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Phlorizin and sorbitol in *Vitis labrusca* grape juices

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Abstract. In Brazil, grape juice is a not fermented beverage, made from *Vitis labrusca* varieties and their hybrids. The most common form of adulteration is by the addition of apple juice. The adulterated samples can be identified by specific analysis, since apple juice has some compounds that grape has not. A more simplified and assessible way to determine this kind of adulteration is the phlorizin analysis by high performance liquid chromatography (HPLC). Phlorizin is a phenolic compound that has been used to identify adulterations in juices. Besides phlorizin, it can be seen that adulteration by quantifying the levels of sorbitol, present in apples, but absent in healthy grapes. The content of this compounds in grape juices can vary due to the variety of grape and harvest.This study aimed to analyze phlorizin and sorbitol, in 141 experimental samples of *Vitis labrusca* grape juices, harvest 2016, of 9 varieties and 43 commercial samples (different vintages). The experimental juices from red grapes showed higher sorbitol content than the white grapes. For sorbitol analysis, the juices of Ives differed statistically of the juices of: Isabella, Concord, Niagara Branca and White Muscat. It was detected the presence of apple juice in 5 commercial grape juices.

1. Introduction

In Brazil, grape juice is the unfermented drink obtained from simple must, sulfited or concentrated, from healthy, fresh and ripe grapes [1]. The consumption of this product, in Brazil, has increased significantly in recent years due to the beneficial health effects showed by products derived from grape. The production of grape juice in Brazil is located primarily in the region of Serra Gaúcha, in southern Brazil. Among the cultivars used for producing grape juice in Brazil, three of the species *Vitis labrusca* are the most important: Concord, Isabella and Ives [2].

The determination of the quality and authenticity of fruit juices has a significant impact on the industry in terms of food safety and consumer protection [3,4]. The most common form of tampering in non-alcoholic fruit beverages is by substituting with another juice of lower commercial value [5].

Among the temperate fruits cultivated in Brazil, apple has shown a great increase in production in recent years. It is mainly directed to the fresh market, but a lot is industrially processed as juices, ciders, jellies, and dried products [6]. Juice is a by-product to be considered since it is an important alternative due to the availability of apples of low commercial value [7], whose total volume increases year after year [8]. The main cultivars grown in Brazil are Gala and Fuji [9].

Different compounds can be useful for identifying adulteration in fruit juices and other beverages, amongst which the phenolic compounds and polyols, which are potentially useful due to their specificity. Phlorizin is a phenolic compound that has been widely used for identifying adulteration of fruit juices and other products [10]. It represents more than 90% of the water-soluble phenolic compounds found in apples. It is found in more than thirty plant families; however, apple has higher amounts when compared to other fruits [11–13].

Polyols are compounds that contribute to the sweetness of musts and wines. Sorbitol, also called glucitol, is the main polyol produced by the Rosaceae family [14], being present in various fruits (apple, pear, cherry, plum, peach, and melon) and normally absent in healthy grapes [15–17]. It is classified among the carbohydrates, since it is an alcohol resulting from the metabolism of sugars, and it is a sweetener [18].

Currently, in our knowledge, there are no studies on the content of phlorizin and sorbitol in grape juices of varieties *Vitis labrusca* and hybrids. Thus, this study aimed to analyze phlorizin and sorbitol, by high-performance liquid chromatography (HPLC) in 141 experimental samples of *Vitis labrusca* grape juices of the harvest 2016 of 9 varieties and 43 commercial samples, from different vintages.

2. Material and methods

2.1. Samples

2.1.1. Experimental samples

One hundred forty-one experimental grape juices from the cultivars Isabella (40), Ives (35), Concord (16), Niagara Rosada (5), Isabel precoce (4), BRS Cora (2), Niagara

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Branca (23), White Muscat (11) and Lorena (5), harvest 2016, were elaborated experimentally and analyzed.

2.1.2. Commercial samples

Forty-three commercial samples of Brazilian whole grape juices from different vintages were purchased from the commercial market and analyzed.

2.2. Standards and reagents

The phlorizin standard used was from Sigma–Aldrich®, while the sorbitol standard was from the European Pharmacopoeia Reference Standard. The reagents used were Milli-Q water (Millipore®), orthophosphoric acid, acetic acid and acetonitrile, gradient grade for liquid chromatography (Merck®).

2.3. Analysis

2.3.1. Phlorizin analysis

Phlorizin analyses were carried out by HPLC with a photodiode array detector, model 1100 series, of Agilent Technologies®. Phlorizin was determined as described by [19]: column Zorbax 300SB-C18, 4.6 × 250 mm 5 μm, 25 °C, and pre-column 300SB-C18 (Agilent Technologies®). The mobile phase was formed by 1.5% acetic acid (v/v) as solvent A, and 1.5% acetic acid and acetonitrile, 60:40 (v/v), as solvent B. The flow used was 1.50 mL min^{-1}, wavelength of 320 nm, and injection volume of 10 μL of sample. The elution gradient of the solvents was as follows: 3% of solvent B for 6 min, 3–100% of solvent B from time 6 for 30 min, then, for 5 min at 100% of solvent B. The results were expressed in mg L^{-1} of phlorizin. The identification of the peak was confirmed and calculated by comparison of retention times with a external calibration curve (n = 7).

2.3.2. Sorbitol analysis

Sorbitol analyses were carried out by HPLC with refractive index (RI), model 1100 series, of Agilent Technologies®. Sorbitol was determined as described by [19]: Aminex® HPX-87C column, 300 × 7.8 mm Bio-Rad® at 80 °C, and Milli-Q water as mobile phase in isocratic flow of 0.60 mL min^{-1}. The injection volume of samples was 20 μL. The results were expressed in mg L^{-1} of sorbitol. The identification of the peak was confirmed and calculates by comparison of retention times with external calibration curve (n = 7).

All juices were diluted twice, filtered through membranes of 13 mm of diameter and 0.8 μm of pore size and the analysis were performed in triplicates.

2.3.3. Statistical analysis

For evaluating the results of sorbitol in the experimental grape juices, the data were presented as means ± standard deviation (SD). The normality test of Kolmogorov–Smirnov was applied and the data were performed using one-way analysis of variance (ANOVA), followed by the Test of Multiple Comparisons, significance level of 5%. To analyze the juices by groups of red and white grapes, was used the Tukey's test, with 95% confidence interval ($p < 0.05$). All analyses were conducted using the statistical software SPSS 21.0 for Windows.

Table 1. Sorbitol values in experimental grape juices, harvest 2016.

Juice	Variety	Mean ± Standard deviation (mg L^{-1})	Total Mean (mg L^{-1})
Purple	Ives	49.9 ± 27.11^{A}	40.6^{A}
	Isabella	35.1 ± 27.03^{B}	
	Concord	30.0 ± 10.09^{B}	
	Niagara Rosada	32.8 ± 16.56^{AB}	
	Isabel Precoce	35.2 ± 3.67^{AB}	
	BRS Cora	53.5 ± 33.70^{AB}	
White	White Muscat	19.1 ± 4.27^{B}	27.9^{B}
	Niagara Branca	30.6 ± 26.00^{B}	
	Lorena	29.7 ± 15.21^{AB}	

For different varieties, means followed by different capital letters in the column differ significantly by ANOVA complemented by the Test of Multiple Comparisons, significance level of 5%. For the different group of grapes, means followed by different capital letters in the column differ significantly by by Tukey's test, significance level of 5%.

3. Results and discussion

3.1. Phlorizin and sorbitol in experimental grape juices

All grape juices elaborated experimentally showed phlorizin content below the quantification limit (0,64 mg L^{-1}), which is consistent with the literature, as it is well known that this compound is not found naturally in grapes [7, 19].

With regard to sorbitol, the experimental purple grape juices showed values between 15.4 mg L^{-1} and 136 mg L^{-1}, while, the experimental white grape juices showed values between 14.7 mg L^{-1} and 102 mg L^{-1} (Table 1). Analyzing the average amount per bunch of grapes (purple and white), the juices elaborated with purple grapes showed a higher value of sorbitol. In relation to the different kind of grapes, there was significant difference between the juices elaborated with the variety Ives and the varieties: Isabella, Concord, Niagara Rosada and White Muscat. In a previous study (Spinelli et al. 2016) sorbitol levels were found higher in experimental juices made with the Ives variety when compared with experimental juices made with the variety Isabella, with average values of 104 ± 38.24 and 71.8 ± 20, 44 mg L $^{-1}$, respectively.

The health status of the grapes influences the concentration of the different polyols [15, 16], whose levels are higher in grapes with higher incidence of rot. In southern Brazil, climatic conditions favor the occurrence of rot in grapes used in the elaboration of juices.

3.2. Phlorizin and sorbitol in commercial grape juices

Among the commercial grape juices analyzed (Table 2), 39 samples were below the quantification limit for phlorizin analysis. Four samples showed presence of phlorizin, with values ranging from 1.92 to 8.39 mg L^{-1}. [20] analyzed phlorizin content in commercial juices and nectars of orange, apple, pineapple, peach, pear, apricot, and grape and found that phlorizin is typical of apple, allowing detection of apple in mixed juices. The average values of sorbitol in commercial grape juices ranged from 47.5 to 1527 mg L^{-1}. Considering the limit of 200 mg L^{-1}, it is assumed that five samples of grape juice were tampered with apple juice. All other samples were considered

Table 2. Results of phlorizin and sorbitol in commercial grape juices.

Juice	Phlorizin (mg L^{-1}) Mean ± Standard Deviation	Sorbitol (mg L^{-1}) Mean ± Standard Deviation
1	8.39 ± 0.18	1527 ± 61.5
2	7.13 ± 0.15	1275 ± 16.1
3	4.54 ± 0.09	1112 ± 27.4
4	1.92 ± 0.08	409 ± 2.41
5	*NQ	276 ± 1.24
6	*NQ	180 ± 6.31
7	*NQ	177 ± 0.99
8	*NQ	175 ± 4.80
9	*NQ	173 ± 3.20
10	*NQ	172 ± 0.30
11	*NQ	162 ± 0.49
12	*NQ	156 ± 4.51
13	*NQ	151 ± 1.00
14	*NQ	141 ± 4.90
15	*NQ	141 ± 0.92
16	*NQ	141 ± 1.50
17	*NQ	133 ± 0.50
18	*NQ	130 ± 0.80
19	*NQ	125 ± 8.30
20	*NQ	123 ± 5.20
21	*NQ	121 ± 4.96
22	*NQ	109 ± 1.17
23	*NQ	109 ± 0.50
24	*NQ	106 ± 0.29
25	*NQ	101 ± 6.90
26	*NQ	100 ± 0.59
27	*NQ	98.7 ± 0.10
28	*NQ	95.7 ± 0.31
29	*NQ	90.1 ± 6.29
30	*NQ	89.0 ± 0.97
31	*NQ	88.3 ± 1.59
32	*NQ	88.2 ± 0.27
33	*NQ	87.6 ± 1.82
34	*NQ	81.6 ± 2.82
35	*NQ	80.3 ± 4.45
36	*NQ	77.8 ± 3.36
37	*NQ	76.4 ± 2.98
38	*NQ	67.9 ± 0.61
39	*NQ	58.5 ± 3.23
40	*NQ	58.2 ± 4.04
41	*NQ	56.4 ± 1.13
42	*NQ	56.1 ± 2.74
43	*NQ	47.5 ± 0.90

*NQ = bellow oh the limit of quantification (LOQ).

genuine. [21] analyzed sorbitol content in commercial wines and found that most wines had sorbitol levels lower than 200 mg L^{-1}. The four juices that presented the highest content of phlorizin also showed higher sorbitol content, confirming the addition of apple juice. In these four cases, the presence of phlorizin was associated with the presence of sorbitol. The juice number 5 showed sorbitol level of 276 ± 1.24 mg L^{-1} but phlorizin bellow of the LOQ. This juice may have been tampered with a clarified apple juice that can affect the phenolic composition. According to [22], the phenolic composition of apple juice is significantly affected by different clarification treatments.

4. Conclusion

All experimental grape juices showed phlorizin content below the quantification limit. A significative difference in the sorbitol content was observed bettwen the juices elaborated with the variety Ives and the varieties Isabella, Concord, Niagara Branca and White Muscat. Juices from red grape varieties showed higher sorbitol content than the white grape varieties. The presence of apple juice was observed in five commercial grape juices.

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