Journal of Food Science and Engineering 3 (2013) 341-348

Effects of Grape Processing on Antioxidant Capacity and ESI-MS Fingerprints of Grape Products

Solange M. Cottica[1], Damila R. de Morais[1], Eliza M. Rotta[1], Sheisa C. Sargi[1], Flamys L. N. Silva[2], Alexandra C. H. F. Sawaya[3], Marcos N. Eberlin[2] and Jesuí V. Visentainer[1]

1. Department of Chemistry, State University of Maringá, Maringá 87020-900, Brazil

2. ThoMSon Mass Spectrometry Laboratory, Institute of Chemistry, University of Campinas, Campinas 13083-970, Brazil

3. Plant Biology Department, Institute of Biology, University of Campinas, Campinas 13083-970, Brazil

Received: April 10, 2013 / Published: July 20, 2013.

Abstract: The antioxidant capacity and changes in chemical composition of two grape varieties, the new hybrid BRS-Carmem and the Bordô grape (*Vitis labrusca*) and of their products (juice, wine and vinegar) were evaluated by several techniques. The DPPH method was used to measure the antioxidant capacity, whereas, the total phenolic contents (TPC) were measured by Folin-Ciocalteau method. Overall chemical composition was also monitored by ESI-MS fingerprints and UPLC-MS analysis. For both grape varieties, the highest (and similar) antioxidant capacity and TPC were observed for the wine and vinegar samples followed by the grapes and then the juices. In addition, ESI-MS fingerprints and UPLC-MS analysis in the negative ion mode indicated substantial changes in chemical composition from grape to juice and wine, and then to vinegar.

Key words: DPPH, total phenolic compounds, Bordô grape (*Vitis labrusca*), BRS-Carmem grape, fruit.

1. Introduction

Interest in food with antioxidant proprieties has increased over the last years [1-3], especially fruits [4, 5]. Grapes are fruits known as an important source of antioxidant compounds, mainly polyphenols [6, 7]. Grapes processed products including juice, wine and vinegar also have many phenolic compounds. These polyphenols are related with protection of plasma lipoproteins from oxidation [8], inhibiting some degenerative diseases, such as cardiovascular diseases, and certain types of cancer [9].

The major classes of phenolic compounds found in grapes and grape products are flavonoids such as catechins, epicatechins, epigallocatechins, quercetin and anthocyanins, and non-flavonoid compounds such as phenolic acids and resveratrol [6]. Tartaric, malic, citric and succinic acids are important examples of such non-phenolic constituents of grapes [10].

The phenolic content of grapes during wine ageing becomes progressively more complex, with substantial changes also in the non-phenolic contents. The variety, climate, soil and processing are also responsible for the final chemical composition of grape products as juices, wines and vinegars. There are many studies about the composition of grapes or their derivatives and about their antioxidant capacity [5, 6, 11-15]. However, the changes in the chemical composition during grape processing to juice, wine, vinegar and the antioxidant capacity of each product seems to have not been systematically investigated yet. In this study, the antioxidant capacity of two varieties of grapes and their juice, wine and vinegar products was evaluated by the DPPH method. The total phenolic contents were determined by the Folin-Ciocalteau method to analyze the changes in chemical composition, whereas, ESI-MS fingerprinting and UPLC-MS analysis were also done for this purpose.

Corresponding author: Solange M. Cottica, Ph.D., researcher fields: food chemistry (fatty acids and antioxidants) and mass spectrometry. E-mail: smcottica@gmail.com.

2. Material and Methods

2.1 Chemicals and Equipments

Folin-Ciocalteau reagent, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), catechin hidrate, epicatechin galate, epigallocatechin, epigallocatechin galate, epicatechin, quercetin, resveratrol and the gallic, malic, tartaric and citric acids were obtained from Sigma-Aldrich. Ammonium hydroxide was obtained from Merck, Darmstadt, Germany, chromatographic grade methanol and acetonitrile were from Tedia, Fairfield, OH, USA and other chemicals were analytical degree.

2.2 Samples

Grape samples of BRS-Carmem hybrid, derived from Muscat Belly A with BRS-Rúbea and developed by the Brazilian Company of Farming Search (Embrapa), were donated by Corol Juice Industry of Corol Cooperative, located at Rolândia City, State of Paraná, Brazil. The Bordô grape samples (*Vitis labrusca*) were acquired from farmers of Santa Catarina State, Brazil. Both grapes varieties were frozen at -20 °C for later analysis.

The juice of both types of grapes was made by maceration. The sugar content of the juice was measured and corrected by addition of sucrose dissolved in pure water (Table 1). After this correction, aliquot proportions were collected and frozen at -20 °C.

For wine production, the grape pulps, seeds and skins were kept in the juice during 7 days' fermentation and were then discarded. After 30 days stored in the dark and at room temperature, the wines were transferred to another recipient, eliminating the deposit formed. This procedure was repeated until no more precipitate at the bottom of the bottle was observed.

Table 1 Sugar content (° Brix) of grape juices before and after addition of sucrose.

Varieties	Before	After
Carmem	13.5°	18.5°
Bordô	13.7°	17.5°

Vinegars from both grape varieties were produced accelerating the wine fermentation process by higher oxygen contact.

2.3 Preparation of Extracts

Methanolic extractions were made for the antioxidant capacity and total phenolic contents analysis of the samples by stirring for 4 h and protecting from light, with a ratio of 1:10 (weight sample:solvent volume). For the liquid samples, the weight value was obtained by their density. The extracts were obtained by a rotary evaporator (Buchi, model RT 210) after filtration.

The liquid samples were diluted for the ESI-MS fingerprints and UPLC/MS analysis, while the grape extracts were prepared with methanol at a ratio of 1:10 (m/v), by sonication for 10 min followed by stirring for 20 min protecting from light. The extracts were dried in a rotary evaporator after filtration.

2.4 Antioxidant Capacity

The free radical scavenging activity was measured using DPPH as already described [16] with some modifications. Briefly, various volumes of samples extract solutions (2.0 mg/mL) were added to 2.0 mL of DPPH methanolic solution (0.1192 mmol/L) and maintained in the dark for 30 min at room temperature. Then, absorbance was measured at 517 nm in a spectrophotometer (Cary 50-Varian). Methanol was used instead of samples extract solutions as a control. The results were expressed by EC_{50}, which determines the extract concentration (μg/mL) that provides 50% inhibition, the lower its value is, the greater the efficiency of the antioxidant. The scavenging capacity of the DPPH radical was calculated with Eq. 1:

$$\%\ Inhibition\ DPPH = \frac{(Abs_{DPPH} - Abs_{sample})}{Abs_{sample}} \times 100 \quad (1)$$

Eq. 1: Percent inhibition of the DPPH radical.

The extract concentration value was plotted versus % inhibition of DPPH· and the EC_{50} value was obtained by linear regression. All treatments were run in triplicate.

2.5 Total Phenolic Contents

The total phenolic contents of the samples were analyzed using gallic acid as a standard by the Folin-Ciocalteau method [17] with some modifications. Methanolic solutions of samples extracts (2.5 mg/mL) were prepared and 250 μL of these solutions or of standard solutions of gallic acid or methanol as blank were added to separate test tubes. To each tube, 250 μL of Folin-Ciocalteau reagent (diluted in water 1:1), 500 μL of Na_2CO_3 saturated solution, and 4 mL of distilled water were added and mixed. The solutions were incubated in the dark at room temperature for 25 min and then centrifuged for 10 min at 3,000 rpm. The sample absorbance was read against the blank at 725 nm using a spectrophotometer (Cary 50-Varian). The total phenolic content of the samples was determined by comparison with a calibration curve of gallic acid as a standard (r^2 = 0.9995) and represented as mg gallic acid equivalents (GAE) 100/g of sample. The analyses were done in triplicate.

2.6 Electrospray Ionization Mass Spectrometry (ESI-MS) Fingerprints

For the negative (ESI(-)-MS) ion mode fingerprints of the samples, 100 μL of each sample, grape extract, juice, wine or vinegar were diluted in 900 μL of HPLC-grade methanol. After, 10 μL of this solution was diluted in 1 mL of HPLC-grade methanol and 1 μL of NH_4OH 0.5% (in methanol). These solutions were directly infused into the ESI source by means of a syringe pump (Harvard Apparatus-Massachusets, USA) at a flow rate of 10 μL/min. Negative ion mode fingerprints were acquired using a Micromass-Waters Q-TOF mass spectrometer (Waters, Manchester, England) in the following conditions: capillary and cone voltages of 3,000 V and 30 V, respectively, desolvation temperature of 100 °C.

ESI-MS fingerprints were acquired and accumulated over 60 s and spectra were scanned in the range between *m/z* 50 and 1,000. However, no important ions were observed below or above the *m*/*z* 80-600 range of the fingerprints. The dissociation patterns of the main ions in the different samples were compared with those of compounds identified in previous studies [12, 18-20] and confirmed with standard solutions by UPLC-MS analysis.

2.7 UPLC-MS

The UPLC-MS analyses were acquired by ultra-high performance chromatography-mass spectrometry equipment (Waters, Acquity UPLC-TQD). The negative ion mode capillary and cone voltages was 3,500 V and 30 V, respectively, the source temperature was 150 °C and the desolvation temperature was 350 °C. The chromatographic conditions were: solvent A (milliQ purified water with 0.1% formic acid) and solvent B (HPLC grade acetonitrile), in a gradient profile with initial condition of 5% B to 8 min 100% B, held to 8.9 min and retuning to initial condition and stabilizing. The total time was of 10 min, the injection was of 5 μL and the column used was an acquity UPLC BEH C18 1.7 micr, 2.1 mm × 50 mm. The oven temperature was 30 °C.

2.8 Statistical Analysis

The results were submitted to variance analysis (ANOVA) and Tukey's test (5% probability) using the software Statistica 5.1 (StatSoft, 1996). Mean values were compared by Tukey's test.

3. Results and Discussion

3.1 Antioxidant Capacity

In DPPH analysis, a lower EC_{50} value indicate greater antioxidant capacity since a smaller mass of extract is required to inhibit 50% of the DPPH radical. According to the results shown in Fig. 1, wine and vinegar display the best (and similar) antioxidant capacity as compared to their precursors grape and juice, that is: vinegar ≅ wine > grape > juice. This is in agreement with previous results that found higher antioxidant activity for wine when compared to grape juice [14]. For Bordô grape, its

vinegar showed a significant better antioxidant capacity than its wine. The EC_{50} values are also similar to previously published results for grape seeds, grape skins and wine [13, 21].

3.2 Total Phenolic Contents

The results of total phenolic contents (TPC) of the samples analyzed indicate a decrease of TPC from the grape to the juice for both grape varieties, followed by an increase from the juice to the wine and vinegar (Fig. 2). This increase was more pronounced for the Carmem vinegar. These TPC are in agreement with previous results, where similar values were observed for fresh grape berries, seeds and skins [6, 13].

The results displayed in Figs. 1 and 2 are also summarized in Table 2 showing significant differences between the grapes and some products ($P < 0.05$).

The correlation between the antioxidant capacity and the TPC of -0.7171 suggests that the antioxidant capacity evaluated by DPPH method is mostly related to TPC. According to the literature, phenolic compounds are the major constituents responsible for the antioxidant capacity in grapes [9].

3.3 Electrospray Ionization Mass Spectrometry Fingerprints in the Negative Ion Mode

Fingerprints (Table 3) were used for the qualitative assessment of the changes in chemical composition of the grape products, from juice to wine and then vinegar.

The detection of the ions of *m/z* 133, 149 and 179 were observed for the grapes and their products in both varieties, and related to the deprotonated molecules of malic, tartaric or caffeic acids or a hexose, respectively [18]. In addition, the ion of *m/z* 191, which may correspond to citric acid or quinic acid [18], was found in all samples, except for that of the Carmem wine. The ions of *m/z* 115 and 313 were observed in the fingerprints of the grapes and the juices samples. The ions of *m/z* 277, 359 and 457 also were found in fingerprints of samples before fermentation, and they have been related to clusters of the hexose of *m/z* 179, to deprotonated dimers of a hexose [20] and to epigallocatechin galate or catechin hidrate, respectively.

Ions of *m/z* 329 and 539 were detected in the juice fingerprints. That of *m/z* 329 and has been related to a cluster of the hexose (*m/z* 179) with a deprotonated organic acid of *m/z* 149 [20], possibly tartaric acid. The ions of *m/z* 277, 313, 329 and 359 have been found to be diagnostic for unfermented must and, during the fermentation process, the sugars are consumed and in wines and vinegars these ions are no longer detected [20]. The (ESI(-)-MS) fingerprints of the wine and vinegar samples detected the ions of *m/z* 117 and of *m/z* 129. The ion of *m/z* 117 has been related to the

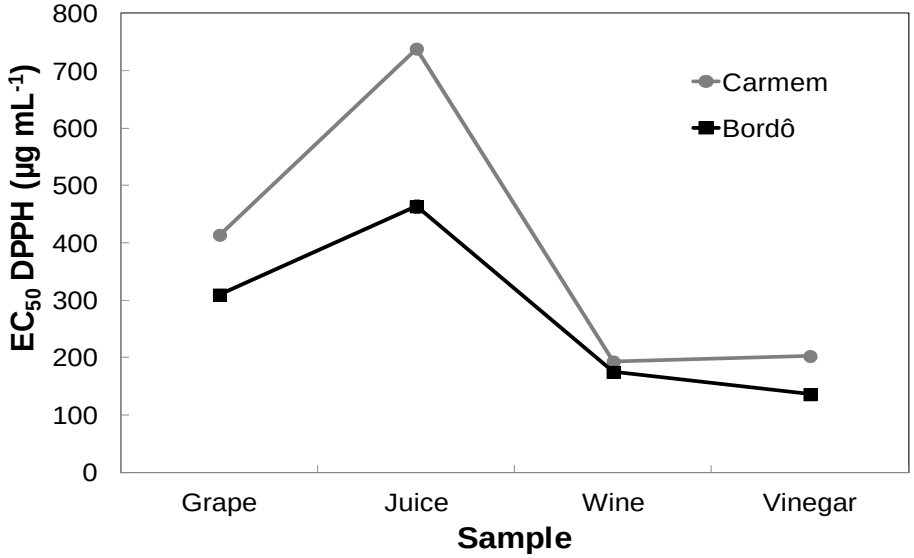

Fig. 1 Antioxidant capacity of grape products by DPPH method.

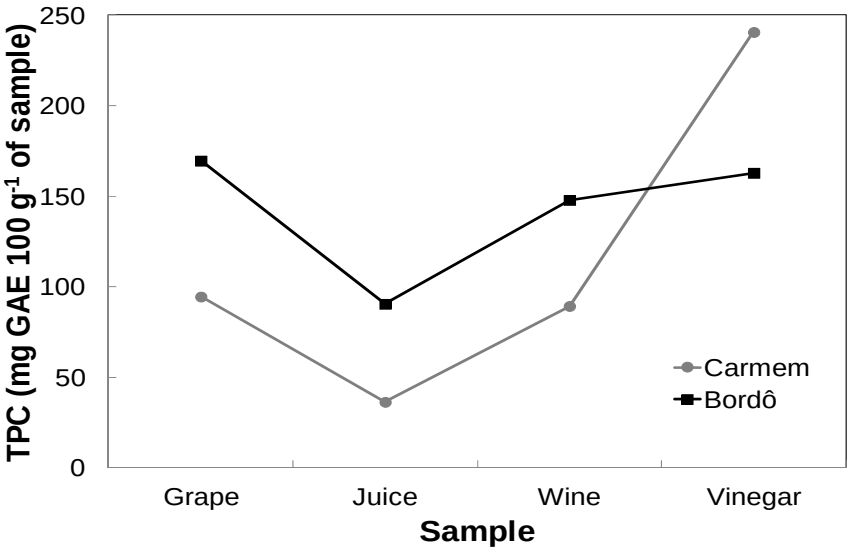

Fig. 2 Total phenolic contents (TPC) of grape products.

Table 2 Total phenolic contents (TPC) and extract concentration (EC_{50}) necessary to reduce by 50% the free radical DPPH.

	Carmem		Bordô	
	TPC*	DPPH#	TPC*	DPPH#
Grape	94.54 b ± 3.10	414.13 b ± 7.13	169.62 a ± 2.13	309.90 b ± 5.53
Juice	36.38 c ± 2.32	738.67 a ± 31.46	90.53 c ± 7.24	463.85 a ± 11.59
Wine	89.35 b ± 2.41	193.24 c ± 5.95	147.92 b ± 5.79	175.20 c ± 2.47
Vinegar	240.68 a ± 3.31	202.80 c ± 3.13	162.91 a ± 5.98	136.23 d ± 0.82
CV (%)	2.11	4.26	3.13	2.42

*Expressed as mg gallic acid equivalents (GAE) 100 g^{-1} of sample; #expressed as EC_{50} (μg/mL); different letters in the same column mean significant difference by Tukey test ($P < 0.05$).

fermentation product succinic acid, which is responsible for a bitter taste, causing salivation [18]. The ions of *m/z* 97 and 175, which has been related to phosphoric acid and ascorbic acid or ethyl cinnamate [18, 22], were also detected in wines and vinegars, except for Carmem wine where the ion of *m/z* 97 was not found and to Bordô vinegar where the ion of *m/z* 175 was not found. Some ions were sporadically detected such as those of *m/z* 193, 227, 255, 283 and 305, which seems to correspond to ferulic acid, resveratrol, palmitic acid, stearic acid and epigallocatechin and cinnamate, respectively [18, 22].

3.3 UPLC-MS

The ultra-high performance chromatography-mass spectrometry analysis using ESI in the negative ion mode using standards confirmed the presence of some of the compounds (Table 4) suggested by ESI-MS fingerprints.

Malic and tartaric acids were found in all samples analyzed, whereas, citric acid was confirmed to be present only in Carmem grape, juice and vinegar and at Bordô juice and vinegar. Also catechin hidrate was confirmed in both grapes and epigallocatechin galate was confirmed in both juices. In addition, the UPLC-MS analysis detected the presence of gallic acid in both varieties of wine and vinegar and resveratrol was found only at Bordô juice. Other flavonoids were found in the samples: epicatechin galate in both juices and Carmem vinegar, epigallocatechin in Bordô grape and epicatechin in both grape varieties. These polyphenols (epigallocatechins) and flavonoids (catechins and epicatechins) are potent inducers of apoptosis in cancer cells [13], inhibit some degenerative diseases and are also regarded as preservatives against oxidation [9].

Table 3 Ions detected by ESI(-)-MS for the grape products.

$(M-H)^-$	Carmem				Bordô				Proposed compound
m/z	Grape	Juice	Wine	Vinegar	Grape	Juice	Wine	Vinegar	
89	d	d	d	d	d	d	d	d	-
97	nd	nd	nd	d	nd	nd	d	d	phosphoric acid
115	d	d	nd	nd	d	d	nd	nd	-
117	nd	nd	d	d	nd	nd	d	d	succinic acid
129	nd	nd	d	d	nd	nd	d	d	-
133	d	d	d	d	d	d	d	d	malic acid
147	nd	nd	nd	d	nd	nd	nd	d	-
149	d	d	d	d	d	d	d	d	tartaric acid
175	nd	nd	d	d	nd	nd	nd	d	ascorbic acid or ethyl cinnamate
179	d	d	d	d	d	d	d	d	caffeic acid
191	d	d	nd	d	d	d	d	d	citric acid
193	nd	nd	d	d	nd	d	d	d	ferulic acid
227	d	nd	d	d	d	nd	nd	nd	resveratrol
255	d	d	d	d	d	nd	nd	d	palmitic acid
277	d	d	nd	nd	d	d	nd	nd	clusters of a hexose (*m*/*z* 179)
283	d	nd	nd	d	D	nd	nd	nd	stearic acid
305	nd	nd	nd	nd	nd	nd	nd	d	epigallocatechin
313	d	d	nd	nd	d	d	nd	nd	-
329	nd	d	nd	nd	nd	d	nd	nd	cluster of an hexose with a deprotonated organic acid of *m*/*z* 149
359	d	d	nd	nd	d	d	nd	nd	deprotonated dimer of the hexose
457	d	d	nd	nd	d	d	nd	nd	epigallocatechin galate or catechin hidrate
539	nd	d	nd	nd	nd	d	nd	nd	-

d = detected; nd = not detected.

Table 4 Compounds identified by UPLC-MS analysis by comparison with standards.

Compound	Carmem				Bordô			
	Grape	Juice	Wine	Vinegar	Grape	Juice	Wine	Vinegar
Malic acid	d	d	d	d	d	d	d	d
Tartaric acid	d	d	d	d	d	d	d	d
Citric acid	d	d	nd	d	nd	d	nd	d
Gallic acid	nd	nd	d	d	nd	nd	d	d
Catechin hidrate	d	nd	nd	nd	d	nd	nd	nd
Epicatechin galate	nd	d	nd	d	nd	d	nd	nd
Epigallocatechin	nd	nd	nd	nd	d	nd	nd	nd
Epigallocatechin galate	nd	d	nd	d	nd	d	nd	nd
Epicatechin	d	nd	nd	nd	d	nd	nd	nd
Resveratrol	nd	nd	nd	nd	nd	d	nd	nd

d = detected; nd = not detected.

Some compounds found at the samples were quantified for grape skins and seeds, as well as for their juices, wines and vinegars (Table 5). Malic acid, which confers a harsh taste, was quantified in the skin of Carmem grape, the seed of Bordô grape and in both juices. Malic acid was not found in either wine or vinegar, maybe because of the malolactic fermentation which transforms malic acid into lactic acid [18]. The most abundant acid in wines, tartaric acid, was quantified for all samples, excepting for the Carmem grape seed and Bordô grape skin, being in agreement with Table 4. However, citric acid that was detected by the UPLC-MS analysis was not quantified due to its low concentration in grapes.

Table 5 Quantification (ppm) of some compounds in Carmem and Bordô grape products.

Compound	Carmem					Bordô				
	Skin	Seed	Juice	Wine	Vinegar	Skin	Seed	Juice	Wine	Vinegar
Malic acid	215.35	nq	296.69	nq	nq	nq	12.28	271.29	nq	nq
Tartaric acid	259.65	nq	177.00	79.6	177.06	nq	50.09	235.45	136.39	302.19
Citric acid	nq	nq	nq	nq	nq	nq	nq	nq	nq	nq
Catechin hidrate	10.37	nq	nq	nq	nq	nq	1080.35	nq	nq	nq
Quercetin	nq	nq	nq	8.02	nq	nq	nq	nq	5.15	nq
Resveratrol	nq	nq	nq	nq	nq	nq	0.10	nq	nq	nq

nq = not quantified.

Quantification of catechin hidrate and quercetin in the samples was possible in Carmem grape skin and Bordô grape seed, and in both wines, respectively. These flavonoids are responsible for many properties including color, flavor and the antioxidant activity [6]. Another very important compound, resveratrol, was quantified in Bordô seeds, and it is related against cardiovascular diseases [6]. As already described at literature [14] and according to the results obtained, it can be inferred that gallic acid, quercetin and resveratrol are related to the antioxidant capacity observed at the samples as well as the other polyphenolic compounds found at all the grape products.

4. Conclusions

Higher and similar antioxidant capacity was observed for wine and vinegar samples followed by the grapes and then the juice for both varieties of grape, BRS-Carmem and Bordô. Similar trends were also found for the total phenolic contents. In addition, analysis by ESI(-)-MS fingerprints and UPLC-MS were able to show significant and important changes in chemical composition that occurs due to sample processing from grape, to juice and wine and up to vinegar.

Acknowledgments

The authors thank the Brazilian Science Foundations CNPq, FAPESP and CAPES for financial support and Corol Cooperative for donation of BRS-Carmem grapes.

References

[1] S. Wang, J.P. Melnyk, R. Tsao, M.F. Marcone, How natural dietary antioxidants in fruits, vegetables and legumes promote vascular health, Food Res. Int. 44 (2011) 14-22.

[2] B.L. Halvorsen, R. Blomhoff, Validation of a quantitative assay for the total content of lipophilic and hydrophilic antioxidants in foods, Food Chem. 127 (2011) 761-768.

[3] S.M. Cottica, A.C.H.F. Sawaya, M.N. Eberlin, S.L. Franco, L.M. Zeoula, J.V. Visentainer, Antioxidant activity and composition of propolis obtained by different methods of extraction, J. Braz. Chem. Soc. 22 (2011) 929-935.

[4] N. Babbar, H.S. Oberoi, D.S. Uppal, R.T. Patil, Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues, Food Res. Int. 44 (2011) 391-396.

[5] L.P. Santos, D.R. Morais, N.E. Souza, S.M. Cottica, M. Boroski, J.V. Visentainer, Phenolic compounds and fatty acids in different parts of *Vitis labrusca* and *V. vinifera* grapes, Food Res. Int. 44 (2011) 1414-1418.

[6] L.T. Abe, R.V. Mota, F.M. Lajolo, M.I. Genovese, Phenolic compounds and antioxidant activity of Vitis labrusca and *Vitis vinifera* cultivars, Ciênc. Tecnol. Aliment. 27 (2007) 394-400.

[7] J.J. Maxcheix, A. Fleuriet, J. Billot, The main phenolics of fruits, in Fruit Phenolics, CRC Press, Boca Raton, FL, 1990, pp. 1-98.

[8] J.K. Jacob, F. Hakimuddin, G. Paliyath, H. Fisher, Antioxidant and antiproliferative activity of polyphenols in novel high-polyphenol grape lines, Food Res. Int. 41 (2008) 419-428.

[9] E.Q. Xia, G.F. Deng, Y.J. Guo, H.B. Li, Biological activities of polyphenols from grapes, Int. J. Mol. Sci. 11 (2010) 622-646.

[10] L.L.A. Lima, A. Schuler, N.B. Guerra, G.E. Pereira, T.L.A. Lima, H. Rocha, Method otimization and validation for determination of organic acids in wine by high performance liquid chromatography, Quim. Nova 33 (2010) 1186-1189. (in Portuguese)

[11] B. Lorrain, K. Chira, P.L. Teissedre, Phenolic composition of merlot and cabernet-sauvignon grapes from Bordeaux vineyard for the 2009-vintage: Comparison to 2006, 2007 and 2008 vintages, Food Chem. 126 (2011) 1991-1999.

[12] R. Flamini, Mass spectrometry in grape and wine chemistry, Part I: polyphenols, Mass Spectrom. Rev. 22 (2003) 218-250.

[13] M. Anastasiadi, H. Pratsinis, D. Kletsas, A.L. Skaltsounis, S.A. Haroutounian, Bioactive non-coloured polyphenols content of grapes, wines and vinification by-products: Evaluation of the antioxidant activities of their extracts, Food Res. Int. 43 (2010) 805-813.

[14] C. Sánchez-Moreno, J.A. Larrauri, F. Saura-Calixto, Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents, Food Res. Int. 32 (1999) 407-412.

[15] F.R.S. Bentlin, F.H. Pulgati, V.L. Dressler, D. Pozebon, Elemental analysis of wines from South America and their classification according to country, J. Braz. Chem. Soc. 22 (2011) 327-336.

[16] W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of a free radical method to evaluate antioxidant activity, Lebensmittel-Wissenschaft und-Technologie 28 (1995) 25-30.

[17] V.L. Singleton, J.A. Jr Rossi, Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents, Am. J. Enol. Vitic. 16 (1965) 144.

[18] A.C.T. Biasoto, R.R. Catharino, G.B. Sanvido, M.N. Eberlin, M.A.A.P. Silva, Flavour characterization of red wines by descriptive analysis and ESI mass spectrometry, Food Qual. Pref. 21 (2010) 755-762.

[19] G. Mazerollesa, S. Preys, C. Bouchut, E. Meudec, H. Fulcrand, J.M. Souquet, et al., Combination of several mass spectrometry ionization modes: A multiblock analysis for a rapid characterization of the red wine polyphenolic composition, Anal. Chim. Acta 678 (2010) 195-202.

[20] R.R. Catharino, I.B.S. Cunha, A.O. Fogac, E.M.P. Facco, E.T. Godoy, C.E. Daudt, et al., Characterization of must and wine of six varieties of grapes by direct infusion electrospray ionization mass spectrometry, J. Mass Spectrom. 41 (2006) 185-190.

[21] G.K. Jayaprakasha, T. Selvi, K.K. Sakariah, Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts, Food Res. Int. 36 (2003) 117-122.

[22] M.N. Bravo, S. Silva, A.V. Coelho, L.V. Boas, M.R. Bronze, Analysis of phenolic compounds in Muscatel wines produced in Portugal, Anal. Chim. Acta 563 (2006) 84-92.