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Detection of addition of apple juice in purple grape juice

F.R. Spinelli [a, b], S.V. Dutra [b], G. Carnieli [b], S. Leonardelli [b], A.P. Drehmer [a, b], R. Vanderlinde [a, b, *]

[a] Universidade de Caxias do Sul (UCS), Instituto de Biotecnologia, Rua Francisco Getúlio Vargas, 1130, 95070-560 Caxias do Sul, Brazil
[b] LAREN/IBRAVIN– Laboratório de Referência Enológica Evanir da Silva, Av. da Vindima, 1855, Caxias do Sul, Rio Grande do Sul, Brazil

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ABSTRACT

Due to increasing grape juice consumption, quality control is a reality in all countries which produce and consume this product. The most common form of adulteration is by substituting grape juice with apple juice. The adulterated samples can be identified by specific analysis, since apple juice has some compounds that grape has not. There are many studies about the phlorizin and sorbitol content in wines, but in grape juice they are scarce. Therefore, analytical methods to identify the composition of grape juices and determine their authenticity are needed to ensure quality control. The present study aimed to the validation and application of analytical methods for the determination of phlorizin and sorbitol, to detect the addition of apple juice in purple grape juice, by high-performance liquid chromatography. Initially, the methods were validated and tests were conducted by additions of percentages of apple juices in grape juices. Finally, experimental and commercial grape and apple juices were analyzed. Our study found that both methods are effective in detecting the addition of apple juice in grape juice. Four of the 39 commercial grape juices analyzed showed adulteration by the addition of apple juice in grape juice.

1. Introduction

Grape juice consumption in Brazil has increased significantly in recent years due to the beneficial health effects showed by products derived from grape. Grape juice is the unfermented drink obtained from simple must, sulfited or concentrated, from healthy, fresh and ripe grapes (BRASIL, 1988). 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 (Rizzon & Meneguzzo, 2007).

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 (Granato, Koot, Schnitzler, & Van Ruth, 2015; Vedeanu, Magdas, Bolojan, & Damian, 2012). Adulteration in non-alcoholic fruit beverages is one of the most studied topics in the area of beverage technology (Figueira, Nogueira, Ducatti, Venturini Filho, & Mischan, 2010). The most common form of tampering is by substituting with another juice of lower commercial value (Thavarajah & Low, 2006).

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 (Fromm, Bayha, Carle, & Kammerer, 2012). The main cultivars grown in Brazil are Gala and Fuji (Fioravanço et al., 2010). Juice is a by-product to be considered since it is an important alternative due to the availability of apples of low commercial value (Stander, Kuhn, & Hiten, 2013), whose total volume increases year after year in view of the growing domestic production (Nogueira, Swiech, Denardi, & Wosiacki, 2006).

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 (Schieber, Keller, & Carle, 2001). Phlorizin is a phenolic compound that has been widely used for identifying adulteration of fruit juices and other products (Schieber et al. 2001). 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 (Dong et al., 2007; Fromm et al., 2012; Gosch, Halbwirth, & Stich, 2010).

Polyols are compounds that contribute to the sweetness of

* Corresponding author. Laboratório de Referência Enológica Evanir da Silva /Instituto Brasileiro do Vinho (IBRAVIN), Avenida da Vindima, 1855, CEP: 95084-470 Caxias do Sul, Rio Grande do Sul, Brazil.
E-mail addresses: fspinelli86@gmail.com (F.R. Spinelli), rvanderl@ucs.br (R. Vanderlinde).

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musts and wines. Sorbitol is an isomer of mannitol (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2003), also called glucitol (Li, Feng, & Cheng, 2012). It is the main polyol produced by the Rosaceae family (Li et al., 2012), being present in various fruits (apple, pear, cherry, plum, peach, and melon) and normally absent in healthy grapes (Flanzy, 2003; Ribéreau-Gayon et al., 2003; Soria-Guerra et al., 2011). It is classified among the carbohydrates, since it is an alcohol resulting from the metabolism of sugars, and it is a sweetener (Silva, Seabra, Andrade, Oliveira, & Ferreira, 1999).

Currently, there are studies to determine sorbitol levels in wine (Dennis, Massey, & Bigwood, 1994; Stander et al., 2013), however, there are few studies on grape and apple juices. Therefore, this study aimed to validate and apply analytical methods for determining phlorizin and sorbitol by high-performance liquid chromatography, aiming to detect the addition of apple juice in grape juice.

2. Material and methods

2.1. Samples

Twenty-eight experimental grape juices from the cultivars Isabella, Ives and Concord, and two clarified apple juices from the cultivars Fuji and Gala were used. In addition, 39 samples of Brazilian commercial whole grape juices and four samples of Brazilian commercial apple juices were also collected.

2.2. Standards and reagents

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

2.3. Chromatographic analysis

Phlorizin analyses were carried out by high performance liquid chromatography (HPLC) with a photodiode array detector, model 1100 series, by Agilent Technologies®. Phlorizin was determined by adapting the method of Tricard, Cazabiel, and Medina (2000): a column Zorbax 300SB-C18, 4.6 × 250 mm 5 μm, 25 °C, and a 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, 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. All samples were filtered through cellulose ester membranes of 13 mm of diameter and 0.8 μm of pore size (Merck S/A). The results were expressed in mg/L of phlorizin. The identification of the peak was confirmed and calculates by comparison of retention times with a external calibration curve (n = 7). The analysis performance was controlled throughout the measurement sequences by analyzing a external reference standard of 5 mg/L of phlorizin every 5 samples analyzed.

The analysis of sorbitol was carried out by HPLC with refractive index (RI), model 1100 series, of Agilent Technologies®. Sorbitol was determined by adapting the official method of the European Community (NF EN 12630, 1999): 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. The injection volume of samples was 20 μL. All samples were diluted twice and filtered through membranes of 13 mm of diameter and 0.8 μm of pore size. The results were expressed in mg/L of sorbitol. The identification of the peak was confirmed and calculates by comparison of retention times with a external calibration curve (n = 7). The analysis performance was controlled throughout the measurement sequences by analyzing a external reference standard of 50 mg/L of sorbitol every 5 samples analyzed.

All juices were diluted twice, and the analysis of phlorizin and sorbitol was performed in triplicate.

2.4. Methods validation

The methods were validated according to the Brazilian National System of Metrology, Standardization and Industrial Quality - INMETRO (2011). For the analysis of phlorizin and sorbitol, following parameters were evaluated: selectivity, linearity, working range, detection limit, quantification limit, accuracy, method robustness, and calculation of measurement uncertainty.

To evaluate the selectivity and accuracy, tests were carried out by adding apple juice to grape juice. For each grape juice cultivar (Isabella, Ives and Concord), juices of apple cultivars Gala and Fuji were added at the following concentrations: 5, 10, 15, 20, 25 and 30%. The accuracy values were evaluated by the AOAC (2012) criteria. To evaluate the linearity and working range, a calibration curve was prepared for each method, by reading seven concentrations of each analyte analyzed in seven replicates. The detection and quantification limits were calculated by dilution tests of a sample containing the matrix. The robustness of the phlorizin and sorbitol methods was evaluated by using different mobile phase flows and different temperatures of the analytical column. Finally, the calculation of measurement uncertainty of the methods was performed using GUM Workbench 2.4 software.

2.5. Statistical analysis

For evaluating the robustness and 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 Tukey's test at $p > 0.05$. To verify if exists a correlation between the results of phlorizin and sorbitol in the commercial apple juices, data analyses were performed using Pearson's correlation. All analyses were conducted using the statistical software SPSS 21.0 for Windows.

3. Results and discussion

3.1. Methods validation

All parameters analyzed for validating the two methods were considered satisfactory (Table 1).

According to Ribani, Bottoli, Collins, Jardim, and Melo (2004), the robustness of a method measures its sensitivity in view of small variations. A method is considered robust when it is not affected by a small modification in its parameters. According to Table 2, the

Table 1
Results of validation parameters of the methods.

Parameter	Phlorizin	Sorbitol
Linear Range (mg/L)	0.64–25	7.09–2000
R^2	0.9996	0.9999
LOD (mg/L)	0.62	6.44
LOQ (mg/L)	0.64	7.09
Percentage recovery (average)	100 ± 0.86	98.2 ± 2.06
Measurement uncertainty	1.05	11.75
Retention time (min)	24.2	22.5