

Polyphenol and Resveratrol Content in Grape Juices Organically and Conventionally Produced in Southern Brazil

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Abstract

The physical and chemical composition of grape juice can vary depending on the type of cultivar, variety, climate, soil and other factors. Due these variations, a mixture of species (blend) is used for the preparation of juices as an alternative to maintain the balance between soluble solids and acidity, as well as visual aspects (color). Grape juice has a high concentration of phenolic compounds that are responsible for different biological activities, including the antioxidant activity. The objective of this study was to quantifying the total polyphenol and resveratrol content in the juices produced from the mixture of varieties of organic grapes and conventional grapes, as well as with respect to the expiration date. Quantification of phenolic compounds and resveratrol was carried out by spectrophotometry and high-performance liquid chromatography (HPLC), respectively. The content of phenolic compounds in juices produced from the blend were twice as large compared to the polyphenol content in the juice produced only with Isabel grape. Resveratrol content resulted in a decrease with increasing storage time, indicating a possible degradation over these one. However, the content of resveratrol may be a delay control indicative of validity grape juices. The mix of species for organic grape juice production is very interesting to obtain a differentiated product content of phenolic compounds, which considered as sources of compounds with biological activity.

Keywords: phenolic compounds, organic farming, expiration date, liquid chromatography, spectrophotometry.

Introduction

There is an annual increase in production and marketing of Brazilian grape juice due to its typicality, quality and nutritional value. Most viticulture region of Brazil is located in the *Serra Gaucha* and more than 80 % of the total grape production are American and hybrid varieties (Rizzon and Miele, 2012). Grape juice is an important source of flavonoids, stilbenes and proanthocyanidins bioactive compounds. The most important are the flavonoids, as anthocyanins (responsible for the colors) and flavonoids, stilbenes that stands out resveratrol, phenolic acids and a wide variety of tannins. Grape derivate products, such as juice, play functions as nutritional and healthy food, that impulsion a growing number of researches focusing biological activity and nutraceutical properties (Georgiev et al, 2014). It is noteworthy that the amount and type of phenolic compounds of grape juice may not be the same that the fresh grape, considering the post-harvest processes such as storage and production of grape juice (Pérez-Magariño and González-Sanjosé, 2004). Even so, the physicochemical composition of grape juice can vary depending on the type of cultivar, variety, climate, soil and other factors such as genetic backgrounds (Freitas et al, 2009; Zhang et al, 2011). Due these variations, a mixture of species (blend) is used as alternative for preparation of juices to maintain balance mainly between soluble solids and acidity, as well as visual aspects (color). Grape juice can be made with any cultivated variety, since that is at a suitable maturation and submitted at adequate sanitary conditions. The variety destined to preparation of juices should necessarily to have features such as a good yield in wine, proper relationship sugar / acidity, aroma, pleasant, defined flavor and ideal conditions for maturation and sanity (Pérez-Magariño and González-Sanjosé, 2004) In the choice of cultivar for juice processing the profile of the consumers must be considered, which changes according to the region. In Brazil, grape juices are produced mainly with grapes of American hybrid group, especially Bordô, Concord and Isabel.

In this study, we adopted a winery in *Serra Gaucha* in southern Brazil as a reference. Interviews were conducted with the winemaker and producers in order to better understand the production process of organic and conventional grape juice. The process of grape juice production in this factory occurs by heat-maceration and enzymatic extraction. Thus, results of this interview led the goal of this study in determining the content of total polyphenols and resveratrol in organic and conventional grape juice by UV/Vis Spectrophotometry and High Performance Liquid Chromatography (HPLC). It were analyzed four lots of juices and among these, two from organic grapes, regarding the period of storage and two from conventional grape, one of them produced with only Isabel species. The other lot, were produced by mixing Bordô, Concord and Isabel species (blend).

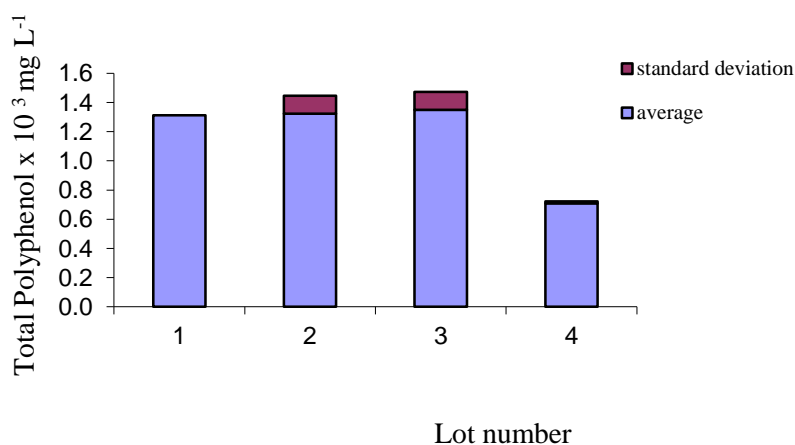
Materials and Methods

Four lots of grape juice were collected in the winery factory. Three among these (Lot 1 and Lot 2), were produced with mixtures of species Bordô, Isabel and Concord. All organically produced, however, Lot 1 with the validity period expired. Lot 3 and Lot 4 were produced with grapes grown conventionally. Lot 4 was produced only with the Isabel specie. It was not added water in juices. Polyphenols concentration, expressed in milligram per liter of gallic acid equivalent (mg GAE L^{-1}), was determined by colorimetric method based on Singleton and Rossi (1965). Calibration curve was prepared with dilutions of 5000 mg L^{-1} gallic acid stock solution in the following concentrations: 0.5; 1.0; 1.5; 2.5 and 5.0 mg L^{-1} . The standards contained 50 % Folin-Ciocalteu reagent (1:9) and 40 % of Na_2CO_3 7.5 %. All solutions were stabilized in water bath at 50°C for 5 minutes. Juices samples were prepared in triplicate with the same reagents of the analytical curve, containing 10 % of diluted grape juice (1:10) and further diluted 1:9 with deionized water. Absorbance data were obtained by a spectrophotometer UV-Vis at 765 nm. Similarly, analyzes to quantify the resveratrol in these samples were carried out by liquid chromatography (HPLC). Chromatographic analyzes were carried out in high-performance liquid chromatography (HPLC) with diode array detector (DAD) and column CLC-ODS (M) MMID $4.6 \times 15 \text{ cm}$. Mobile phase A:B, 75:25. A: milliQ H_2O + 0.1% phosphoric acid B: Acetonitrile. To quantify the content of resveratrol, a calibration curve was performed with standard addition in resveratrol $\geq 99\%$ (GC) and the peak area was evaluated.

Results and Discussion

The content of phenolic compounds in the blend juices samples were within the range obtained by Mullen et al (2007). Analytical curve shows a linear correlation coefficient of 0.9997 and the data obtained by spectrophotometry were plotted in content of polyphenols x Lot n (Figure 1).

Figure 1. Content of total polyphenols in four Lots analyzed by Spectrophotometry at 760 nanometer.



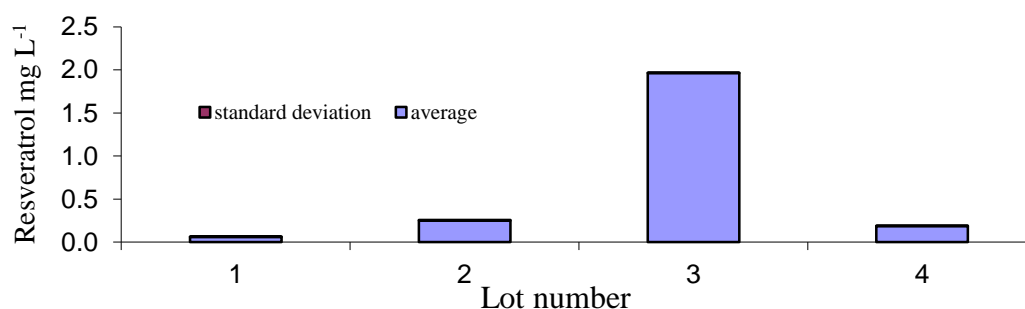
Polyphenols contents covers a range of $1350 \pm 122 \text{ mg L}^{-1}$ for conventional grape blend juice, meaning twice as compared with the juice produced only with Isabel grape, which covers a range of $709 \pm 14 \text{ mg L}^{-1}$, according to

Table 1A.

Lot number	(A) Polyphenols (mg L^{-1}) \pm SD	(B) Resveratrol (mg L^{-1}) \pm SD
1	1313 ± 1	$0,051 \pm 0,001$
2	1324 ± 121	$0,181 \pm 0,002$
3	1350 ± 122	$1,071 \pm 0,016$
4	709 ± 14	$0,152 \pm 0,001$

Table 1: Average content in organic and conventional grape juices. (A) Polyphenols and (B) Resveratrol. Polyphenol contents, obtained by HPLC, showed a greater variation in different Lots compared to the results of total polyphenol contents found by spectrophotometry. For Lot 1 (blend juice with organic grape) the results showed a resveratrol content range of $0.051 \pm 0.001 \text{ mg L}^{-1}$, meantime, the blend juice produced by conventional grape (Lot 3) showed a content of twenty times greater, $1.071 \pm 0.016 \text{ mg L}^{-1}$, as shown in Table 1B. These results are within the range obtained by Souto et al (2001). The low content of resveratrol founded in Lot 1 may be associated to the period of validity, which was more than two years expired. This highlights that resveratrol is degraded with respect to the storage time. Lot 3 showed a higher content in polyphenols, as viewed in Figure 2.

Figure 2. Results of resveratrol content in grape juice by high-performance liquid chromatography (HPLC) whit diode array detector (DAD), CLC-ODS column (M) 4.6 MMID x 15cm and mobile phase A: B, 75:25. A: milliQ H_2O + 0.1 % phosphoric acid B: Acetonitrile.



This plot was within the expiration date, but produced three months after the Lot 2. They are within the validity period. Probably ripest grapes were utilized for production of this juice, once that in southern Brazil, grape cultivation takes place in January and February, and consequently this possible variation of resveratrol content. The content of resveratrol in Isabel juice is lesser. However, it follows the same behavior regarding the content of polyphenols, shown in Figure 1.

There was no information on the label about the variety and crop used in production of these juices. According to interview with the Winemaker of the winery, grape juices are processed with different varieties. The ratios are not observed and this may be a strongly factor related to the change in the total polyphenol content, and consequently resveratrol, from one lot to another. Furthermore, we cannot ignore the fact that during the vacuum application by Flash Détente process, losses may occur in content of phenolic compounds, mainly due to high temperature ($90 \text{ }^\circ\text{C}$) in which the juice is subjected (Lee et al, 2014; Genova et al, 2016). The mix of species for grape juice production is very interesting to obtain a differentiated product content of phenolic compounds, which can be consider as sources of compounds with

biological activity. The shelf life may influence the decrease of the content of resveratrol, although this trend, not observed on the content of polyphenols, indicating a possible restructuring of resveratrol molecule in other phenolic compounds. The resveratrol content can be an indicative of control of validity of grape juice, but more results are needed to confirm a possible mechanism for their degradation.

Acknowledgments

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