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The influence of different time durations of thermal processing on berries quality☆

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ABSTRACT

Bioactive compounds (polyphenols, flavonoids, flavanols, tannins, anthocyanins and ascorbic acid) and the level of antioxidant activity by ABTS, DPPH, FRAP and CUPRAC of water, acetone and hexane extracts of Chilean 'Murtilla' (*Ugni molinae* Turcz) and 'Myrteola' berries (Myrtaceae, *Myrteola nummularia* (Poiret) Berg.), Chilean and Polish blueberries (*Vaccinium corymbosum*), Chilean raspberries (*Rubus idaeus*), and Polish black chokeberry (*Aronia melanocarpa*) were determined and compared. It was found that the contents of the bioactive compounds and the levels of antioxidant activities in used extracts differ significantly ($P < 0.05$). The correlation between the total polyphenols, flavanols and the antioxidant activities was significantly the highest in water, average in acetone and the lowest in hexane extracts. Fourier transform infrared (FTIR) spectroscopy was applied as an additional tool for the characterization of the water polyphenol extracts. Aqueous extracts of investigated berries were subjected to different times of thermal processing. Bioactive compounds and the levels of antioxidant activities by 2,2-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{+}$); 1,1-Diphenyl-2-picrylhydrazyl method (DPPH); Ferric-reducing/antioxidant power (FRAP) and Cupric reducing antioxidant capacity (CUPRAC) after 10, 20, 40 and 60 min of thermal processing were determined and compared with non processed samples. It was found that the antioxidant activity only of berries subjected to thermal processing for 10 and 20 min did not differ from the non thermally processed studied berries, showing high correlation between the total polyphenols, flavanols and the antioxidant activities. In conclusion, thermal treatment of studied berries influences their quality: only berries after 10 and 20 min of thermal processing preserved their bioactivity.

1. Introduction

Polyphenolic compounds, which present in berries, fruits and vegetables important not only in terms of quality, as they influence the visual appearance and taste, but also from a therapeutical point of view, as they appear to be associated with the prevention of different diseases (Arancibia-Avila et al., 2011; Borowska & Mazur, 2008; Fredes, 2009; Gorinstein et al., 2009; Piasek et al., 2011).

The bioactive nutrients and antioxidants present in fruits and berries are responsible for their perception as healthy foods (Dean, Leavens, & Boyd, 2010). Lugasi, Hovari, Kadar, and Denes (2011) determined phenolics in raspberry, blackberry and currant cultivars. Two cultivars of conventionally and organically grown red raspberries and blueberries were analyzed for total anthocyanins, total and specific phenolic compounds and total antioxidant activity (Sablani et al., 2010). From a big number of cited references above it can be concluded that the subject of different berries was investigated intensively. Chilean berries were also studied (Fredes, 2009). We were interested to investigate a new kind of Chilean berry known by the name of 'Myrteola' and to compare its composition with the wide consumed berries, which was described in our recent report (Arancibia-Avila et al., 2011).

☆ This research is dedicated to the memory of Prof. Simon Trakhtenberg, who encouraged and supported our research group during all his life.

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However, fresh berries are not available all year around. Therefore, it was important to find a proper substitute for fresh berries, which could be used when fresh berries are not available. Now berries are processed into a number of liquid forms for use in beverages and dairy products. This includes single strength berry juice, purees, jams and concentrates of different brix levels. However, processing of fruits and vegetables leads to decrease in their bioactivity (Bushra, Farooq, & Shahid, 2008; Cisse, Vaillant, Acosta, Dhuique-Mayer, & Dornier, 2009; Ferracane et al., 2008; Jimenez-Montral, Garcia-Diz, Martinez-Tome, Mariscal, & Murzia, 2009; Piasek et al., 2011). In the present study we use different time durations for berries thermal processing in order to find the optimal one which better preserves their quality.

In order to evaluate the bioactivity of the berries before and after processing the contents of polyphenols, flavonoids, flavanols, tannins, anthocyanins and ascorbic acid and the antioxidant activity were determined by ABTS DPPH, FRAP and CUPRAC. FTIR spectroscopy was used for the characterization of polyphenols in water extracts.

As far as we know no results of such investigations were published.

2. Material and methods

2.1. Reagents

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent (FCR), lanthanum (III) chloride heptahydrate, $FeCl_3 \times 6H_2O$, $CuCl_2 \times 2H_2O$, 2,9-dimethyl-1,10-phenanthroline (neocuproine) were purchased from Sigma Chemical Co., St Louis, MO, USA. 2, 4, 6-Tripyridyl-s-triazine (TPTZ) was purchased from Fluka Chemie, Buchs, Switzerland. All reagents were of analytical grade. Deionised and distilled water was used throughout.

2.2. Samples

Chilean 'Murtilla' (*Ugni molinae* Turcz) and 'Myrteola' berries (Myrtaceae, Myrteola nummularia (Poiret) Berg.), Chilean and Polish blueberries (*Vaccinium corymbosum*), raspberries (*Rubus idaeus*) and Polish black chokeberry (*Aronia melanocarpa*) were investigated. The fruits were harvested at their maturity stage and 'Murtilla' and 'Myrteola' berries in two stages of ripening. 'Myrteola' ripe was harvested in May 2008. 'Myrteola' non-ripe was harvested in February 2010, in Chiloé. 'Myrteola' non-ripe was collected in Puerto Varas, Chile, in February 2010, and 'Murtilla' ripe was purchased in May 2010 at the market in Puerto Mont. Arandano (blueberries) and raspberries were purchased at the local market in Chillan, Chile; and blueberries and chokeberries—at the local market in Warsaw, Poland. For the investigation were used five replicates of five berries each. Their edible parts were prepared manually without using steel knives. The prepared berries were weighed, chopped and homogenized under liquid nitrogen in a high-speed blender (Hamilton Beach Silex professional model) for 1 min. A weighed portion (50–100 g) was then lyophilized for 48 h (Virtis model 10–324), and the dry weight was determined. The samples were ground to pass through a 0.5 mm sieve and stored at −20° C until the bioactive substances were analyzed.

2.3. Determination of bioactive compounds and antioxidant activities (AA)

The contents of polyphenols, flavonoids, flavanols, tannins, anthocyanins and ascorbic acid in three different extracts of the studied samples were determined as previously described (Gorinstein et al., 2010). Phenols were extracted from lyophilized berries with water, 100% of acetone and 100% of hexane (concentration 25 mg/mL) at room temperature twice during 3 h. The polyphenols were determined by Folin-Ciocalteu method with measurement at 750 nm using spectrophotometer (Hewlett-Packard, model 8452A, Rockvile, USA). The results were expressed as mg of gallic acid equivalents (GAE) per g DW (Singleton, Orthofer, & Lamuela-Raventos, 1999). Flavonoids, extracted with 5% $NaNO_2$, 10% $AlCl_3 \times 6H_2O$ and 1 M NaOH, were measured at 510 nm. The total flavanols were estimated using the *p*-dimethylaminocinnamaldehyde (DMACA) method, and then the absorbance at 640 nm was read. The extracts of condensed tannins (procyanidins) with 4% methanol vanillin solution were measured at 500 nm (+)-Catechin served as a standard for flavonoids, flavanols, and tannins, and the results were expressed as catechin equivalents (CE). Total ascorbic acid was determined by CUPRAC assay (Ozyurek, Guclu, Bektasoglu, & Apak, 2007) in water extract (100 mg of lyophilized sample and 5 mL of water). The absorbance of the formed bis (Nc)-copper (I) chelate was measured at 450 nm. The total anthocyanins were measured by a pH differential method. Absorbance was measured in a Beckman spectrophotometer at 510 nm and at 700 nm in buffers at pH 1.0 and 4.5, using $A = [(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}]$. Results were expressed as mg of cyanidin-3-glucoside equivalent (CGE)/g of DW (Cheng & Breen, 1991).

The AA was determined by four complementary assays:

(1) 2,2-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^+$) method for the screening of antioxidant activity is reported as a decolorization assay applicable to both lipophilic and hydrophilic antioxidants, including flavonoids, hydroxycinnamates, carotenoids, and plasma antioxidants. The pre-formed radical monocation ABTS is generated by oxidation of ABTS with potassium persulfate and is reduced in the presence of such hydrogen-donating antioxidants. The influences of both the concentration of antioxidant and duration of reaction on the inhibition of the radical cation absorption are taken into account when determining the antioxidant activity. $ABTS^{\cdot +}$ radical cation was generated by the interaction of ABTS (7 mM/L) and $K_2S_2O_8$ (2.45 mM/L). This solution was diluted with methanol until the absorbance in the samples reached 0.7 at 734 nm ABTS (Re et al., 1999).

(2) Ferric-reducing/antioxidant power (FRAP) assay measures the ability of the antioxidants in the investigated samples to reduce ferric-tripiridyltriazine (Fe^{3+}-TPTZ) to a ferrous form (Fe^{2+}), which absorbs light at 593 nm (Benzie & Strain, 1996).

(3) Cupric reducing antioxidant capacity (CUPRAC): This assay is based on utilizing the copper (II)-neocuproine [Cu (II)-Nc] reagent as the chromogenic oxidizing agent. The absorbance at 450 nm was recorded against a reagent blank (Apak, Guclu, Ozyurek, & Karademir, 2004).

(4) Scavenging free radical potentials were tested in a methanolic solution of 1,1-Diphenyl-2-picrylhydrazyl method (DPPH). The degree of decoloration of the solution indicates the scavenging efficiency of the added substance. In its radical form, DPPH has an absorption band at 515 nm which disappears upon reduction by an antiradical compounds. DPPH solution (3.9 mL, 25 mg/L) in methanol was mixed with the samples extracts (0.1 mL), then the reaction progress was monitored at 515 nm until the absorbance was stable (Brand-Williams, Cuvelier, & Berset, 1995).

2.4. Fourier transform infrared (FTIR) spectra

The presence of polyphenols in the investigated fruit samples was studied by Fourier Transform Infrared (FTIR) spectroscopy. A

Nicolet iS 10 FTIR Spectrometer (Thermo Scientific Instruments LLC, Madison, WI, USA), with the smart iTR™ ATR (Attenuated Total Reflectance) accessory was used to record IR spectra (Sinelli, Spinardi, Di Egidio, Mignani, & Casiraghia, 2008).

2.5. The method of thermal processing

All berries can be used either in infusion or decoction. Therefore lyophilized samples of three berries ('Murtilla', Chilean and Polish blueberries) were extracted twice in water at room temperature during 3 h and then were placed in oven at 100 °C for different periods of time as 10, 20, 40 and 60 min. Bioactive compounds and antioxidant activities were determined by the methods described above.

2.6. Statistical analyses

To verify the statistical significance, mean ± SD of five independent measurements were calculated. Differences between groups were tested by two ways ANOVA. In the assessment of the antioxidant activity, Spearman correlation coefficients (R) were used. Linear regressions were also calculated. P-values of <0.05 were considered significant.

3. Results and discussion

3.1. Bioactive compounds and antioxidant activity

The results of the determination of the contents of the bioactive compounds of all studied samples in water and organic solvents are summarized in the Tables 1 and 2. As can be seen, the significant highest content ($P < 0.05$) of polyphenols, flavonoids, flavanols, tannins and ascorbic acid, was in water extract of 'Murtilla' non-ripe (MURTANR) sample (84.81 ± 3.9 mg GAE/g, 11.47 ± 0.6 mg CE/g, 4241.2 ± 189.8 μg CE/g, 8.91 ± 0.4 mg CE/g and 9.12 ± 0.4 mg/g, respectively). Only the content of anthocyanins (mg CGE/g) was significantly higher ($P < 0.05$) in blueberries (BLUEBPOL) from Poland (323.2 ± 16.1 mg CGE/g). The contents of most bioactive compounds in blueberries (BLUEBCH) and raspberries (RASBER) in water, acetone and hexane extracts are comparable with the data in 'Murteola' ripe (MURTEOR). The bioactive compounds (polyphenols, flavonoids, flavanols, tannins and ascorbic acid, Tables 1 and 2) are significantly higher in water and significantly lower in hexane extracts, respectively ($P < 0.05$).

The following order of the value of polyphenols was obtained in different extracts such as (Tables 1 and 2):

MURTANR > MURTAR > CHOKEB > BLUEBPOL > BLUEBCH > MURTEONR > RASBER > MURTEOR in water extract;

MURTANR > MURTAR > BLUEBPOL > MURTEONR > BLUEBCH > CHOKEB > RASBER > MURTEOR in acetone extract;

Table 1
Bioactive compounds in water extract of the studied berries.[a,b,c]

Samples	POL, mg GAE/g	FLAVON, mg CE/g	FLAV, μg CE/g	TAN, mg CE/g
Murtilla-like NR	23.44 ± 1.9a	7.68 ± 0.5b	4280 ± 213.3d	4.40 ± 0.2b
Murtilla NR	77.99 ± 6.1d	27.30 ± 2.3d	7788 ± 424.3e	15.73 ± 1.2b
Raspberry	30.12 ± 2.4c	2.08 ± 0.1a	177 ± 13.3a	5.87 ± 0.4a
Chilean Blueberry	19.81 ± 1.2a	9.93 ± 0.6c	1855 ± 112.3b	23.47 ± 1.9c
Polish Blueberry	31.16 ± 2.4c	11.07 ± 0.8c	3191 ± 198.2c	36.00 ± 2.3e

Abbreviations: POL, polyphenols; FLAVON, flavonoids; FLAV, flavanols; TAN, tannins; CE, catechin equivalent; GAE, gallic acid equivalent; NR, non-ripe.
[a] Values are means ± SD of 5 measurements.
[b] Values in columns with different letters are significantly different ($P < 0.05$).
[c] per g dry weight.

Table 2
Bioactive compounds extracted with organic solvents [acetone (Ac) and hexane (He)].[a,b,c]

	POL, mg GAE/g	FLAVON, mg CE/g	FLAV, μg CE/g	TAN, mg CE/g
MURTEONRAc	2.20 ± 0.1a	0.83 ± 0.01a	332.5 ± 15.1c	4.08 ± 0.3c
BLUEBCHAc	2.01 ± 0.07a	0.53 ± 0.04a	182.8 ± 9.1b	2.15 ± 0.07a
MURTEORAc	1.54 ± 0.07a	0.65 ± 0.02a	125.6 ± 5.9b	1.18 ± 0.07a
MURTANRAc	10.62 ± 0.5c	1.33 ± 0.08b	359.3 ± 15.9c	11.52 ± 0.6d
RASBERAc	1.57 ± 0.07a	0.39 ± 0.01a	64.0 ± 3.1a	1.48 ± 0.07b
MURTARAc	7.15 ± 0.4b	1.23 ± 0.08b	301.5 ± 15.9c	3.00 ± 0.07c
CHOKEBAc	1.83 ± 0.07a	0.81 ± 0.07a	125.1 ± 5.9 b	1.96 ± 0.07a
BLUEBPOLAc	6.43 ± 0.3b	1.18 ± 0.07b	322.1 ± 15.1c	4.54 ± 0.3c
MURTEONRHe	0.19 ± 0.01b	0.44 ± 0.01a	17.9 ± 0.9a	1.08 ± 0.07d
BLUEBCHHe	0.21 ± 0.01b	0.43 ± 0.02a	ND	0.52 ± 0.03b
MURTEORHe	0.03 ± 0.001a	0.23 ± 0.01a	ND	0.32 ± 0.02a
MURTANRHe	0.33 ± 0.02c	1.11 ± 0.08b	24.8 ± 1.9b	3.52 ± 0.2e
RASBERHe	0.54 ± 0.03d	0.37 ± 0.01a	ND	0.35 ± 0.02a
MURTARHe	0.13 ± 0.01b	0.74 ± 0.01b	ND	0.71 ± 0.03c
CHOKEBHe	0.38 ± 0.02c	0.25 ± 0.01a	ND	0.45 ± 0.03b
BLUEBPOLHe	0.31 ± 0.02c	0.48 ± 0.02b	ND	1.15 ± 0.03d

Abbreviations: POL, polyphenols; FLAVON, flavonoids; FLAV, flavanols; TAN, tannins; CE, catechin equivalent; GAE, gallic acid equivalent; CE, catechin equivalent; CGE, cyanidin-3-glucoside equivalent; MURTEONR, Murteola non-ripe; BLUEBCH, blueberries from Chile; MURTEOR, Murteola ripe; MURTANR, Murtilla non-ripe; RASBER, Raspberries; MURTAR, Murtilla ripe; CHOKEBPOL, chokeberry; BLUEBPOL, blueberries from Poland; ND, not detected.
[a] Values are means ± SD of 5 measurements.
[b] Values in columns for every bioactive compound with the same solvent bearing different letters are significantly different ($P < 0.05$).
[c] per g dry weight.

RASBER > CHOKEB > MURTANR > BLUEBPOL > BLUEBCH > MURTEONR > MURTANR > MURTEOR in hexane extract.

Our data are consistent with others (Lugasi et al., 2011), that the antioxidant properties of berries depend on several factors including species, cultivars, soil and climate conditions, water and nutrition supply.

3.2. FTIR spectra

The spectra of commonly used berries were compared with 'Murtilla' and 'Myrteola'. The comparison of three berries (Fig. 1) Raspberry [RASBER (a)], 'Murtilla' non-ripe [MUTRANR (b)], and 'Myrteola' ripe [MURTEOR (c)] showed that all polyphenols extracted with water and then lyophilized had the following common bands from 1700 to 800 cm^{-1} (1712, 1581, 1397, 1230, 1025, 866 cm^{-1}), but the most intensive showed the 'Murtilla' non-ripe sample. The other two berries were similar and overlaid in the same area. The wavelength numbers of FTIR spectra for catechin at 831, 1040, 1112, 1144, 1285, 1478, 1512 and 1611 cm^{-1} were assigned to −C−H alkenes, −C−O alcohols, C−O−H alcohols, −OH aromatic, C−O alcohols, C−H alkanes, C═C aromatic ring and C═C alkenes, respectively. Gallic acid showed the following wavelength numbers (cm^{-1}): 866, 1026, 1237, 1451, 1542 and 1619. A shift in the difference between the standards and the investigated samples can be explained by the method of extraction of the main polyphenols. The correlation of the peaks is the following (4000–700 cm^{-1}): RASBER:MURTANR = 0.669; RASBER:MURTEOR = 0.862; MURTANR:MURTEOR = 0.551; RASBER:Quercetin = 0.047; MURTANR:Quercetin = 0.037; MURTEOR:Quercetin = 0.094. Other berries were compared (Fig. 2) as well: MURTANR, MURTEONR, and BLUEBPOL. The matching between the spectra were the following: MURTANR:MURTEONR = 0.014; MURTANR:BLUEBPOL = 0.551; MURTEONR:BLUEBPOL = 0.014; MURTANR:catechin = 0.149; MURTEONR:catechin = 0154; BLUEBPOL:catechin = 0.172. As can be seen that flavonoid catechin has significantly higher matching than quercetin in the samples of polyphenols extracted with water. Application of the FTIR data and matching of the peaks in the region

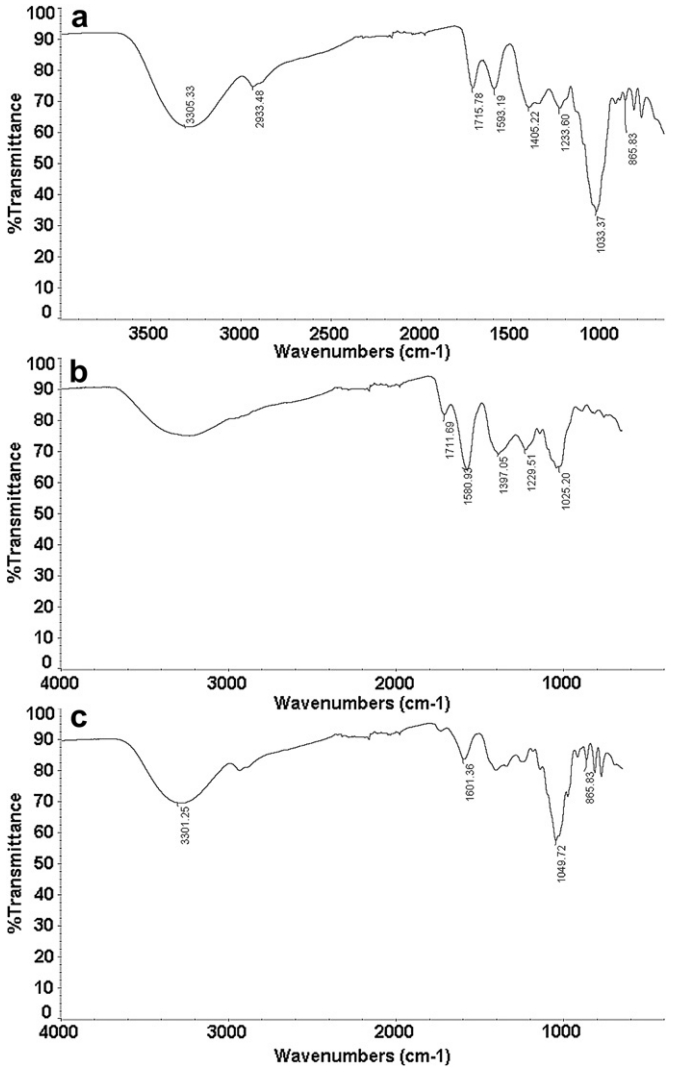

Fig. 1. FTIR spectra of dry matter of polyphenols extracted with water from: Raspberry [RASBER (a)], 'Murtilla' non-ripe [MUTRANR (b)], and 'Myrteola' ripe [MURTEOR (c)].

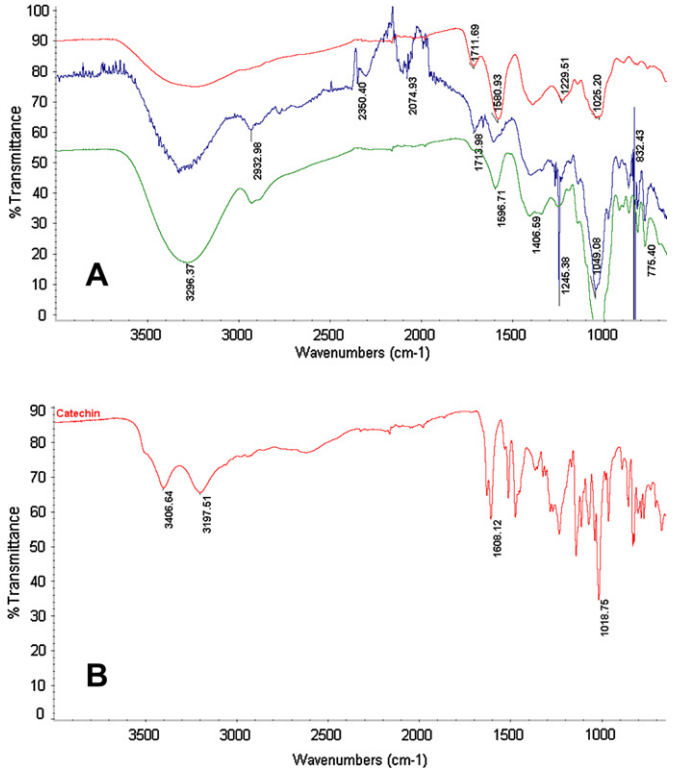

Fig. 2. FTIR spectra of dry matter of polyphenols extracted with water from (A): 'Murtilla' non-ripe (MURTANR, upper line); 'Myrteola' non-ripe (MURTEONR, middle line); Blueberries from Poland (BLUEBPOL, lower line); (B): catechin.

of polyphenols is in correspondence with the data of polyphenols (Table 1). FTIR is used as a rapid method for comparison of water extracts from the studied berries as an additional indicator of similarity or difference between the studied samples, based on the bands and peaks in the polyphenol region. These analytical techniques can be recommended for any plant extracts.

3.3. Antioxidant activity (μMTE/g) before and after thermal processing

The results of the determination of the levels of antioxidant activities (AA) of all studied samples are shown in the Tables 3 and 4. As can be seen, the AA of MURTANR as determined by ABTS, DPPH, FRAP and CUPRAC assays (620.7 ± 30.4, 334.7 ± 15.2, 327.3 ± 15.9 and 600.5 ± 27.3 μMTE/g, respectively) in all used extracts is significant higher than in other studied berries ($P < 0.05$). The data of AA water extract are significantly higher than in other two extracts (acetone and hexane, Table 4). The antioxidant activity of blueberries is higher than of raspberries, and comparable with AA of 'Myrteola' (Table 3). It was revealed that the fruits contained superior levels of anthocyanins (146–2199 mg/100 g FW) to those previously reported for other raspberry and blackberry species (Bowen-Forbes, Zhang, & Nair, 2010), and their hexane, EtOAc and MeOH extracts showed good antioxidant activity. The majority of the extracts exhibiting over 50% lipid peroxidation inhibitory activity at 50 μg/mL. These results are in accordance with our findings that in hexane extracts raspberry (Table 4), where this sample was significantly higher with the values of antioxidant activity by ABTS, DPPH, FRAP and CUPRAC than all other samples. As was calculated, a very good correlation was found between the antioxidant activity and the contents of total polyphenols and other bioactive compounds (Table 5, R^2 from 0.96 to 0.83) in water and acetone extracts and lower in hexane (Table 6, R^2 from 0.85 to 0.76). Other polyphenolics (flavonoids, flavanols, tannins) showed lower correlation. The correlation

Table 3
The antioxidant activity of all studied berries (μMTE/g) in water extracts.[a,b,c]

Samples	ABTS	DPPH	FRAP	CUPRAC
MURTEONR	144.4 ± 7.2b	64.6 ± 3.1a	43.0 ± 2.1b	82.9 ± 3.9b
BLUEBCH	197.7 ± 7.2c	94.5 ± 3.9b	73.3 ± 3.1c	154.0 ± 7.2c
MURTEOR	200.6 ± 8.7c	102.4 ± 4.9b	76.0 ± 3.7c	116.8 ± 5.7b
MURTANR	620.7 ± 30.4e	334.7 ± 15.2d	327.3 ± 15.9d	600.5 ± 27.3e
RASBER	82.5 ± 3.8a	77.6 ± 3.1a	27.7 ± 1.3a	30.4 ± 1.4a
MURTAR	446.6 ± 19.8d	210.6 ± 9.8c	208.9 ± 9.8c	428.5 ± 21.1d
CHOKEB	219.3 ± 10.2c	87.2 ± 3.1b	57.4 ± 2.3b	212.9 ± 9.8c
BLUEBPOL	254.8 ± 11.9c	75.1 ± 3.1a	177.3 ± 8.6a	250.9 ± 11.2c

Abbreviations: MURTEONR, Murteola non-ripe; BLUEBCH, blueberries from Chile; MURTEOR, Murteola ripe; MURTANR, Murtilla non-ripe; RASBER, Raspberries; MURTAR, Murtilla ripe; CHOKEBPOL, chokeberry; BLUEBPOL, blueberries from Poland, ABTS, 2,2-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt; CUPRAC, Cupric reducing antioxidant capacity; DPPH, Radical Scavenging Activity using 1,1-diphenyl-2-picrylhydrazyl; FRAP, Ferric-reducing/antioxidant power.

[a] Values are means ± SD of 5 measurements.

[b] Values in columns for every value of antioxidant activity with the same solvent bearing different letters are significantly different ($P < 0.05$).

[c] per g dry weight.

Table 4
The antioxidant activity of all studied berries (μMTE/g) extracted with organic solvents [acetone (Ac) and hexane (He)].[a,b,c]

Samples	ABTS	DPPH	FRAP	CUPRAC
MURTEONRAc	19.79 ± 0.8b	9.32 ± 0.4b	8.38 ± 0.4b	14.11 ± 0.6b
BLUEBCHAc	9.79 ± 0.4a	3.01 ± 0.2a	5.59 ± 0.3b	13.45 ± 0.6b
MURTEORAc	10.08 ± 0.5a	4.00 ± 0.2a	2.94 ± 0.1a	7.72 ± 0.4a
MURTANRAc	56.42 ± 2.7d	23.76 ± 0.2d	41.46 ± 2.1e	38.79 ± 1.9d
RASBERAc	7.61 ± 0.3a	3.29 ± 0.2a	2.80 ± 0.07a	6.09 ± 0.3a
MURTARAc	29.89 ± 1.4c	16.43 ± 0.2c	20.87 ± 0.9 d	28.65 ± 1.4c
CHOKEBAc	15.14 ± 0.7b	3.55 ± 0.2a	2.84 ± 0.1a	7.16 ± 0.3a
BLUEBPOLAc	26.59 ± 1.3c	15.01 ± 0.7a	11.59 ± 0.6c	33.27 ± 1.7c
MURTEONRHe	1.54 ± 0.07b	0.98 ± 0.04c	0.52 ± 0.03c	2.86 ± 0.1b
BLUEBCHHe	1.11 ± 0.07b	0.37 ± 0.02b	0.58 ± 0.03c	2.68 ± 0.1c
MURTEORHe	0.60 ± 0.07a	0.18 ± 0.009a	0.16 ± 0.008a	0.12 ± 0.06a
MURTANRHe	2.30 ± 0.1c	1.15 ± 0.05c	0.89 ± 0.04d	3.18 ± 0.2c
RASBERHe	3.25 ± 0.07d	2.14 ± 0.1d	1.99 ± 0.09e	4.56 ± 0.2d
MURTARHe	1.10 ± 0.07b	0.35 ± 0.01b	0.30 ± 0.02b	0.77 ± 0.03b
CHOKEBHe	2.80 ± 0.07b	1.19 ± 0.05c	0.89 ± 0.04d	3.12 ± 0.1c
BLUEBPOLHe	0.98 ± 0.07b	0.44 ± 0.02b	0.31 ± 0.02b	2.78 ± 0.1c

Abbreviations: MURTEONR, Murteola non-ripe; BLUEBCH, blueberries from Chile; MURTEOR, Murteola ripe; MURTANR, Murtilla non-ripe; RASBER, Raspberries; MURTAR, Murtilla ripe; CHOKEBPOL, chokeberry; BLUEBPOL, blueberries from Poland, ABTS, 2,2-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diamonium salt; CUPRAC, Cupric reducing antioxidant capacity; DPPH, Radical Scavenging Activity using 1,1-diphenyl-2-picrylhydrazyl; FRAP, Ferric-reducing/antioxidant power.

[a] Values are means ± SD of 5 measurements.

[b] Values in columns for every value of antioxidant activity with the same solvent bearing different letters are significantly different ($P < 0.05$).

[c] per g dry weight.

between the antioxidant activity and ascorbic acid was lower than with polyphenols (Table 5, R^2 from 0.84 to 0.50). As can be seen, also the significantly highest antioxidant activity was in MURTANR following partially by BLUEBPOL and BLUEBCH. Therefore it was decided to subject to thermal processing these 3 kinds of berries.

3.4. Bioactive compounds before and after thermal processing

The changes in the contents of the bioactive compounds before and after thermal processing are summarized in the Table 7. As can be seen, only thermo-processing for 10 and 20 min preserves the content of the bioactive compounds of the studied berries. The changes in the antioxidant activity before and after thermal processing are summarized in the Table 8. As can be seen, the same relationship was registered as in the case of bioactive compounds: only thermal processing for 10 and 20 min preserves the level of the antioxidant activity. Most of the investigators found that the thermal processing of fruits and vegetables decreases the contents of their bioactive compounds and the level of antioxidant activity (Bushra et al., 2008; Cisse et al., 2009; Ferracane et al., 2008; Jimenez-Montral et al., 2009). The preservation of these bioactive

Table 5
Correlation coefficients between bioactive compounds and the overall antioxidants activities in water extracts of investigated berries.

Assays	POL × AA	FLAVON × AA	FLAV × AA	TAN × AA	AC × AA
ABTSW	0.916	0.803	0.929	0.815	0.748
DPPHW	0.825	0.610	0.823	0.654	0.505
FRAPW	0.844	0.778	0.863	0.644	0.819
CUPRACW	0.960	0.822	0.916	0.851	0.842

Abbreviations: POL × AA, polyphenols vs antioxidant activities; FLAVON × AA, flavonoids vs antioxidant activities; FLAV × AA, flavanols vs antioxidant activities; TAN × AA, tannins vs antioxidant activities; ABTS, 2,2-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt; CUPRAC, Cupric reducing antioxidant capacity; DPPH, Radical Scavenging Activity using 1,1-diphenyl-2-picrylhydrazyl; FRAP, Ferric-reducing/antioxidant power; W, water extract; AC, ascorbic acid.

Table 6
Correlation coefficients between bioactive compounds and the overall antioxidants activities in organic solvents extracts of investigated berries.

Assays	POL × AA	FLAVON × AA	FLAV × AA	TAN × AA	AC × AA
ABTSAc	0.918	0.774	0.612	0.898	0.918
DPPHAc	0.927	0.862	0.731	0.749	0.927
FRAPAc	0.884	0.647	0.522	0.858	0.884
CUPRACAc	0.893	0.834	0.725	0.642	0.893
ABTSHe	0.777	0.003	0.005	0.019	0.777
DPPHHe	0.765	0.001	0.002	0.011	0.765
FRAPHe	0.772	0.001	0.013	0.001	0.772
CUPRACHe	0.855	0.004	0.107	0.041	0.855

Abbreviations: POL × AA, polyphenols vs antioxidant activities; FLAVON × AA, flavonoids vs antioxidant activities; FLAV × AA, flavanols vs antioxidant activities; TAN × AA, tannins vs antioxidant activities; ABTS, 2,2-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt; CUPRAC, Cupric reducing antioxidant capacity; DPPH, Radical Scavenging Activity using 1,1-diphenyl-2-picrylhydrazyl; FRAP, Ferric-reducing/antioxidant power; Ac, acetone extract.

compounds is very important. As was already cited, the studied fresh berries are not available all year around. Therefore, in order to please consumers, part of berries is subjected to thermal processing. In the present investigation we tried to find a thermal processing regime, which maximum preserves the berries bioactivity. We found that thermal processing for 40 and 60 min shows significant decrease in the berries bioactivity. Also other investigators reported similar results (Im et al., 2011; Mpiana et al., 2009; Wawire, Makule, Oey, Loey, & Hendrickx, 2010; Wawire et al., 2011). Im et al. (2011) studied thermostability of Korean lotus roots (KLR) and Polish white onion. They reported that the loose of water KLR extract's of polyphenols, flavanols, flavonoids, anthocyanins and tannins after 60 min of boiling was 60.0, 57.7, 49.5, 58.6 and 59.0% of their contents, respectively. The same patterns were registered for antioxidant activity: according to DPPH, FRAP, ABTS and CUPRAC assays after 60 min of boiling the water extracts of KLR lost 59.4, 57.7, 53.7 and 56.4% of the AA, respectively. Mpiana et al. (2009) have shown the decrease in the content of anthocyanins with duration of time at 100 °C and 120 °C. Heating of this fraction at 100 °C drastically modified its absorption spectrum showing the degradation of the anthocyanin fraction. A total modification was observed after only 45 min of heating.Our data are consistent with Wawire et al. (2010) and with Wawire et al. (2011). They reported that temperature treatment at 30–90 °C for 10 min decreased the total vitamin C content whereas total vitamin C in 8-week-old cowpea leaves was more than 80%. So a high retention of the total vitamin C can be obtained even after heating and/or reheating (30–90 °C for 10 min) before consumption. The results indicated that the stability of total vitamin C in situ was strongly dependent on the plant maturity stage and the processing conditions applied. Our data show that thermal processing for 10 and 20 min preserved the bioactivity of the studied berries. It was found that even after 20 min of thermal processing the contents of bioactive compounds were highly preserved: polyphenols–97.5, 97.0 and 97.2%, flavonoids–95.7, 91.5 and 94.6%, flavanols–98.7, 96.4 and 94.4%, tannins–91.1, 95.4 and 96.8%, anthocyanins–95.2, 93.8 and 99.85% and ascorbic acid–97.4, 93.2 and 91.9% for MURTANR, BLUEBPOL and BLUEBCH, respectively (Table 7). The same patterns were registered for antioxidant activity (Table 8). Our data can be compared with others (Piasek et al., 2011), where fruit juices that are rich sources of anthocyanins, obtained from aronia and blueberried honeysuckle were subjected to heat treatment. The rapid decline of anthocyanin content accompanied by lowered antioxidant activity was observed when juices were submitted to heating at 100 °C. The changes in chemical composition were reflected in altered biological activity. Our results were in accordance with Sablani et al. (2010), where fresh berries were thermally processed

Table 7
Bioactive compounds before and after thermal processing in MURTANR, BLUEBPOL and BLUEBCH.

Time of treatment, min	POL, mg GAE/g	FLAVON, mg CE/g	FLAV, μg CE/g	TAN, mg CE/g	Anthocya, mg CGE/g	Ascorbic acid, mg/g
MURTANR						
Before	84.81 ± 3.9^{c}	11.47 ± 0.6^{c}	4241.2 ± 19.1^{c}	8.91 ± 0.4^{c}	16.7 ± 0.8^{c}	9.12 ± 0.4^{c}
After 10	82.98 ± 3.9^{c}	11.16 ± 0.6^{c}	4201.2 ± 19.1^{c}	8.79 ± 0.4^{c}	16.3 ± 0.8^{c}	9.02 ± 0.4^{c}
After 20	82.69 ± 3.9^{c}	10.98 ± 0.6^{c}	4188.2 ± 19.0^{c}	8.11 ± 0.4^{c}	15.9 ± 0.8^{c}	8.88 ± 0.4^{c}
After 40	69.99 ± 3.2^{b}	7.88 ± 0.4^{b}	3222.1 ± 16.1^{b}	6.42 ± 0.3^{b}	12.8 ± 0.6^{b}	6.94 ± 0.3 ^{b}
After 60	54.97 ± 2.6^{a}	5.99 ± 0.3^{a}	2343.2 ± 11.2^{a}	4.94 ± 0.2^{a}	9.9 ± 0.5^{a}	4.34 ± 0.2^{a}
BLUEBPOL						
Before	41.42 ± 2.1^{b}	6.68 ± 0.3^{c}	1762.2 ± 77.2^{c}	5.00 ± 0.3^{c}	323.2 ± 16.1^{c}	7.65 ± 04^{c}
After 10	40.62 ± 2.1^{b}	6.29 ± 0.3^{c}	1711.3 ± 77.1^{c}	4.89 ± 0.3^{c}	311.2 ± 16.1^{c}	7.22 ± 0.4^{c}
After 20	40.19 ± 2.1^{b}	6.11 ± 0.3^{c}	1699.3 ± 77.1^{c}	4.77 ± 0.3^{c}	303.2 ± 16.1^{c}	7.13 ± 0.4^{c}
After 40	31.92 ± 1.5^{a}	4.68 ± 0.2^{b}	1512.2 ± 75.2^{b}	3.33 ± 0.2^{b}	213.2 ± 9.1^{b}	5.95 ± 0.3^{b}
After 60	30.12 ± 1.4^{a}	3.88 ± 0.2^{a}	1363.0 ± 67.3^{a}	2.12 ± 0.1^{a}	173.2 ± 8.2^{a}	4.33 ± 0.2^{a}
BLUEBCH						
Before	30.69 ± 1.4^{c}	1.68 ± 0.07^{c}	1832.3 ± 81.4^{c}	3.85 ± 0.2^{c}	270.5 ± 13.4^{c}	3.71 ± 0.2^{c}
After 10	30.11 ± 1.4^{c}	1.61 ± 0.07^{c}	1782.7 ± 81.2^{c}	3.78 ± 0.2^{c}	270.3 ± 13.4^{c}	3.59 ± 0.2^{c}
After 20	29.84 ± 1.4^{c}	1.59 ± 0.06^{c}	1730.0 ± 81.2^{c}	3.73 ± 0.2^{c}	270.2 ± 13.4^{c}	3.41 ± 0.2^{c}
After 40	23.19 ± 1.2^{b}	1.39 ± 0.07^{b}	1623.3 ± 79.3^{b}	2.85 ± 0.1^{b}	211.0 ± 10.3^{b}	2.21 ± 0.1^{b}
After 60	20.62 ± 1.0^{a}	1.08 ± 0.07^{a}	1512.3 ± 78.1^{a}	1.98 ± 0.1^{a}	189.2 ± 8.7^{a}	1.71 ± 0.1^{a}

Values are means ± SD of 5 measurements. Values in columns with the different superscript letters are significantly different ($P < 0.05$).
Abbreviations: Anthocya, Anthocyanins; POL, polyphenols; FLAVON, flavonoids; FLAV, flavanols; TAN, tannins; CE, catechin equivalent; GAE, gallic acid equivalent; CE, catechin equivalent; CGE, cyanidin-3-glucoside equivalent; MURTANR, Murtilla non-ripe; BLUEBPOL, blueberries from Poland; BLUEBCH, blueberries from Chile.

into cans and juice/puree with and without blanching, and the changes in phytochemicals were monitored. After canning, total anthocyanins decreased by up to 44%, while phenolic contents and antioxidant activity of both berries generally increased by up to 50 and 53% respectively. The level of changes in phytochemicals during berry puree/juice processing was influenced by blanching and type of berries. Flavonol aglycons were formed during processing as a result of heat treatment in cranberries. Drying of cranberry pomace resulted in increase of flavonols and procyanidin oligomers (White, Howard, & Prior, 2011). Heating and pasteurization cause flavor changes and losses of antioxidant compounds in blueberries (Dean et al., 2010). Our results were in accordance with others who used heating of berries and other kinds of processing. Whole fresh and dried fruits were assessed for phenolics (anthocyanins, flavanols, hydroxycinnamic acids, and flavonols), ascorbic acid, and antioxidant activity. Such analytical data show that ellagic acid and flavanol changes were affected by drying techniques and cultivar. Drying destroyed anthocyanins, flavanols, and ascorbic acid, and there was a significant decrease in antioxidant activity (Wojdylo, Figiel, & Oszmianski, 2009). Every heat treatment caused a significant reduction in the content of flavonoids. Our results are also in accordance with Borowska and Mazur (2008): the thermal berry processing decreased polyphenols and anthocyanins. These changes varied and were attributed to cultivars and can be used as important criteria in the selection of raw berry material for processing. This conclusion can be applied to our findings as well. Our results can be compared with Hager, Howard, Prior, and Brownmiller (2008), where thermal processing resulted in marked losses in total anthocyanins ranging from 37% in puree and from 69% to 73% in juices and antioxidant capacity losses were from 38% to 41% in nonclarified and clarified black raspberry juices. In blueberries the losses were in total anthocyanins from 28% to 59% and antioxidant values from 43% to 71% in all products, with the greatest losses occurring in clarified juices (Brownmiller, Howard, & Prior, 2008).

Table 8
The changes in the antioxidant activity before and after thermal processing of the studied berries (μMTE/g).[a]

Time of treatment, min	ABTS	DPPH	FRAP	CUPRAC
MURTANR				
Before	620.7 ± 30.4c	334.7 ± 15.2c	327.3 ± 15.9c	600.5 ± 27.3c
After 10	611.8 ± 30.4c	318.9 ± 15.2c	312.9 ± 15.9c	588.5 ± 27.3c
After 20	601.9 ± 30.4c	309.9 ± 15.2c	305.0 ± 15.9c	5.72.5 ± 27.2c
After 40	498.9 ± 24.1b	203.1 ± 10.1b	211.3 ± 10.1b	344.2 ± 17.7b
After 60	344.8 ± 17.2a	122.7 ± 6.3a	144.4 ± 7.2a	276.6 ± 13.8a
BLUEBPOL				
Before	254.8 ± 11.9c	75.09 ± 3.1c	177.3 ± 8.6c	250.9 ± 11.2c
After 10	239.9 ± 11.9c	74.66 ± 3.1c	169.3 ± 8.6c	244.8 ± 11.1c
After 20	228.9 ± 11.8c	73.98 ± 3.0c	162.0 ± 8.6c	239.9 ± 11.0c
After 40	154.8 ± 7.2b	55.66 ± 2.6b	106.2 ± 8.6b	162.7 ± 8.1b
After 60	92.8 ± 4.9a	40.16 ± 2.1a	76.3 ± 8.6a	112.3 ± 5.2a
BLUEBCH				
Before	197.7 ± 7.2c	94.53 ± 3.9c	73.33 ± 3.1c	154.0 ± 7.2c
After 10	169.7 ± 7.1c	92.23 ± 3.9c	72.98 ± 3.1c	153.7 ± 7.2c
After 20	167.2 ± 7.0c	91.59 ± 3.9c	72.44 ± 3.0c	153.5 ± 7.2c
After 40	108.9 ± 5.1b	74.53 ± 3.1b	59.54 ± 2.8b	111.0 ± 5.2b
After 60	91.1 ± 4 0.2a	52.22 ± 2.5a	43.11 ± 2.1a	84.21 ± 4.1a

Values are means ± SD of 5 measurements. Values in columns bearing different letters are significantly different ($P < 0.05$).
Abbreviations: ABTS, 2,2-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diamonium salt; CUPRAC, Cupric reducing antioxidant capacity; DPPH, Radical Scavenging Activity using 1,1-diphenyl-2-picrylhydrazyl; FRAP, Ferric-reducing/antioxidant power; MURTANR, Murtilla non-ripe; BLUEBPOL, blueberries from Poland; BLUEBCH, blueberries from Chile.
[a] dry weight (DW).

4. Conclusions

The aim of this investigation was to find a thermal processing regime (heating to 100 °C), which maximum preserves the bioactivity of the studied Chilean and Polish berries. These fruits were subjected to thermal processing for 10, 20, 40, 60 min. After every process the bioactivity of the studied berries was assessed. It was found that berries subjected to thermal processing not more than 20 min maximally preserved the bioactivity.

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