Correlation coefficients showed that the $ORAC_{FL}$ value of the jelly is mainly due to the presence of flavonols and ACNs with *ortho-*

dihydroxy structure, which are excellent radical scavengers. However, given that the $ORAC_{FL}$ assay is an in vitro method, it is recommended to apply in vivo assays to test the radical scavenging ability of *V. meridionale* jelly.

Since agraz jelly is a highly valued product only among a selected circle of consumers, this new knowledge could promote further use of this underutilized wild species from the tropical Andes. Furthermore, these findings can contribute to the utilization of *V. meridionale* pomace, which retains high levels of health-promoting polyphenolics and therefore, can be used as a raw material for extracting natural antioxidants.

Data availability

Data will be made available on request.

CRediT authorship contribution statement

Luke Howard: Writing – review & editing, Methodology. **Cindi R. Brownmiller:** Data curation. **G. Astrid Garzón:** Writing – review & editing, Writing – original draft, Project administration, Formal analysis, Conceptualization.

Declaration of competing interest

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

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