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Physicochemical composition, minerals, and pesticide residues in organic grape juices

Alberto MIELE^{1*}, Luiz Antenor RIZZON¹, Sonia Claudia do Nascimento de QUEIROZ², Clésio GIANELLO³

Abstract

Demand for organic products is intensified in many countries each year. Following this trend, Brazil produces increasing volumes of organic grape juice. In this way, a survey of organic grape juices made from grapes produced according to this system was carried out where physicochemical composition, minerals, trace elements, and pesticide residues were determined. Variables related to grape juice composition were performed by physicochemical procedures; minerals and trace elements, by inductively plasma optical emission spectrometry; pesticide residues, by liquid chromatography-mass spectrometry. Main results show that the physicochemical composition of organic grape juices was in general in accordance to the Brazilian legislation. The mean concentrations of trace elements were very low, varying from 0.002 (Cd) to 0.970 (Ba) mg L⁻¹. Pesticide residues were not detected in any sample analyzed (MRL= 10 µg L⁻¹). These results show that the Serra Gaúcha viticultural region present conditions to produce organic grape juices, despite the adverse climate factors that occurs in some years. Nevertheless, these products should be made with grape varieties, such as the labrusca ones, less susceptibles to the main grapevine pathogens.

Keywords: organic viticulture; agrobiolgy; minerals; heavy metals.

Practical Application: This survey allows knowing the presence of heavy metals and pesticides in organic grape juice.

1 Introduction

The development of science and technology in the last decades had considerable effects on the increasing production and productivity of most crops. To achieve this goal, there was the contribution of many areas especially since the beginning of the 20th century. However, sometimes this development was followed by negative impacts to the environment and to the human being, directly or indirectly. For this, many international institutions, officials or not, are acting today to mitigate the negative impact that some technological practices have on the environment.

Among these institutions, there are many organizations spread all over the world related to agrobiolgy. In Brazil, there is also a considerable demand for organic products, what can be seen by the increase of commercialized vegetables, fruits, and crops, estimated from 15% to 20% a year. These products are supplied by 90 thousand farms where 85% are small ones practicing family farming. Regarding the grape and wine chain, there is also an increasing demand for organic grapes. Indeed, there is a considerable number of grape growers and winemakers around the world with the commitment to produce organic products.

The international bibliography concerning the production of organic grapes covers different aspects of this field, such as those related to a) minimize the use and effect of copper application (Dagostin et al., 2011); b) search alternative products for different diseases (Crisp et al., 2006); c) control nematodes (Coll et al., 2012); d) fertilization and mineral nutrition

(Coll et al., 2011); e) the impact and evaluation of the organic viticulture (Reeve et al., 2005; Fragoulis et al., 2009).

Serra Gaúcha, the most important Brazilian viticultural region was also influenced by this scientific and technological development with the use of different agricultural inputs – such as fungicides, insecticides, herbicides, growth regulators, fertilizers –, and enological – such as yeasts, enzymes, stabilizers, antioxidants. This region is humid and hot during the summer time, climatic conditions that promote the development of diseases caused by a series of fungi species. Among the most important ones are antracnose, downy mildew, *Botrytis*, and grape ripe rot that should be controlled by specific fungicides. The application of these products, and other pesticides used in vineyards, can result in residues on grape and grape juice. In addition, it is necessary to emphasize the possibility to cause problems to the environment – air, soil, and groundwater contamination, – and to the human being. However, it is important to consider that organic products could also have residues of undesirable substances used in vineyards. For example, copper content in the soil and in grape juice must be low because it is a trace element and component of the Bordeaux mixture, the main fungicide used to control diseases in vineyards conducted according to the organic system in Brazil.

However, researches related to organic grape juices are relatively scarce. In general, most works on grape juices emphasizes composition related to sugar, acidity, and resveratrol (Freitas et al., 2010) and phenolic compounds (Dani et al., 2007).

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Considering the increase in consciousness of the conditions of life on Earth and of the health problems caused by pesticides in many grape growers, a group of viticulturists of Serra Gaúcha is adopting organic procedures in their vineyards. In this way, some organic grapes are directed to the market to be consumed as table grapes and another part goes to the industry to make wine, sparkling wine, vinegar, and jelly. However, most of them go to the grape juice industry to cope with the increasing demand for organic juices.

Despite the approval of these products by certified organizations, there was a demand to carry out a survey to determine the physicochemical composition of grape juices from the Serra Gaúcha viticultural region made with grapes produced according to the organic principles, as well as to determine the concentration of minerals, trace elements, and the presence or not of residues of pesticides.

2 Materials and methods

The work was made on 27 samples of organic grape juices, which were taken from wineries of the Serra Gaúcha viticultural region. At that time, they represented the majority of organic grape juices made in this region and were made with different cultivars, where those from the labrusca group predominated, such as Isabella (n=13), Ives (local name is Bordô) (n=7), Concord (n=2), White Niagara (n=2), Rosy Niagara (n=1), Goethe (n=1), and Muscat Bailey (n=1).

Five bottles of each sample were acquired in markets or wineries of this region and analyses were related to physicochemical composition, concentration of minerals and trace elements, and presence or not of residues of pesticides.

2.1 Physicochemical

In grape juices, the following variables were evaluated in duplicate: titratable acidity, volatile acidity, Brix, pH, reducing sugars, Brix/titratable acidity ratio, absorbance at 420 nm, 520 nm, 620 nm, color intensity, hue, total polyphenols index, anthocyanins, and tannins. These variables were determined by physicochemical methods (Ribéreau-Gayon et al., 1982); anthocyanins, by pH difference; tannins, by acid hydrolysis; absorbance at 420 and 520 nm, by UV/VIS spectrophotometry using a 1-mm path length cell and at 620 nm using a 10-mm path length cell (Ribéreau-Gayon & Stonestreet, 1965, 1966).

2.2 Minerals and trace elements

The minerals and trace elements evaluated in grape juices and wines were phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), sodium (Na), aluminum (Al), cadmium (Cd), chromium (Cr), nickel (Ni), lead (Pb), molybdenum (Mo), cobalt (Co), arsenic (As), selenium (Se), tin (Sn), barium (Ba), and vanadium (V).

The determination of these elements was performed by a nitric acid-perchloric digestion of samples using a specific POP of the Laboratório de Solos, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul. Analyses were done

by an inductively coupled plasma optical emission spectrometer (Franson, 1995).

2.3 Pesticide residues

The following pesticides were analyzed in grape juices in the Laboratório de Resíduos e Contaminantes of Embrapa Meio Ambiente, in Jaguariúna (SP): a) fungicides – azoxystrobin, cyproconazole, difenoconazole, fenarimol, myclobutanil, pyraclostrobin, tebuconazole, thiophanate-methyl, and triadimefon; b) insecticides – fenthion, carbaryl, and fenitrothion; c) herbicides – simazine and diuron.

The method used was the QuEChERS (Anastassiades et al., 2003). A short description is as follows: 10 g of sample were weighed into a 50 mL centrifuge tube, 10 mL acetonitrile was added and the tube was closed and shaken for 1 min. After that a mixture of 4 g of magnesium sulfate anhydrous, 1 g of sodium chloride, 1 g of trisodium citrate dehydrate and 0.5 g of disodium hydrogencitrate sesquihydrate were added. The tube was closed and shaken and centrifuged. Then, 1 mL was taken, the solvent evaporated and resuspended in mobile phase. The extract was filtered and injected in a LC-MS/MS.

The pesticides were analyzed using a Varian 1200L LC-MS/MS (triplequadrupole) equipped with an ESI source. The column used was a Phenomenex RP 80, 4.6 mm x 150 mm, 4 µm. The separation in the analysis of grape juice samples was performed by means of a linear gradient elution (eluent A, methanol; eluent B, acetic acid 0.1%). The gradient was as follows: 60% A for 1 min, 60-70% A in 2 min, 70% A for 37 min, 70-60% A in 3 min, 60% A for 5 min. The volume of injection was 20 µL and the flow rate was 0.4 mL min⁻¹. Detection and quantification of the pesticides by the mass spectrometer was performed using two transitions.

Data of the grape juices physicochemical and mineral contents show maximum, minimum, mean values, and standard deviation. Mean values of the physicochemical and mineral composition were submitted to the Principal Component Analysis (PCA).

3 Results and discussion

Results of the Brazilian organic grape juices, which are related to physicochemical composition, minerals, trace elements, and pesticide residues, are shown in Tables 1 to 3.

3.1 Physicochemical composition

Considering the mean values of the grape juices evaluated, all variables had parameters (Table 1) in accordance to the Brazilian legislation. Some results, such as those related to sugar and acidity, varied considerably in certain cases. In this way, Brix varied 4.8 and titratable acidity 96 meq L⁻¹ between the lowest and the highest values, consequently the difference for the Brix/titratable acidity ratio was 31. The mean of 22.1 for this variable could be considered a very nice value for Brazilian consumers. However, some people prefer sweeter grape juices, but others less sweet ones or slightly acid. If the maximum and minimum

Table 1. Data of the physicochemical composition of grape juices made with grapes (*Vitis* spp.) produced according to the organic system.

Variable	Grape juice			Standard deviation
	Maximum	Minimum	Mean	
Density at 20°C (g mL ⁻¹)	1.0717	1.0510	1.0619	0.0060
Titrateable acidity (meq L ⁻¹)	144	48	96	23
Volatile acidity (meq L ⁻¹)	8	2	3	2
Brix	17.5	12.7	15.0	1.4
pH	3.62	3.17	3.36	0.12
Reducing sugars (g L ⁻¹)	154	94	127	19
Brix/Titrateable acidity	45.8	14.4	22.1	6.8
Absorbance at 420 nm	1.150	0.192	0.568	0.280
Absorbance at 520 nm	2.100	0.095	0.780	0.552
Absorbance at 620 nm	0.524	0.008	0.201	0.151
Color intensity	3.737	0.338	1.548	0.969
Hue	0.55	2.02	0.73	0.51
Total polyphenols index (I280)	126.5	32.7	64.5	26.5
Anthocyanins (mg L ⁻¹)	801.2	22.4	305.9	256.2

Table 2. Data of minerals in grape juices made with grapes (*Vitis* spp.) produced according to the organic system.

Variable (mg L ⁻¹)	Grape juice			Standard deviation
	Maximum	Minimum	Mean	
P	212.6	47.0	114.7	32.5
K	2792	895	1436	388
Ca	114.6	45.4	80.6	18.2
Mg	103.3	39.1	77.7	15.5
S	55.5	26.0	40.8	8.1
Cu	12.38	0.23	2.91	3.00
Zn	1.01	0.23	0.51	0.20
Fe	4.75	0.64	2.15	1.10
Mn	2.85	0.39	1.25	0.63
Na	11.67	1.07	2.33	2.00
Al	1.66	0.12	0.53	0.39

Table 3. Data of trace elements in grape juices made from grapes (*Vitis* spp.) produced according to the organic system.

Variable (mg L ⁻¹)	Grape juice		
	Maximum	Minimum	Mean
Cd	0.003	nd	0.002
Cr	0.037	0.001	0.023
Ni	0.082	nd	0.016
Pb	0.033	<0.02	0.020
Mo	0.023	<0.002	0.009
Co	0.007	0.003	0.005
As	0.138	<0.020	0.025
Se	<0.030	0.020	0.029
Sn	0.12	0.01	0.06
Ba	2.70	0.31	0.97
V	0.003	<0.003	0.010

nd= not detected.

values are considered, there are variables with parameters that are not in accordance to the Brazilian legislation.

Bibliography about organic grape juices is scarce. Nonetheless, there are some researches comparing the physicochemical composition of conventional and organic

products. Freitas et al. (2010), working with Concord and Rúbea grape juices, found contents below or above the values specified by the Brazilian legislation, depending on the system used, conventional or organic ones. These authors found that Brix and Brix/titrateable acidity ratio were higher for both juices in the organic system, but for titrateable acidity it was higher in the organic system for Concord and in the conventional for Rúbea. These authors found that the Concord and Rúbea conventional grape juices had higher concentrations (+32%) of *trans*-resveratrol but there was no considerable difference between both cultivars. However, opposite results were found by Dani et al. (2007), where they concluded that organic Ives grape juice had higher values for total phenolic compounds. These authors also showed that organic products presented higher levels of total anthocyanins, among them cyanidin, delphinidin, peonidin, and malvidin, and lower of catechin and epicatechin. In addition, they showed that the composition of organic grape juices in phenolic compounds lead them to a considerable antioxidant activity, which is an important point for the human health.

Because results concerning the composition of organic grape juices are so few, some data of conventional Brazilian products are compared with them. In general, results of the

present research are in accordance to those where 49 whole grape juices and four reprocessed ones were evaluated, where some samples did not have parameters of Brix, density, and titratable acidity according to the Brazilian legislation (Rizzon & Miele, 1995). Homemade juices from Isabella, Concord, Ives, and Cabernet Sauvignon showed parameters varying according to the cultivar, where juice of Cabernet Sauvignon, a *Vitis vinifera* variety, had lower concentration of methanol (Rizzon & Link, 2006). In another work, 24 grape juices and derivative products – whole, sweetened, reprocessed, nectar, and beverage – were analyzed and results show that whole and reprocessed juices had the highest values for all physicochemical variables evaluated (Rizzon & Miele, 2012). Three grape juice samples from no Brazilian traditional-producer regions – Sudeste and Centro-Oeste – showed differences among them and some parameters that are not in accordance to the Brazilian legislation (Santana et al., 2008).

Another important point to be considered is related to the phenolic compounds, such as color intensity, hue, anthocyanins, and total polyphenols index. There was a considerable variation among the parameters found, which were mainly due to the characteristics of the white, rosé, and red grape juices. Regarding red cultivars, the values of the absorbance in general were high, which was due to cultivars having high anthocyanin synthesis. The highest value for the absorbance at 420 nm was found in white grape juices. The absorbance at 280 nm, which is related to the total polyphenols, was also very high – 126.5 – because it represents about 7 g L⁻¹ of tannins, what is not normal for a grape juice. A priori, this result could be due to the cultivar or mainly to the technological process used. Indeed, some practices could increase the absorbance at 280 nm, such as berry grinding – especially the peel – maceration time, high temperature, and enological products used.

As early as in the 1970s decade, Pezzi & Fenochio (1976) evaluated 12 commercially available grape juices from the south of Brazil, which represented almost the totality of juices

produced in the country at that time. They concluded that 16.7% of them had Fe concentrations above 15 mg L⁻¹ and 41.7% SO₂ higher than 200 mg L⁻¹, maximum concentrations then permitted. Much later, evaluating conventional grape juices from the State of Rio Grande do Sul, Rizzon & Miele (1995) showed that the absorbance at 520 nm varied from 0.268 to 0.734 (mean of 0.407) and anthocyanins from 21.0 to 380.0 mg L⁻¹ (mean of 144.3 mg L⁻¹). In a more detailed work, Rizzon and Miele (2012) compared the composition in phenolic compounds of grape products, whose results showed that whole, reprocessed, and sweetened grape juices had higher values for the nine variables of phenolic compounds evaluated than grape nectar and grape beverage. Comparing these results with those of the present work it is shown that they are lower for most variables, i.e., absorbance at 420 nm, 520 nm, and 620 nm, anthocyanins, color intensity, and total polyphenols index, but higher hue. The differences between both works were due to some factors, such as grape cultivars, climatic conditions, and enological practices used during grape juice elaboration. Analyzing 20 red grape juices, simple and reconstituted, Malacrida & Motta (2005) found concentrations of total phenolic compounds varying from 0.27 to 2.41 g L⁻¹ and of anthocyanins from 1.17 to 66.8 mg L⁻¹. They still showed that anthocyanins degradation was higher with the reconstituted juices. Studying the effect of the heater system in homemade Isabella and Ives grape juices, it was showed that the later had higher contents of phenolic compounds than Isabella, no matter the heater system used. However, the concentration of resveratrol was dependent on the heater system used (Marcon et al., 2013). This substance, an important antioxidant, was already detected in Brazilian grape juices in the concentrations varying from 0.19 to 0.90 mg L⁻¹ in both *cis* and *trans*-resveratrol (Sautter et al., 2005).

Data of the 27 organic grape juices submitted to the PCA (Figure 1) show that PC1 discriminated Ives, Rosy Niagara, and Goethe grape juices; PC2, White Niagara; and PC3, Concord. Juices of Isabella and Muscat Bailey did not show discrimination.

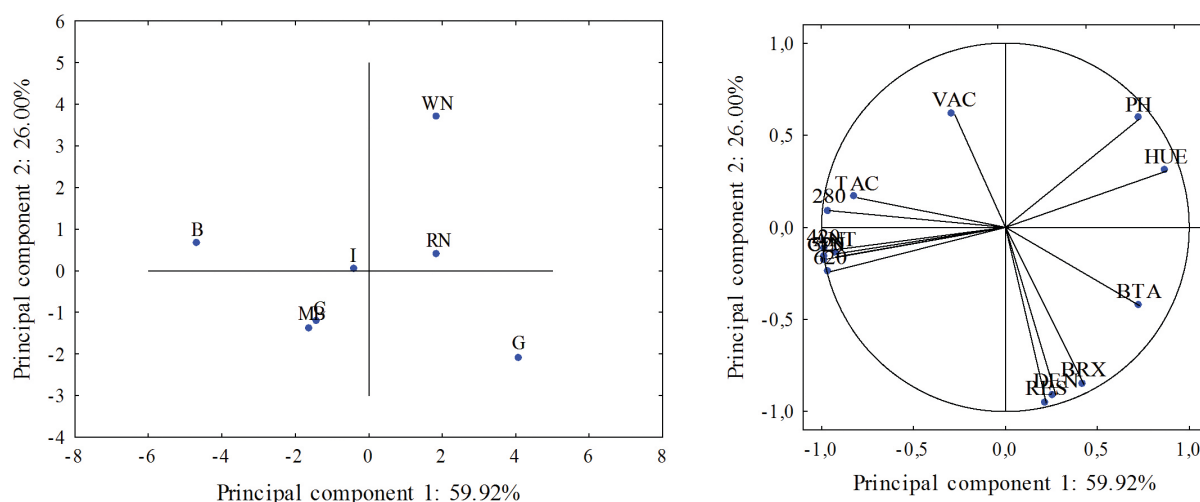


Figure 1. Projection of grape juices and physicochemical variables on the principal components 1 x 2. Legend: C= Concord; G= Goethe; I= Isabella; IV= Ives; MB= Muscat Bailey; RN= Rosy Niagara; WN= White Niagara; DEN= density; TAC= titratable acidity, VAC= volatile acidity, BRX= Brix, PH= pH, RES= reducing sugars, BTA= Brix/titratable acidity, 420= absorbance at 420 nm, 520= absorbance at 520 nm, 620= absorbance at 620 nm, CIN= color intensity, HUE= hue, ANT= anthocyanins, TPI= total polyphenols index.

Ives juice was mainly characterized by high values of substances related to phenolic compounds, such as absorbance at 420 nm, 520 nm, and 620 nm, anthocyanins, color intensity, and total polyphenols index, but low pH and Brix/titratable acidity ratio; Goethe, by high Brix and residual sugars and low volatile acidity; Rosy Niagara, by high volatile acidity and low density, Brix, and residual sugars; Concord, by high volatile acidity too, titratable acidity, absorbance at 420 nm and 520 nm, and low Brix/titratable acidity ratio.

White Niagara and Goethe juices did not show concentrations of anthocyanins and very small contents of these pigments were detected in Rosy Niagara juice. Furthermore, the four red cultivars – Isabella, Ives, Concord, and Muscat Bailey – showed, as expected, hue values smaller than 1, and those from the white cultivars – White Niagara – and rosy – Goethe and Rosy Niagara – greater than 1. This result was due to the fact that red juices had higher values of absorbance at 520 nm (red color) than at 420 nm (yellow color), however white ones showed inverse results.

3.2 Minerals

Macronutrient concentrations (Table 2) in grape juices in general had the same order of importance of those mentioned in literature (Rizzon & Link, 2006), which means that K predominated followed by P, Ca, Mg, and S. In relation to the micronutrients, the concentration of Mn, a raw material of some synthetic fungicides, which can not be used in organic systems, was also low. This means that growers apparently used good ecological practices during grape growing, at least for fungicides having Mn in their composition.

Another element detected in high concentration was K, a cation present in the highest concentration in most juice of grapes. This was probably due to the process used to make grape juice which extracts more K, especially from the peel. Other two elements present in somewhat high concentrations were those of P and Na. The last one was probably derived from products

used during the process. Although Brazilian legislation does not specify the limit of Na concentration, it is important that grape juices contain low contents of Na and appreciable levels of K. Copper had the highest concentration, which can be supplied by the soil and especially by sprays of the Bordeaux mixture, the main fungicide used by organic growers. However, it is important to emphasize that the mean concentrations of this cation was low. This is an important point because this fungicide is fundamental for the organic grape production considering the humid and hot conditions of Serra Gaúcha. However, the maximum value observed was more than two times greater than 5 mg L^{-1} , the limit permitted by the Brazilian legislation. It seems to be the most important element to be worried about in the Serra Gaúcha viticultural region. Indeed, it is largely used as an important component of a fungicide, where it can be present on the raisin surface and, consequently, in the grape juice where it does not go to precipitation such as occurs in wines. Besides, when sprayed on the grapevines it falls on the soil and remains there for long time because it is non-degradable and can contaminate groundwater sources. To overcome, or at least to minimize this negative effect, there are some researches that focused on the biologic control (Schmid et al., 2011), alternative products to copper (Dagostin et al., 2011), and models to use copper in the vineyards (Kuflik et al., 2009).

Data of the mineral composition submitted to the PCA (Figure 2) show that the three most important components of the PCA discriminated the juice of White Niagara, with low concentrations of Ca, Mg, Cu, and Fe; PC2, juices of Ives and Rosy Niagara, with high values of P and S, and low of Zn and Al; PC3, juices of Concord and Muscat Bailey, the first being characterized by high concentrations of Na and the second one by high levels of K.

3.3 Trace elements

The Brazilian legislation specifies the Maximum Tolerance Limits for the following trace elements in grape juice, in ppm: As (0.50), Cd (0.50), Cr (0.10), Cu (30.00), Hg (0.01), Ni (3.00),

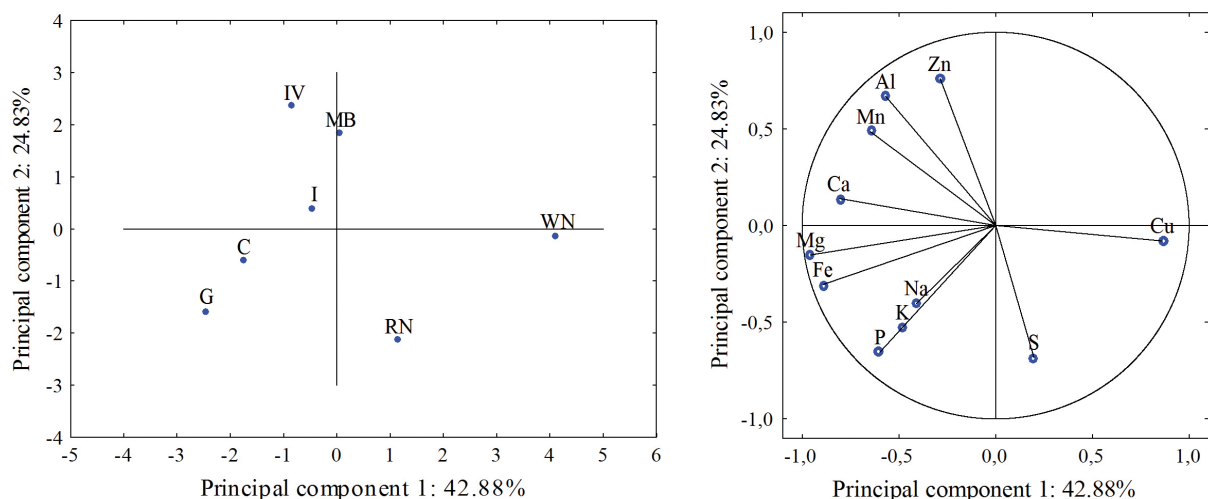


Figure 2. Projection of grape juices and minerals on the principal components 1 x 2. *Legend:* C= Concord; G= Goethe; I= Isabella; IV= Ives; MB= Muscat Bailey; RN= Rosy Niagara; WN= White Niagara; P= phosphorus, K= potassium, Ca= calcium, Mg= magnesium, S= sulfur, Cu= copper, Zn= zinc, Fe= iron, Mn= manganese, Na= sodium, Al= aluminum.

Pb (0.40), Sb (1.00), Se (0.05), Sn (250.00), and Zn (25.00) (Brasil, 1965). The trace elements detected in the present work had much lower concentrations than those mentioned by the Brazilian Health Ministry. From the 11 trace elements evaluated in this work, the minimum mean concentration was found with Cd and the maximum mean with Ba (Table 3).

Prior works showed that Brazilian grape juice had low levels of trace elements. One of them evaluated Cd, Cr, Ni, and Pb in four grape juices from the State of Rio Grande do Sul (Mirlean et al., 2005) and another one, 20 Brazilian grape juices which analyzed nine trace elements, i.e., As, Cd, Cr, Cu, Na, Ni, Pb, Sb, Zn (Assis et al., 2008).

3.4 Residues of pesticides

From the 16 active ingredients evaluated, exception to simazin and diuron, 14 are commonly used in the Brazilian vineyards, i.e., azoxystrobin, ciproconazol, difenoconazole, fenarimol, mancozeb, miclobutanil, piraclostrobin, tebuconazole, thyophanate-methyl, and triadimefon; b) insecticides – fention and fenitroriona; c) herbicides – glyphosate and simazin. However, residues of these pesticides (LMR= 10 µg kg⁻¹) were not detected in any of the 27 grape juices.

4 Conclusions

Mean values of the grape juices physicochemical composition are within the limits described in the Brazilian legislation, although some maximum or minimum parameters are outside these limits. The concentrations of the trace elements analyzed are very low and residues of pesticides are not detected. In this way, despite the adverse climatic conditions during the grapevine vegetative cycle, which in general lead to grape infections by fungi diseases and reduction of vineyards yield, it is possible to make organic grape juice in Serra Gaúcha with good quality. For this, labrusca type varieties are recommended because they are less susceptible to the most important diseases occurring in this region. Among them it should be mentioned Ives and Concord, which are adapted to the conditions of this region and their juices present organoleptic characteristics accepted by consumers. In addition, some grape cultivars released by Embrapa Uva e Vinho can still be used with this purpose.

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