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Phenolic content and antioxidant activities of white and purple juices manufactured with organically- or conventionally-produced grapes

C. Dani, L.S. Oliboni, R. Vanderlinde, D. Bonatto, M. Salvador, J.A.P. Henriques *

Instituto de Biotecnologia, Universidade de Caxias do Sul, Caxias do Sul (UCS), Rio Grande do Sul, Brazil

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Abstract

Although the beneficial effects of moderate wine intake are well-known, data on antioxidant capacity of grape juices are scarce and controversial. The purpose of this study was to quantify total polyphenols, anthocyanins, resveratrol, catechin, epicatechin, procyanidins, and ascorbic acid contents in grape juices, and to assess their possible antioxidant activity. Eight *Vitis labrusca* juices – white or purple, from organically- or conventionally-grown grapes, and obtained in pilot or commercial scale – were used. Organic grape juices showed statistically different ($p < 0.05$) higher values of total polyphenols and resveratrol as compared conventional grape juices. Purple juices presented higher total polyphenol content and *in vitro* antioxidant activity as compared to white juices, and this activity was positively correlated ($r = 0.680$; $p < 0.01$) with total polyphenol content. These results indicate that white and purple grape juices can be used as antioxidants and nutritional sources.
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keywords: Grape juice; Antioxidant; Nutritional; Phenolic compounds

1. Introduction

Experimental data have increasingly suggested that cellular oxidative damage induced by reactive species (RS) has a relevant pathophysiological role in several types of human diseases, such as atherosclerosis and cancer (Ames et al., 1993). In order to neutralize these RS, our cells have developed a complex biochemical redox mechanism, consisting of both enzymatic and non-enzymatic components (Park et al., 2003). Moreover, foods, particularly fruits and vegetables, also have an important role in maintaining physiological redox equilibrium. These foods supply several antioxidants, such as vitamin C and several polyphenolic compounds, to the body. Grapes are rich in phenolic compounds, such as flavonoids (catechin, epicatechin, quercetin, anthocyanins and procyanidins), and resveratrol (3,5,4′-trihydroxy-stilbene), which are mainly found in red grape products (Wang et al., 2002; Soleas et al., 1997; Fuleki and Ricardo-da-Silva, 2003). It has been already reported that grape juice compounds can prevent: (i) platelet aggregation, (ii) LDL oxidation and oxidative damage to DNA, (iii) coronary diseases and atherosclerosis (Frankel et al., 1998; Osman et al., 1998; Day et al., 1997; Singletary et al., 2003).

As grape juices are a relevant source of polyphenolic compounds, many people are becoming aware of the importance of their consumption in their daily diet. There is an increasing public concern as to developing healthy habits and eating quality food. Some consumers are also taking into account agricultural methods (conventional or organic) when purchasing their food. Organic agriculture, among other practices, does not use pesticides during the cultivation (IFOAM, 2005). Organic strawberries and tomatoes present higher content of secondary metabolites

* Corresponding author. Tel.: +55 54 218 2105; fax: +55 54 218 2664.
E-mail address: henriques@cbiot.ufrgs.br (J.A.P. Henriques).

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(e.g. polyphenolic compounds), as they suffer more fungal infections, thereby producing a higher level of these metabolites for their defense (Asami et al., 2003; Lombardi-Boccia et al., 2004). To our knowledge, there are no studies in literature on how organic cultivation of grapes may change the chemical characteristics of grapes and their products (wine and juices). In addition, we did not find any studies comparing the biological activities of organic and conventional, white and purple grape juices.

This study aimed at assessing the antioxidant capacity of different types of grape juices (white or purple juices from organically- or conventionally-grown grapes) using standard *in vitro and ex vivo* assays. In addition, (+)-catechin, (−)-epicatechin, *trans*-resveratrol, anthocyanidin, and individual procyanidin contents of juices produced from different *Vitis labrusca* varieties were analyzed by HPLC.

2. Materials and methods

2.1. Chemicals

DPPH·, *trans*-resveratrol, (+)-catechin, (−)-epicatechin, gallic acid, and procyanidins B1, B2, B3 and B4 were obtained from Sigma–Aldrich (St. Louis, MO). The anthocyanin pigments cyanidin-3-glucoside, delphinidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside were obtained from Extrasynthese (Gennay, France). Methyl-parathion was obtained from Bayer, and the acetylcolinesterase kit was purchased from UFRJ (Rio de Janeiro, Brazil). All other chemicals were purchased from E. Merck (Damstadt, Germany).

2.2. Grapes and grape juices

Grape juice samples produced from *V. labrusca,* varieties Bordo and Niagara, were analyzed according to eight different groups (Table 1). Organic grapes cultivated with no pesticides, and commercial organic juices were obtained from Cooperativa Aecia (Antonio Prado, Rio Grande do Sul, RS, Brazil). Conventional grapes, cultivated using traditional methods, and commercial conventional juices were obtained from Vinhos Monte Reale (Flores da Cunha, RS, Brazil). Validity periods were observed, and the same brands were used for the entire study.

Grapes were cultivated in 2005, and all juices were manufactured during the same year. Purple juices were heat-extracted using pulp, seeds, and skin, whereas the skin was removed before extraction in the white juices. Commercial conventional juices were manufactured by heat extraction (approximately 50 °C), with a subsequent pressing in order to separate the pulp, and then submitted to pasteurization (at 85°). All other juices, purple and white, were manufactured by heat extraction, immediately followed by bottling, both at 80 °C.

2.3. Grape juice chemical analysis and nutritional evaluation

Alcoholic grade, total acidity, volatile acidity, pH, total SO_2, and ascorbic acid were determined using the methods described by Zoecklein et al. (2000). All analyses were performed in duplicate. Carbohydrates, food fiber, saturated fats, proteins, and humidity levels, as well as calorie values were determined according to AOAC International official methodologies (AOAC, 1998).

2.4. Pesticide determination

Organophosphorus and carbamate pesticides were determined in juice samples as methyl parathion-equivalent activity, which causes inhibition of the enzyme acetylcholinesterase (AChE), as previously described by Bastos et al. (1991) and Lima et al. (1996). Methyl-parathion (Folidol 600® – Bayer, Brazil) calibration curve was used to express AChE activity in ppm of methyl-parathion.

2.5. Phenolic compound content

Total phenol content was measured using Singleton and Rossi's modification of Folin–Ciocalteau's colorimetric method (Singleton et al., 1999). High performance liquid chromatography (HPLC) analysis was used to quantify the presence of individual phenolic compounds. Before HPLC analysis, 5 mL of each sample were filtered through a 0.20-mm cellulose membrane. The equipment consisted of a liquid-gradient chromatographic system, LC-DAD Series 1100 (Palo Alto, CA), with diode array (DAD) detector system. Zorbax 300 SB C18 pre-column (12 mm × 4.6 mm × 5 μm) and C18-ODS column (150 mm × 4 mm × 5 μm) (Agilent Technologies, USA) were used.

2.5.1. trans-Resveratrol analysis

In order to quantify *trans*-resveratrol, ultra-pure water and acetonitrile mobile phase (75:25 v/v), pH 3.0, in constant flow of 1.0 mL min^{-1} for 20 min in a controlled-temperature room at 20 °C, was used. The peak was detected at 306 nm, after injection of 20-μL samples (Jeandet et al., 1995).

2.5.2. Anthocyanins analysis

In order to determine cyanidin-3-glucoside, delphidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside levels, mobile phase with solvents A: ultra-pure water, formic acid, acetonitrile (87:10:3 v/v/v), and B: ultra-pure water, formic acid, acetonitrile (40:10:50 v/v/v), in constant flow of 0.8 mL min^{-1}, in a temperature-controlled room at 25 °C, was used. The peak was detected at 518 nm, after injection of 50-μL samples (Office International de la Vigne et du Vin, 2003).

2.5.3. Procyanidins analysis

In order to determine procyanidins B_1, B_2, B_3, and B_4, (+)-catechin, (−)-epicatechin, and gallic acid, mobile phase with solvent A (ammonium hydroxide diphosphate 50 mmol L^{-1}, pH 2.6), solvent B (20% of solvent A and 80% of acetonitrile), and solvent C (orthophosphoric acid

Table 1
Analyzed grape juices ($n = 8$)

Name	Cultivar	Agriculture practice	Production scale	Heat treatment
BCC	Bordo	Conventional	Commercial	Pasteurized at 85 °C[a]
BCP	Bordo	Conventional	Pilot	Hot bottled at 80 °C[b]
BOC	Bordo	Organic	Commercial	Hot bottled at 80 °C
BOP	Bordo	Organic	Pilot	Hot bottled at 80 °C
NCC	Niagara	Conventional	Commercial	Pasteurized at 85 °C
NCP	Niagara	Conventional	Pilot	Hot bottled at 80 °C
NOC	Niagara	Organic	Commercial	Hot bottled at 80 °C
NOP	Niagara	Organic	Pilot	Hot bottled at 80 °C

[a] Juice pasteurized at 85 °C, for 3 min in pasteurization unity for fresh juices EFC-250 l/h, ETAL®, before being bottled.
[b] Juice hotted at 80 °C and immediately bottled.

0.2 mol L^{-1}, pH 1.5), in a constant flow of 0.5 mL min^{-1}, in a temperature-controlled room at 40 °C, was used. The peak was detected at 204 nm, after injection of 5-μL samples. Elution conditions were standardized according to Lamuela-Raventós and Waterhouse (1994).

2.6. Antioxidant activity

Juice antioxidant activity was measured by *in vitro* (DPPH· radical-scavenging activity, and sod and cat-like activities) and *ex vivo* (inhibition of serum lipid peroxidation).

2.6.1. Chemical measurement of DPPH·-radical scavenging activity

DPPH· radical scavenging was measured using a modified Yamaguchi et al. (1998) method, in which white and purple grape juice solutions were added to obtain final concentrations of 0.1, 1.0, 10.0, 50.0, and 100.0% v/v. Tubes were stored in the dark for 20 min, after which absorbance was measured at 517 nm. Results were expressed as the amount of juice necessary to scavenge 50% of DPPH· radical (IC_{50}). Distilled water was used instead of antioxidant solutions as control. Catechin was used as standard.

2.6.2. Superoxide dismutase- and catalase-like activities

Superoxide dismutase-like activity was spectrophotometrically determined in grape juice samples by measuring the inhibition of self-catalytic adrenochrome formation rate at 480 nm, in a reaction medium containing 1 mmol L^{-1} adrenaline (pH 2.0), and 50 mmol L^{-1} glycine (pH 10.2). This reaction was performed at 30 °C for 3 min (Bannister and Calabrese, 1987). Results were expressed as the amount of grape juice needed to reduce 50% of adenochrome. Catalase-like activity assay was performed according to the method described by Aebi (1984), by determining hydrogen peroxide decomposition rate at 240 nm. Results were expressed in micromoles of H_2O_2 decomposed per minute.

2.6.3. Inhibition of serum lipid peroxidation assay

Inhibition of serum lipid peroxidation was determined using a modification of the method described by Durak et al. (1999). Pooled fresh human serum of 1 mL, 150 μL of grape juices sample, and 15 μL of $CuSO_4$ (5 mM; positive control) were incubated at 37 °C for 1 h. Oxidative stress levels were then spectrophotometrically measured as thiobarbituric acid reactive substances concentration (TBARS), as described by Wills (1996). TBARS results were expressed as nmol/mL.

2.7. Statistical analyses

Values were determined as being parametrical or non-parametrical by the Kolmogorov–Smirnoff test. All assays were performed in triplicate. Data were submitted to analysis of variance (ANOVA), and means were compared using the test of Tukey. Groups were compared using Student's *t*-test and Mann–Whitney *U*-test. Relationships between variables were assessed using Pearson's product–moment correlation coefficient. SPSS 12.0 software package was used in all statistical analysis.

3. Results

3.1. Grape juice chemical analysis and nutritional evaluation

Grape juice alcohol levels were between 0.03% and 0.3% (v/v), with total acidity between 0.40 and 0.96 g/100 mL tartaric acid. No volatile acidity was detected in any juice. pH values varied from 3.21 to 3.60, and sulfur dioxide from 0.027 to 0.029 g/L. Table 2 shows nutritional analyses and ascorbic acid content. Juices produced at pilot scale presented the highest calorie value and carbohydrate content. Purple grape juices had high vitamin C (ascorbic acid) levels. Except for the purple grape juice produced at pilot scale (BOP), organic juices presented higher ascorbic acid values as compared to conventional juices.

3.2. Pesticide determination

No organophosphorus or carbamate pesticides were detected in the analyzed grape juice samples.

3.3. Total phenolic content

Purple juices presented higher total phenolic content as compared to white juices (Fig. 1). Within agricultural method, organic juices presented higher polyphenol content as compared to juices manufactured with conventionally-grown grapes (Fig. 1).

3.4. Resveratrol and anthocyanins contents

Resveratrol and anthocyanins contents were only measured in purple grape juices, which were heated with the skin, allowing phenolic compound to be transferred to the juice (Fuleki and Ricardo-da-Silva, 2003). Organic juices presented higher resveratrol (Fig. 2) and anthocyanins contents (Table 3) as compared to conventional juices.

Table 2
Nutritional analyses and acid ascorbic level in different grape juices ($n = 8$)

Sample[A]	Calorie values (kJ)	Carbohydrates (%)	Protein (%)	Fiber (%)	Moisture (%)	Ashes (%)	Ascorbic acid (mg %)
BCC	$39.04 \pm 0.05^{d,B}$	9.43 ± 0.01^{d}	0.317 ± 0.05^{d}	0.010 ± 0.00^{e}	90.02 ± 0.02^{b}	0.199 ± 0.00^{b}	30.8 ± 0.40^{c}
BCP	53.68 ± 0.10^{a}	12.93 ± 0.02^{a}	0.487 ± 0.05^{a}	0.250 ± 0.01^{a}	86.12 ± 0.02^{d}	0.197 ± 0.00^{b}	44.0 ± 0.13^{b}
BOC	32.47 ± 0.02^{e}	7.82 ± 0.07^{e}	0.240 ± 0.05^{f}	0.105 ± 0.05^{d}	91.65 ± 0.08^{a}	0.132 ± 0.00^{c}	57.2 ± 0.70^{a}
BOP	48.36 ± 0.08^{b}	11.76 ± 0.02^{b}	0.332 ± 0.05^{b}	0.120 ± 0.00^{c}	87.56 ± 0.01^{c}	0.216 ± 0.00^{a}	30.8 ± 0.90^{c}
NCC	46.03 ± 0.01^{c}	11.19 ± 0.03^{c}	0.310 ± 0.05^{e}	0.271 ± 0.01^{a}	88.10 ± 0.03^{c}	0.110 ± 0.00^{d}	17.6 ± 0.30^{e}
NCP	52.12 ± 1.72^{a}	$12.68 \pm 0.46^{b'}$	0.316 ± 0.05^{d}	0.093 ± 0.01^{d}	86.79 ± 0.46^{d}	0.106 ± 0.00^{d}	4.4 ± 0.10^{g}
NOC	35.07 ± 0.09^{e}	8.43 ± 0.02^{e}	0.327 ± 0.01^{c}	0.170 ± 0.05^{b}	90.84 ± 0.02^{ab}	0.224 ± 0.00^{a}	22.0 ± 0.80^{d}
NOP	50.92 ± 0.08^{a}	12.48 ± 0.02^{b}	0.327 ± 0.05^{c}	0.120 ± 0.00^{c}	86.88 ± 0.55^{d}	0.226 ± 0.00^{a}	8.8 ± 0.21^{f}

[A] BCC: Bordo conventional commercial; BCP: Bordo conventional pilot scale; BOC: bordo conventional organic; BOP: Bordo organic pilot scale; NCC: niagara conventional commercial; NCP: Niagara conventional pilot scale; NOC: Niagara conventional organic; NOP: Niagara organic pilot Scale.

[B] Different letters correspond to mean values statistically different by analysis of variance (ANOVA) and Tukey post hoc test, for $p < 0.01$, that means a, b, c, d, e, f, g at same column are statistically different.

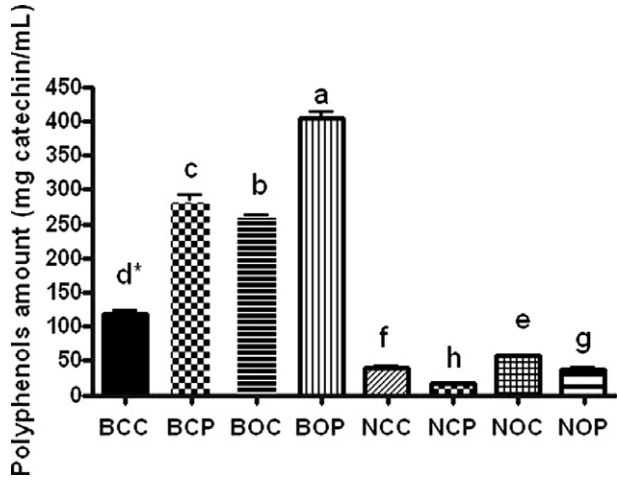

Fig. 1. Polyphenols amount in different grape juices. Values are the mean ±SD of three replicates. BCC: Bordo conventional commercial; BCP: Bordo conventional pilot scale; BOC: Bordo conventional organic; BOP: Bordo organic pilot scale; NCC: Niagara conventional commercial; NCP: Niagara conventional pilot scale; NOC: Niagara conventional organic; NOP: Niagara organic pilot scale. Different letters correspond to mean values statistically different by analysis of variance (ANOVA) and Tukey post hoc test, for $p < 0.01$, that means a, b, c, d, e, f, g, h at same figure are statistically significantly different.

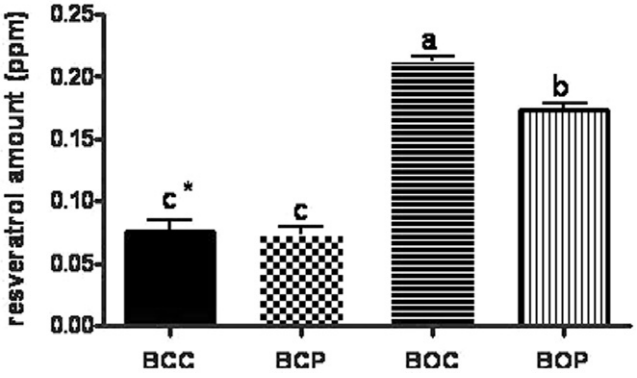

Fig. 2. Resveratrol amount in different purple grape juices. BCC: Bordo conventional commercial; BCP: Bordo conventional pilot scale; BOC: Bordo conventional organic; BOP: Bordo organic pilot scale. Different letters correspond to mean values statistically different by analysis of variance (ANOVA) and Tukey post hoc test, for $p < 0.01$ that means a, b, c at same figure are statistically significantly different.

3.5. Catechins and procyanidins content

Many differences were observed in the contents of catechins and procyanidins between purple and white juices (Table 4). Except for NCC, purple juices presented higher catechin and epicatechin contents as compared to white juices. Among all evaluated procyanidins, the B_3 fraction presented the highest level in all analyzed juices (Table 4).

3.6. Scavenging of DPPH· radical

Except for NCP and NOP, all juices showed higher or similar antioxidant activity as compared to the standard solution, which contained catechin (Fig. 3). Among all assayed samples, BCP, BOC, BOP, and NOC presented higher antioxidant activity. This activity was positively correlated with total phenolic ($r = 0.616$; $p \leqslant 0.05$), procyanidins B_1 ($r = 0.689$; $p \leqslant 0.05$), B_3 ($r = 0.521$; $p \leqslant 0.05$), and catechin ($r = 0.545$; $p \leqslant 0.05$) contents. Purple juice antioxidant activity was positively correlated with malvidin, cyanidin, peonidin, and delphinidin ($r = 0.781$; $p < 0.01$), catechin ($r = 0.741$; $p < 0.01$), and procyanidin B1 ($r = 0.781$; $p < 0.01$) contents.

3.7. Serum lipid peroxidation inhibition assay

$CuSO_4$-induced lipid peroxidation inhibition was tested for the eight grape juices samples (Table 5). Except for NCP, all grape juices were able to suppress serum lipid peroxidation induced by $CuSO_4$. Among all tested samples, BCP showed the highest lipoperoxidation protection activity (2.94 ± 0.03 nmol/mL) as compared to the $CuSO_4$ control (4.88 ± 0.03 nmol/mL).

3.8. Superoxide dismutase and catalase-like activities

In the present study, superoxide dismutase and catalase-like activities of different grape juices were tested. Among all tested samples (Table 6), BOP showed the highest superoxide-like activity, with the lowest IC50 value (3.52 ± 0.00 ml). There was a positive correlation between sod-like activity and total phenolic content ($r = 0.838$, $p < 0.01$), epicatechin content ($r = 0{,}824$, $p < 0.01$), and ascorbic acid level ($r = 0.625$, $p < 0.01$). NCP presented the highest

Table 3
Anthocyanins content in different purple grape juices ($n = 4$)

Sample[A]	Cyanidin (ppm)	Delphynidin (ppm)	Peonidin (ppm)	Malvidin (ppm)
BCC	$0.76 \pm 0.04^{B,c}$	4.10 ± 0.40^{c}	8.59 ± 0.82^{a}	95.26 ± 1.95^{d}
BCP	12.98 ± 0.51^{b}	30.22 ± 1.35^{b}	22.82 ± 1.18^{b}	308.76 ± 4.20^{b}
BOC	11.79 ± 0.42^{b}	26.30 ± 1.15^{b}	19.21 ± 1.43^{b}	232.46 ± 4.25^{c}
BOP	20.91 ± 0.83^{a}	49.51 ± 1.80^{a}	32.60 ± 1.78^{c}	425.96 ± 6.36^{a}

Values are the average of three replicates ±SD.
[A] BCC: Bordo Conventional Commercial; BCP: Bordo Conventional Pilot Scale; BOC: Bordo Conventional Organic; BOP: Bordo Organic Pilot Scale.
[B] Different letters correspond to mean values statistically different by analysis of variance (ANOVA) and Tukey post hoc test, for $p < 0.01$, that means a, b, c, d, at same column are statistically different.

Table 4
Catechins and procyanidins content in different grape juices ($n = 8$)

Sample[a]	Catechins (ppm)		Procyanidins (ppm)			
	Catechin	Epicatechin	B1	B2	B3	B4
BCC	$2.06 \pm 0.15^{d,*}$	22.13 ± 1.92^{a}	1.33 ± 0.18^{d}	1.83 ± 0.16^{a}	7.95 ± 0.58^{d}	4.66 ± 0.36^{a}
BCP	86.43 ± 2.31^{a}	2.11 ± 0.20^{c}	7.98 ± 0.67^{b}	1.88 ± 0.15^{a}	27.04 ± 1.32^{a}	2.27 ± 0.27^{b}
BOC	33.89 ± 1.82^{c}	2.72 ± 0.22^{c}	7.53 ± 0.52^{b}	2.32 ± 0.15^{a}	10.03 ± 0.51^{c}	0.64 ± 0.13^{e}
BOP	76.69 ± 2.70^{b}	4.91 ± 0.21^{b}	14.0 ± 0.82^{a}	1.13 ± 0.34^{a}	25.68 ± 1.17^{a}	2.93 ± 0.38^{a}
NCC	7.39 ± 0.52^{d}	5.95 ± 0.32^{b}	7.53 ± 0.31^{b}	1.32 ± 0.42^{a}	13.06 ± 0.62^{c}	2.45 ± 0.26^{b}
NCP	0.79 ± 0.05^{d}	0.97 ± 0.14^{d}	0.93 ± 0.06^{d}	0.94 ± 0.23^{a}	14.78 ± 0.34^{b}	1.68 ± 0.27^{c}
NOC	0.90 ± 0.06^{d}	1.81 ± 0.10^{c}	3.45 ± 0.43^{c}	1.58 ± 0.38^{a}	18.5 ± 0.7^{b}	3.59 ± 0.50^{a}
NOP	0.38 ± 0.02^{d}	0.92 ± 0.16^{d}	0.76 ± 0.11^{d}	0.61 ± 0.10^{b}	7.47 ± 0.28^{d}	1.23 ± 0.24^{d}

Values are the average of three replicates ±SD.
[*] Different letters correspond to mean values statistically different by analysis of variance (ANOVA) and Tukey post hoc test, for $p < 0.01$, that means a, b, c, d, e at same column are statistically different.
[a] BCC: Bordo conventional commercial; BCP: Bordo conventional pilot scale; BOC: Bordo conventional organic; BOP: Bordo organic pilot scale; NCC: Niagara conventional commercial; NCP: Niagara conventional pilot scale; NOC: Niagara conventional organic; NOP: Niagara organic pilot scale.

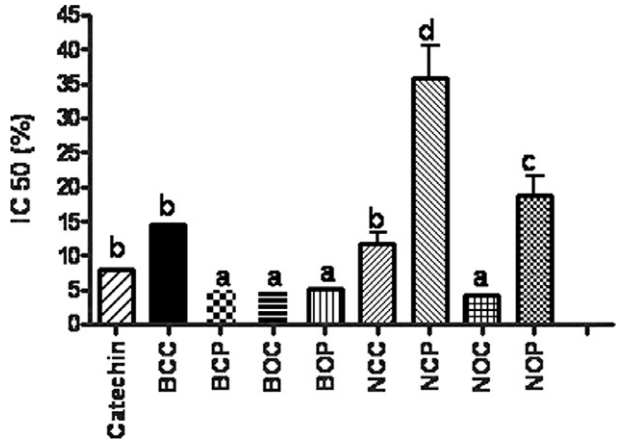

Fig. 3. IC 50 (amount of samples needed to reduce 50% of DPPH.) of different grape juices. Values are the mean ± SD of three replicates. BCC: Bordo conventional commercial; BCP: Bordo conventional pilot scale; BOC: Bordo conventional organic; BOP: Bordo organic pilot scale; NCC: Niagara conventional commercial; NCP: Niagara conventional pilot scale; NOC: Niagara conventional organic; NOP: Niagara organic pilot scale. Different letters correspond to mean values statistically significantly different by analysis of variance (ANOVA) and Tukey post hoc test, for $p < 0.01$, that means a, b, c at same figure are statistically.

Table 5
Peroxidation levels of serum treated with or without $CuSO_4$ or grape juice ($n = 8$)

Sample[a]	TBARS (nmol/mL)
Control	$3.29 \pm 0.03^{*}$
$CuSO_4$	$4.88 \pm 0.03^{**}$
$CuSO_4$ + BCC	$4.41 \pm 0.00^{*}$ (**)
$CuSO_4$ + BCP	$2.94 \pm 0.03^{*}$ (**)
$CuSO_4$ + BOC	$4.58 \pm 0.00^{*}$ (**)
$CuSO_4$ + BOP	$3.51 \pm 0.03^{*}$
$CuSO_4$ + NCC	$3.77 \pm 0.03^{*}$
$CuSO_4$ + NCP	$4.90 \pm 0.00^{**}$
$CuSO_4$ + NOC	$4.33 \pm 0.06^{*}$ (**)
$CuSO_4$ + NOP	$4.01 \pm 0.00^{*}$ (**)

Values are the average of three replicates ±SD.
[*] $p < 0.05$ compared to $CuSO_4$.
[**] $p < 0.05$ compared to control.
[a] BCC: Bordo conventional commercial; BCP: Bordo conventional pilot scale; BOC: Bordo conventional organic; BOP: Bordo organic pilot scale; NCC: Niagara conventional commercial; NCP: Niagara conventional pilot scale; NOC: Niagara conventional organic; NOP: Niagara organic pilot scale.

catalase-like activity (34.37 ± 0.62 μmol of H_2O_2 decomposed/min).

4. Discussion

4.1. Grape juice phenolic compounds, vitamin C, and nutritional analyses

Phenolic compounds are secondary metabolites produced and accumulated in plant tissues. Changes in phytopathogenesis, among others factors, may result in different concentrations of these compounds in plant organs. Organic farming is currently practiced world wide, and does not use chemical substances, such as pesticides and synthetic fertilizers, for growing crops. As pesticides are not used, plants are more susceptible to the action of phytopathogens, and this causes the plant to produce higher amounts of phenolic compounds as a means to defend itself (Soleas et al., 1997). In the present study, it was observed that the choice of agricultural method (organic versus conventional) resulted in different amounts of resveratrol, anthocyanins, and tannins in grape juices (Fig. 2; Tables 3 and 4). It should be noted that the present study is the first to report resveratrol content differences between organic and conventional grape juices. This difference is due to the fact that no pesticides are used in organic vineyards (Carbonaro et al., 2002).

Moreover, different methodologies are applied in grape juice manufacturing. When purple juices are produced, the pulp is heated along with the skin, resulting in the incorporation of phenolic compounds into the juice (Fuleki and Ricardo-da-Silva, 2003). In the present study, we also observed that purple juices presented higher phenolic compound content as compared to white juices, in which manufacturing process the grape skin is not heated (Fig. 1).

Table 6
Sod-like and catalase-like activity in different grape juices ($n = 8$)

Sample[A]	Sod-like activity (IC 50)[B]	Cat-like activity (μmol of H_2O_2 decomposed/min)
BCC	$5.40 \pm 0.05^{a,C}$	3.77 ± 0.02^{a}
BCP	11.64 ± 0.01^{d}	20.27 ± 0.25^{f}
BOC	6.47 ± 0.01^{c}	7.60 ± 0.10^{b}
BOP	3.52 ± 0.02^{b}	9.37 ± 1.87^{c}
NCC	20.53 ± 0.17^{e}	12.12 ± 0.87^{d}
NCP	22.98 ± 0.01^{f}	34.37 ± 0.62^{g}
NOC	87.00 ± 0.25^{h}	15.00 ± 0.10^{e}
NOP	30.42 ± 0.04^{g}	18.87 ± 0.125^{e}

Values are the average of three replicates ±SD.
[A] BCC: Bordo conventional commercial; BCP: Bordo conventional pilot scale; BOC: Bordo conventional organic; BOP: Bordo organic pilot scale; NCC: Niagara conventional commercial; NCP: Niagara conventional pilot scale; NOC: Niagara conventional organic; NOP: Niagara organic pilot scale.
[B] Amount of samples (ml) necessary to reduce 50% the adrenochrome formation.
[C] Different letters correspond to mean values statistically significantly different by analysis of variance (ANOVA) and Tukey post hoc test, for $p < 0.01$, that means a, b, c, d, e, f, g at same column are statistically significantly different.

In addition to phenolic compounds, vitamin C is also present in grape juices. In plants, vitamin C provides protection against RS generated during photosynthesis and respiration processes. Vitamin C is also involved in cell growth, and it is a co-factor of several enzymes participating in the synthesis of anthocyanidins and several secondary metabolites (Barata Soares et al., 2004). Vitamin C levels were positively correlated to total polyphenol ($r = 0.878$; $p < 0.01$), procyanidins B1 ($r = 0.676$; $p < 0.01$) and B2 (0.852; $p < 0.01$), and catechin (0.799; $p < 0.01$) contents. Except for BCP juice, organic juices presented higher vitamin C content as compared to conventional juices (Table 2). Similar results were found by Carbonaro et al. (2002) with organic and conventionally-grown peaches. Significant variation in vitamin C levels was observed between purple and white juices, and this is probably due to grape variety, ripening grade, and hours of sun exposure (Wang et al., 1996).

Several factors may influence grape juice nutritional analyses, such as grape variety, soil, climate, processing methods, etc. (Fuleki and Ricardo-da-Silva, 2003). In the present study, we observed that grape juices produced at pilot scale presented high carbohydrate levels. Although further studies are needed to confirm this hypothesis, this fact may be attributed to manufacturing process (Table 2).

4.2. Grape juice antioxidant properties

Except for one study that showed *in vitro* antioxidant activity of a white grape juice (Dávalos et al., 2005), all other studies on antioxidant activity of grape juices used purple grape juices elaborated from *Vitis vinifera* (Castilla et al., 2006; Rho and Kim, 2006; Day et al., 1997). This is the first study that showed the antioxidant activity of grapes juices produced with *V. labrusca* varieties (Bordo and Niagara), which are frequently used to produce grape juice in South America.

All studied grape juices showed significant *in vitro* antioxidant activity (Fig. 3). In the *ex vivo* assay, only one grape juice (NCP) did not prevent $CuSO_4$-induced peroxidation in serum (Table 5). Indeed, this grape juice presented the lowest total phenolic and ascorbic acid contents. This result suggests that these compounds provide protection by inhibiting lipid peroxidation, as shown by Carbonaro et al. (2002) in peaches. Although the measurement of other possible chemicals found in juices would be important, grape juices are a very complex mixture of compounds. The biologically active compounds are mainly phenolic and ascorbic acid. To our knowledge, the presence of alkaloids and aflatoxins in grapes juices is rare. No references on this issue were found in a recent literature database revision.

It was observed that white juices presented higher catalase-like activity antioxidant response as compared to purple juices (Table 6). In fact, some polyphenols are able to decompose H_2O_2 (Ferguson, 2001), thereby reducing the damage induced by this oxidative agent (Halliwell and Gutteridge, 1999).

This study showed that grape juices elaborated from *V. labrusca* are good antioxidant sources. This biological activity can be influenced by agricultural methods and polyphenols and ascorbic acid levels present in the juices.

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