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Evolution of major phenolic components and radical scavenging activity of grape juices through concentration process and storage

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ABSTRACT

The total phenolic (TP) and radical scavenging activity (RSA) of concentrated grape juices during process and storage were characterized and quantified. TP were determined using the Folin–Ciocalteu method and RSA by the DPPH assay. The main phenolic components of juices were investigated by direct infusion electrospray ionization-mass spectrometry (ESI-MS) in the negative ion mode. Concord juice (CJ) demonstrated higher RSA and TP contents than Isabel juice (IJ) with some differences at each processing step. During storage, retention of TP and RSA were 90% and 77% in CJ and of 81% and 86% in IJ, respectively. During processing, peonidin and peonidin-3-*O*-glucoside gave place to malvidin and dimethoxy-flavylium as major significant components; piceatannol-*O*-glucoside became significant after concentration and malvidin decreased after 8 months of storage. Concentrated and refrigerated storage were effective in preserving total phenolics and antioxidative status of grape juices, although changes in compounds were observed.

1. Introduction

Phenolic-rich foods have received increasing attention due to recent findings on their association with disease prevention (Arts & Hollman, 2005; Knet et al., 2002; Sesso, Gaziano, Liu, & Buring, 2003). Studies have identified dietary sources of polyphenols as being mainly fruits, fruit juices and beverages such as wine, tea and coffee (Beecher, 2003; Bravo, 1998; Scalbert, Johnson, & Saltmarsh, 2005). Average daily intake has been difficult to estimate for reasons mostly related to diversity in polyphenol structures and variations in the content of particular foodstuffs influenced by cultivar and manufacturing processes (Scalbert & Williamson, 2000). Some of the new compounds formed during processing and storage of fruit beverages are often overlooked in studies addressing food composition, although they may show particular properties different from their precursors (Cheynier, 2005).

The efficacy of natural antioxidants is related to the protection of the food itself against oxidative damage and also to the continued action in animal fluids and tissues (Sánchez-Moreno, Larrauri, & Saura-Calixto, 1999). Several assays have been used to measure free radical scavenging capacity and the International Organisation of Vine and Wine has recommended the DPPH· assay as a rapid and precise method for grape products. Previous studies have examined the antioxidant properties of wine (de Beer, Joubert, Gelderblom, & Manley, 2003; Katalinic, Milos, Modun, Music, & Boban, 2004; Muñoz-Espada, Wood, Bordelon, & Watkins, 2004). The antioxidant capacity of fruit juices is not always the same as that of fresh fruits and its measurement in a wide range of food matrices raises discrepancies due to differences in plant cultivars (Karakaya, El, & Tas, 2001). Such inconsistencies reveal that certain properties of phenolic-rich products are influenced by polyphenolic composition, which is affected by vintage, grape cultivar, production techniques and aging.

Electrospray ionization-mass spectrometry (ESI-MS) with direct infusion of sample has appeared as a new alternative for the fingerprinting characterization of chemical mixtures, offering a fast and robust technique for typification of several beverages such as fruit juices (Roesler, Catharino, Malta, Eberlin, & Pastore, 2007), yerba matte and green tea (Bastos et al., 2007), whisky (Moller, Catharino, & Eberlin, 2005), wine (Catharino et al., 2006) and cachaça (de Souza et al., 2007). ESI-MS fingerprinting has therefore greatly expanded the applicability of mass spectrometry to perform fast, selective and reliable characterization of products of different origins (Moller, Catharino, & Eberlin, 2007). It has also been proven as a powerful technique for the characterization and quality

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control of essential oils, fatty acids, organic acids and pigments in foods (Catharino et al., 2005; Moller, Adamsen, Catharino, Skibsted, & Eberlin, 2005). ESI-MS fingerprinting is also convenient for direct analysis of grape juices, as most molecules bearing acidic or basic sites should be detected (Fig. 1), whereas MS/MS with collision-induced dissociation (CID) could be used for more detailed structural elucidation. This paper describes the characterization and quantification of total phenolic and radical scavenging properties of concentrated grape juices during processing and storage and the first ESI-MS fingerprinting investigation of these juices.

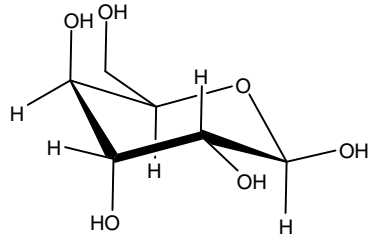
2. Materials and methods

2.1. Samples

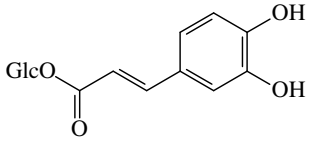
Concentrated grape juice samples of Concord and Isabel (known as "Isabella" in North-America) cultivars were received in February and March of 2006, respectively. These cultivars belong to *Vitis labrusca* species and are the most used cultivars for grape juice production in Brazil, with variations on their soluble solids contents: Concord between 14 and 16° Brix and Isabel between 15 and 19° Brix. Concentrated grape juices were provided by a national producer from Rio Grande do Sul – Brazil. Concentrated juices are produced during harvest and supplied along the year to other manufacturers for the production of reconstituted grape beverages. Samples of both cultivars were also obtained at each step of the industrial process which consists of hot pressing of grapes and pasteurization of must (80 °C, 30 s) followed by filtration and concentration of juice to 68° Brix by evaporation (highest temperature of 98 °C for 5 s). Concentrated juices were stored at 5 °C in the dark, simulating industrial storage conditions. Every 30 days two samples of each grape cultivar were removed and placed under −18 °C for subsequent analysis, with maximum aging time of 10 months. Prior to analysis concentrated juices were reconstituted to 17° Brix.

2.2. Determination of total phenols

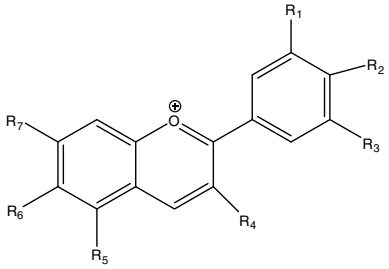
Total phenols were measured by the Folin–Ciocalteu assay (Singleton & Rossi, 1965) using gallic acid (Sigma–Aldrich, St. Louis, MO, USA) for the standard curve and the results being expressed in mg gallic acid equivalents (GAE)/L. Floating particles were



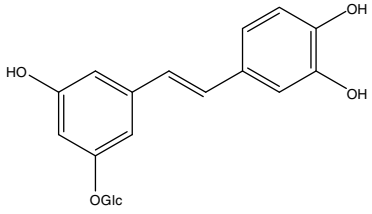
Hexose (**1**)



Caffeoyltartaric acid (**2**)



Anthocyanidin (**3**)



Piceatannol-O-glucoside (**4**)

Glc=glucose

Anthocyanidin	R_1	R_2	R_3	R_4	R_5	R_6	R_7
Cyanidin	-OH	-OH	-H	-OH	-OH	-H	-OH
Malvidin	$-OCH_3$	-OH	$-OCH_3$	-OH	-OH	-H	-OH
Peonidin	$-OCH_3$	-OH	-H	-OH	-OH	-H	-OH
Peonidin-3-O-glucoside	$-OCH_3$	-OH	-Glc	-OH	-OH	-H	-OH

Glc=glucose

Fig. 1. Structures of hexose (**1**), caffeoyltartaric acid (**2**), derivative anthocyanidin (**3**) and piceatannol-*O*-glucoside (**4**).

removed by centrifugation and juice samples diluted 1:100 with deionized water. The readings (in duplicate) were taken at 760 nm using a Beckman spectrometer.

2.3. Determination of radical scavenging activity

The DPPH (1,1-diphenyl-2-picrylhydrazil) (Sigma–Aldrich, Steinheim, BW, Germany) assay was used based on the methods of Brand-Williams, Cuvelier, and Berset (1995), as modified by Kim, Lee, Lee, and Lee (2002). The absorbance was measured with a Beckman spectrometer at 517 nm before addition of samples and after 30 min; the difference was plotted on a Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) (Sigma–Aldrich, St. Louis, MO, USA) standard curve. Analyses were carried out in duplicates and the results expressed in mM Trolox equivalents (mM TE)/L.

2.4. Electrospray ionization-mass spectrometry fingerprinting

Grape juices were diluted in a solution containing 50% (v/v) chromatographic grade methanol (Tedia, Fairfield, OH, USA) and 50% (v/v) deionized water and 0.5% of ammonium hydroxide (Merck, Darmstadt, Germany). ESI-MS fingerprints in the negative ion mode of juices were acquired and accumulated over 60 s and spectra were scanned in the range between *m/z* 250 and 600 to investigate processing and in the range of *m/z* 250–900 for cultivar comparisons, using a Micromass-Waters Q-TOF mass spectrometer (Waters, Manchester, England). Capillary and cone voltages were set to −3000 V and −40 V, respectively, with a desolvation temperature of 100 °C. ESI-MS was performed by direct infusion with typical flow rate of 10 μl min^{-1} using a syringe pump (Harvard Apparatus, MA, USA). Structural analysis of selected ions from the grape juices was performed by ESI-MS/MS. The ion of interest was selected and submitted to 15–45 eV collisions with argon in the collision hexapole. The collision gas pressure was optimized to produce extensive fragmentation of the ion under investigation. The grape juice constituents were identified by comparison of their MS/MS data with data from the literature (Ye, Han, Chen, Zhen, & Guo, 2007) and from our previous works (Catharino et al., 2006).

2.5. Statistical analysis

The *t*-test was applied to compare total phenols and radical scavenging activity averages between cultivars. To verify the relationships between parameters, Pearson correlation coefficients were calculated. Data analyses were conducted using Excel 97 (Microsoft Corporation, Washington, USA).

3. Results and discussion

3.1. Total phenol (TP) and radical scavenging activity (RSA)

TP and RSA contents found during processing of concentrated grape juice are shown in Fig. 2. Concord juice (CJ) and Isabel juice (IJ) demonstrated diverse amounts of TP and RSA throughout the process steps. A stable behaviour of parameters for both juices was observed during processing with some variation after heat treatment (pasteurization). Although CJ showed ca. 50% higher contents of TP, its RSA was on the average 25% higher. Such disparities could be attributed to different phenolic composition, which would yield the radical scavenging activity. Yildrin, Akçai, Güvenç, Altindisli, and Sözmen (2005) reported variations in total phenols and antioxidant activity during the steps of wine making (grape, pomace, juice, must and wine). The authors observed that although total phenolic contents did not differ during some steps, antioxidant activity was enhanced after fermentation. These findings confirmed the theory of higher antioxidant action of individual phenolic compounds in food matrices with similar total phenolic contents.

TP contents and RSA of CJ and IJ displayed a fairly stable behaviour during aging (Fig. 3). TP contents varied from 2872.9 to 2587.6 GAE in CJ and from 1756.8 to 1428.9 GAE in IJ. RSA went from 9.68 to 7.45 mM TE in CJ and from 7.40 to 6.33 mM TE in IJ. On average, Concord grape juice presented higher TP contents and RSA during 10 months of storage ($p < 0.001$) and a positive and significant correlation was found between TP and RSA for Concord juice ($r = 0.78$, $p = 0.005$) and Isabel juice ($r = 0.88$, $p < 0.001$). TP retention percentage was 90% and 81% for CJ and IJ, respectively, while RSA retention was 77% and 86% for CJ and IJ, respectively. IJ portrayed higher RSA retention in spite of the higher TP loss. Pérez-Vicente, Serrano, Abellán, and García-Vigueira (2004) observed similar behavior regarding TP contents of pomegranate juice: a 2% loss during process and ca. 20% decrease after 5 months aging. However, contrary to our findings, no correlation was observed between TP and RSA, which increased by 10% after heat treatment and by 30% after the storage period. To investigate the contribution of a "phenolic unit" to the RSA of both juices, we calculated the mean ratio TP:RSA and found a value of 0.0045 for IJ and 0.0032

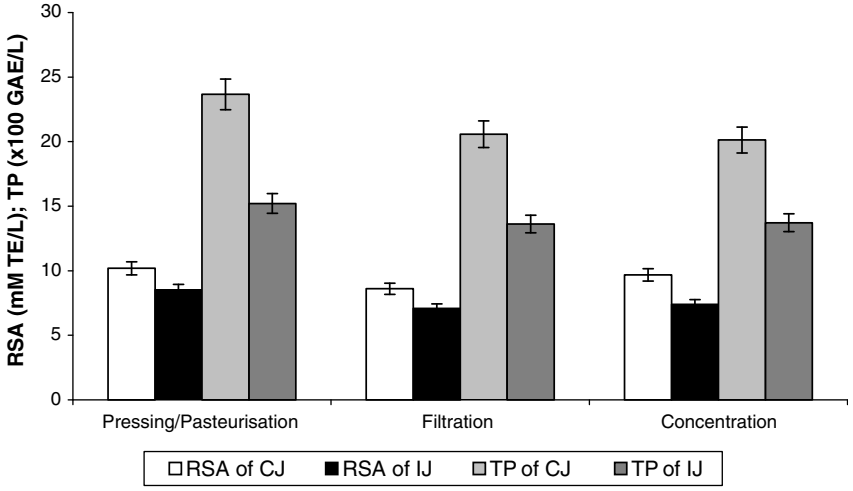


Fig. 2. Radical scavenging activity (RSA) and total phenol contents (TP) during processing of Concord (CJ) and Isabel (IJ) grape juices. Variation between duplicates <5%.

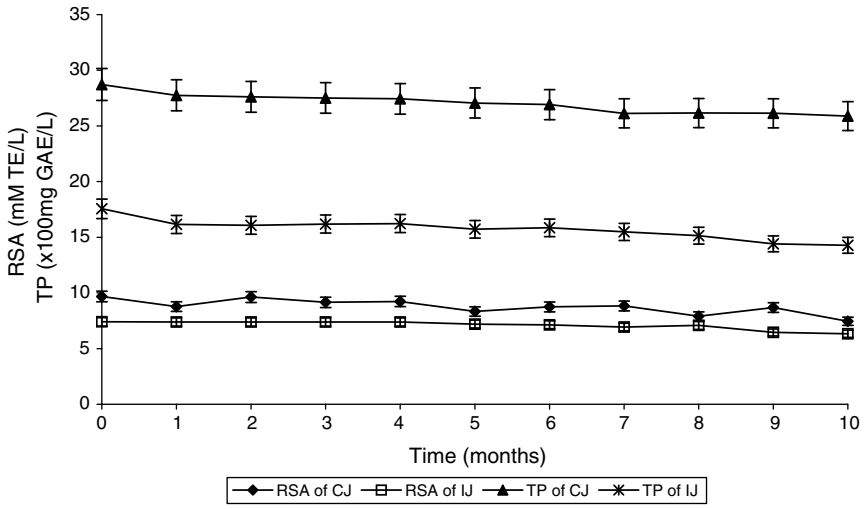


Fig. 3. Radical scavenging activity (RSA) and total phenols content (TP) of Concord (CJ) and Isabel (IJ) grape juices stored at 68° Brix and 5 °C. Variations between duplicates <5%.

for CJ. These ratios indicate therefore that specific phenolic compounds or synergy among them were relevant for the greater radical scavenging power "per unit" of IJ.

3.2. ESI(−)-MS fingerprinting

Fig. 4 shows ESI(−)-MS fingerprints of the Concord grape juices in different stages of processing and storage, which reveal significant and interesting composition changes. Given that Isabel juice demonstrated equivalent behavior, ESI-MS fingerprints of this cultivar are not shown. Note that these spectra display characteristic profiles of mainly polyphenols and eventually hexose. The ESI(−)-MS fingerprints of juice samples show characteristic distributions of mainly the following compounds tentatively identified as: dimethoxy-flavylium (DF), malvidin (M), dimer of the hexose (H) and piceatannol-*O*-glucoside (PG) detected as the deprotonated molecules of *m/z* 313, 329, 359 and 405, respectively (Table 1). Prior to heat treatment (Fig. 4A), characteristic compounds such as peonidin (P), caffeoyltartaric acid (CA) and peonidin-3-*O*-glucoside (P3G) identified as the marker ions of *m/z* 299, 311 and 461, respectively were detected. The juice composition as identified by ESI-MS changes significantly after pasteurization (Fig. 4B), being characterized by the predominance of three significant marker ions of *m/z* 313 (DF), *m/z* 329 (M) and *m/z* 359 (H), in a ratio of ca. 4:2:5. The ESI-MS fingerprint of the juice after concentration (Fig. 4C) also changes with the clear detection of new major polar components (note those detected by the anions of *m/z* 293, 457 for instance), being characterized by the predominance of five significant major ions of *m/z* 293 (unknown), *m/z* 313 (DF), *m/z* 329 (M), *m/z* 359 (H) and *m/z* 405 (PG), in a ratio of ca. 4:10:5:6:3. Fig. 4D shows that after 8 months of storage in concentrated and refrigerated conditions, the ESI-MS fingerprint of grape juice changes slightly being characterized now by two major marker ions of *m/z* 313 (DF) and *m/z* 359 (H) in a ratio of ca. 9:10. Earlier, we had identified the ions of *m/z* 313 (DF), *m/z* 329 (M), *m/z* 359 (H) as diagnostic ions for the must of six varieties of grapes (Catharino et al., 2006). The present results revealed that peonidin and peonidin-3-*O*-glucoside were the major phenolic components of grape juice before heat treatment; malvidin and dimethoxy-flavylium became most significant after pasteurization. Piceatannol-*O*-glucoside was a significant compound after the concentration step and malvidin decreased after 8 months of storage. These findings confirm that methylated anthocyanins show higher stability to oxidative and thermal conditions than glucosylated and highly hydroxylated anthocyanins (Talcott & Lee, 2002). In bottled wines, Muñoz-Espada et al. (2004) have observed the presence of several anthocyanin aglycons in higher amounts than glucosides, depending on the grape cultivar.

The ESI-MS fingerprints of Fig. 5 show similarities and some important differences between Isabel and Concord cultivars (after concentration). IJ (Fig. 5A) is characterized mainly by three

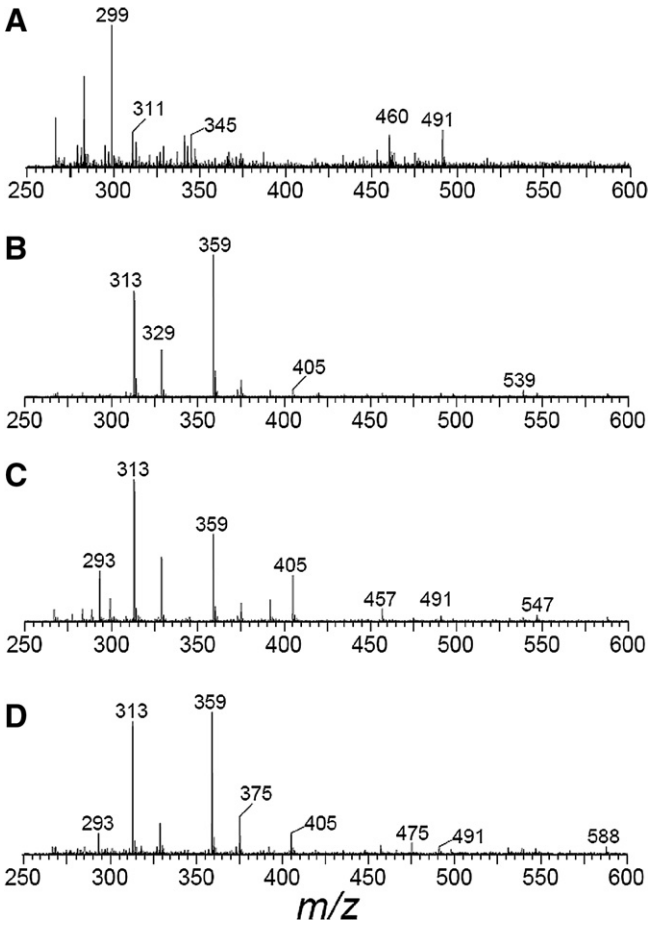


Fig. 4. ESI(−)-MS fingerprints of Concord grape juice: (A) at pressing; (B) after pasteurization and filtration; (C) after concentration; and (D) after 8 month storage.

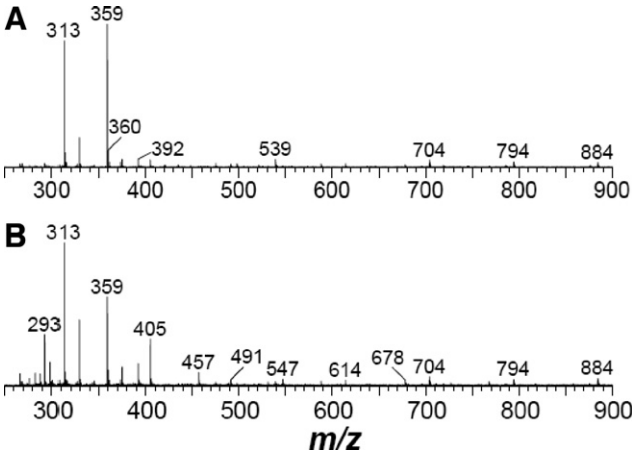


Fig. 5. ESI(−)-MS fingerprints of (A) Isabel; and (B) concord grape juices after concentration.

Table 1
Identified compounds in grape juice using ESI(−)-MS/MS

Compound	Deprotonated ions $[M-H]^-$ *m/z*	MS/MS ions *m/z*
Peonidin	299	283
Caffeoyltartaric acid	311	179
Dimethoxy-flavylium	313	295
Malvidin	329	313
Dimmer hexose	359	162
Piceatannol-*O*-glucoside	405	243
Peonidin-3-*O*-glucoside	461	299

abundant ions of *m/z* 313 (DF), *m/z* 329 (M), *m/z* 359 (H) in a ratio of ca. 9:3:10. CJ (Fig. 5B) also produces a quite characteristic ESI-MS showing the predominance of five ions of *m/z* 293 (unknown), *m/z* 313 (DF), *m/z* 329 (M), *m/z* 359 (H), and *m/z* 405 (PG) in a ratio of ca. 4:10:5:6:3. Concord juice presented higher proportion of malvidin and lower of hexose dimers than Isabel, which is in agreement to the intense colour of CJ and higher sweetness of IJ. Piceatannol glucoside, tentatively identified as a major component of CJ was found only in CJ. The substance, a tetrahydroxystilbene, is known as a potent inducer of apoptosis in many cancer cell lines (Potter et al., 2002). It is considered to be present in small amounts in foodstuffs and only recently improved methods for its quantification have been proposed (Lin, Lien, Cheng, & Ku, 2007). In our study, piceatannol glucoside appeared as a major component of grape juice after the concentration process (Fig. 4C).

4. Conclusions

The investigated grape juices revealed similar TP and RSA values, as also reported for red wine (2036 GAE and 6-12 TE, respectively) and green tea infusion (1029 GAE and 8 TE, respectively), according to Gil, Tomás-Barberán, Hess-Pierce, Holcroft, and Kader (2000). Phenolic composition of Isabel grape juice presented greater contribution to antioxidant activity, indicating that particular alterations occurred during processing and storage. Specific compositions should be taken into account when assessing the intake and the antioxidative properties of fruit beverages. The ESI-MS technique with direct infusion provided information about product compositions, particularly the bioactive components. Further studies on the presence of piceatannol glucoside in concentrated grape juice should be carried out in order to investigate the mechanisms of formation during processing. Processing and storage conditions of grape juices were shown to be effective in preserving juice quality with respect to phenolic compounds and oxidative status.

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