



Rapid determination of the aromatic compounds methyl-anthranilate, 2'-aminoacetophenone and furaneol by GC-MS: Method validation and characterization of grape derivatives



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ABSTRACT

A methodology for the rapid determination of the aromatic compounds methyl anthranilate (MA), 2'-aminoacetophenone (2-AAP) and furaneol by GC-MS was validated and used to characterize grape juice and wine elaborated with the new Brazilian grape varieties cultivated in northeastern Brazil, and Brazilian grape nectars. The method presented linearity ($R^2 > 0.9952$), good accuracy ($CV < 12.9\%$), recovery (76.6% to 106.3%), limit of detection ($23 \mu\text{g L}^{-1}$ to $94 \mu\text{g L}^{-1}$) and limit of quantification ($96 \mu\text{g L}^{-1}$ to $277 \mu\text{g L}^{-1}$) acceptable in only 20 min of running. The methodology was considered satisfactory for the purpose, being a simple and rapid method for the determination of these compounds in grape derivatives drinks. In the characterization of the nectars the compound that stood out was the MA, being its presence attributed to the addition of flavorings in these products. It was evidenced a significant contribution of furaneol in the aroma of grape juice and wines elaborated with the new Brazilian grape varieties.

1. Introduction

Aromas are important chemical compounds that influence the quality and typicality of grapes and derivatives such as juices and wines. Aromatic substances are complex molecules belonging to distinct chemical classes such as volatile esters, higher alcohols and aldehydes (Hadi, Zhang, Wu, Zhou, & Tao, 2013). The aroma of grape and its derivatives depends on several factors, including grape variety and winemaking practices (Morris & Striegler, 2005; Slegers, Angers, Ouellet, Truchon, & Pedneault, 2015). These molecules are formed during the maturation of the fruit, and in *Vitis labrusca* L. and *Vitis rotundifolia* varieties the presence of the methyl anthranilate and/or 2'-aminoacetophenone compounds predominate in high quantities, which are the characteristic aromas of American grape juice (Meyers, Sacks, & Vanden Heuvel, 2013; Morris & Striegler, 2005; Panighel, Vedova, De Rosso, Gardiman, & Flamini, 2010). Another volatile compound that is associated with the characteristic aroma of *V. labrusca* and hybrid varieties in grape-derived beverages is 4-hydroxy-2,5-dimethyl-3 (2H)-

furanone (furaneol) (Kobayashi et al., 2013).

Beverages derived from grapes *V. labrusca* and hybrids are widely consumed in Brazil, mainly in the form of table wines, juices and nectars (Marinelli et al., 2016). The largest Brazilian production of grape derivatives is in the state of Rio Grande Sul, Southern Brazil. However, a new producing region has been highlighting in the last years, the region of the Submédio São Francisco Valley (SFV), an atypical region (Coelho et al., 2018). The SFV, in northeast Brazil, is a new world region for the production of tropical wines. It differs from other regions in terms of its climatic conditions, with an average temperature of 26 °C, high sunlight intensity (3000 h/year), and low annual rainfall (around 500 mm per year). The growth of grapes associated with crop irrigation management makes it possible for each vine to produce two harvests per year in this region (Padilha, Biasoto, Corrêa, Lima, & Pereira, 2017). The SFV has been investing in the production of juices of high quality grapes produced with the new Brazilian cultivars: Isabel Precoce (*V. labrusca*) and hybrids (*V. labrusca* × *V. vinifera*) BRS Violeta, BRS Cora and BRS Magna (Lima et al., 2014; Padilha et al., 2017). The juice and

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Table 1
Classical analysis of grape juice and wines elaborated with the new Brazilian varieties of grapes cultivated in the Northeast of Brazil, and of Brazilian grape nectars.

Grape juices, wines and nectars	Classical analysis					
	°Brix	TA (%)	Ratio °Brix/TA	pH	CI	TON
GJA	20.5 ± 0.2	0.67 ± 0.01	30.8 ± 0.5	3.56 ± 0.005	5.25 ± 0.1	0.67 ± 0.007
GJB	20.3 ± 0.0	0.73 ± 0.02	27.6 ± 0.9	3.41 ± 0.005	7.45 ± 0.3	0.95 ± 0.003
GJC	18.3 ± 0.05	0.65 ± 0.008	28.2 ± 0.3	3.24 ± 0.05	3.58 ± 0.004	0.71 ± 0.002
GJD	19.0 ± 0.4	0.76 ± 0.02	24.8 ± 1.3	3.37 ± 0.04	4.95 ± 0.1	0.64 ± 0.01
GJE	18.8 ± 0.1	0.83 ± 0.002	22.8 ± 0.2	3.37 ± 0.01	14.74 ± 0.5	1.11 ± 0.006
WF	–	0.89 ± 0.008	–	3.52 ± 0.0	5.28 ± 0.4	0.79 ± 0.02
WG	–	0.75 ± 0.0	–	3.24 ± 0.05	5.49 ± 0.0005	1.01 ± 0.01
NH	12.0 ± 0.4	0.90 ± 0.008	13.3 ± 0.4	3.23 ± 0.05	2.15 ± 0.04	1.22 ± 0.1
NI	12.2 ± 0.1	0.78 ± 0.01	15.6 ± 0.2	3.46 ± 0.03	3.17 ± 0.03	0.91 ± 0.004
NJ	8.3 ± 0.1	0.36 ± 0.008	22.7 ± 0.3	2.90 ± 0.08	1.48 ± 0.01	1.38 ± 0.001
NL	12.9 ± 0.1	0.63 ± 0.02	20.5 ± 0.7	2.70 ± 0.02	1.90 ± 0.04	1.64 ± 0.02
NM	10.8 ± 0.1	0.66 ± 0.01	16.2 ± 0.2	2.74 ± 0.005	2.46 ± 0.02	1.12 ± 0.01
NN	5.2 ± 0.0	0.63 ± 0.01	8.2 ± 0.2	2.77 ± 0.03	3.21 ± 0.084	1.11 ± 0.01
NO	11.1 ± 0.0	0.74 ± 0.008	14.8 ± 0.2	3.50 ± 0.06	3.65 ± 0.01	1.17 ± 0.007
IPO	18.7 ± 0.3	0.68 ± 0.02	27.4 ± 1.3	3.36 ± 0.01	5.20 ± 0.2	0.89 ± 0.004
IP	21.2 ± 0.8	0.53 ± 0.008	39.7 ± 1.9	3.60 ± 0.0	6.67 ± 0.7	0.88 ± 0.03
BVO	18.3 ± 0.5	0.92 ± 0.02	19.8 ± 0.8	3.21 ± 0.005	21.48 ± 0.3	0.44 ± 0.008
BV	21.2 ± 0.8	0.57 ± 0.002	37.3 ± 3.1	3.88 ± 0.005	20.94 ± 0.7	0.76 ± 0.007
BC	20.0 ± 0.3	0.85 ± 0.002	23.5 ± 0.8	3.54 ± 0.01	31.97 ± 2.9	0.69 ± 0.05
BM	16.3 ± 0.1	0.51 ± 0.01	32.0 ± 1.1	3.73 ± 0.01	21.78 ± 1.7	0.72 ± 0.01

The results are expressed as mean ± standard deviation of 3 repetitions. TA: titratable acidity; CI: color intensity; TON: tonality. GJA-GJE: commercial grape juices; WF-WG: wines; NH-NO: grape nectars; IPO, IP, BVO, BV, BC e BM: varietal grape juices.

wines (*V. labrusca* and hybrids) produced in this region stand out for being a functional beverage with high bioactive content, good ability to inhibit reactive oxygen species and high in vitro antioxidant activity associated with the significant content of phenolic compounds (Padilha, Miskinis, et al., 2017). The studies carried out with the new Brazilian grape varieties are usually related to the phenolic composition, antioxidant activity, sugars and organic acids, and bioactive properties (Coelho et al., 2018; Natividade, Correa, Souza, Pereira, & Lima, 2013; Padilha, Miskinis, et al., 2017; Silva et al., 2015; Toscano et al., 2015), and no studies were found to evaluate the volatile compounds of beverages derived from these grapes.

The main methods for determining the aromatic compounds methyl anthranilate (MA), 2'-aminoacetophenone (2-AAP) and furaneol in juices and wines are by gas chromatography coupled to mass spectrometry (GC-MS), where the separation of the compounds is made in columns of the type fused silica capillary column, length 30 m with 0.25 mm internal diameter and 0.25 µm film thickness. The main extraction techniques of these compounds in wines and juices for later characterization are by liquid-liquid extraction (LLE) with ethyl acetate or dichloromethane solvents, solid phase extraction (SPE) and head-space solid phase microextraction (HS-SPME) (Fan, Tsai, & Qian, 2007; Guedes de Pinho & Bertrand, 1995; Iyer, Sacks, & Padilla-Zakour, 2012; Schmar, Keiser, & Krautwald, 2016; Zulj, Maslov, Tomaz, & Jeromel, 2015).

In this context, the objective of this work was to validate a simple methodology for the rapid determination of MA, 2-AAP and furaneol aromatic compounds by GC-MS and to characterize juices and wines produced with new Brazilian grape varieties (*V. labrusca* and hybrids), and nectars of Brazilian grapes.

2. Material and methods

2.1. Standards and reactants

Standards of 2-octanone, methyl anthranilate, 2-aminoacetophenone and 4-hydroxy-2,5-dimethyl-3(2H)-furanone were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethyl acetate, anhydrous sodium sulfate, ethyl alcohol and potassium persulfate were acquired from Merck (Darmstadt, Alemanha). The ultrapure water was obtained in a purification system from Marte Científica (São Paulo, SP, Brazil).

2.2. Samples

The validated methodology was used to characterize grape juices and wines produced with new Brazilian grape varieties (*V. labrusca* and hybrids) and Brazilian grape nectars. All the juices were elaborated in triplicate and each commercial label was composed of three bottles, where each bottle corresponded to a repetition. A total of 20 products were analyzed in three replicates, divided into 6 single grape juices, 5 commercial grape juices, 2 red wines and 7 grapes nectars labels, totaling 60 analyzed samples.

Monovarietal grape varieties of the Isabel Precoce organic and conventional varieties (IPO and IP, respectively), BRS Cora (BC), BRS organic and conventional violet (BVO and BV, respectively) and BRS Magna (BM) were handcrafted by the hot press method (Morris & Striegler, 2005) with grapes from the São Francisco Valley (SFV), Petrolina PE, at latitude 09°21'S and longitude 40°40'W; and Lagoa Grande PE at latitude 8°59'S and longitude 40°16'W, Brazil. Five samples of SFV commercial grape juice were donated by local companies and produced by mixing the grapes: two Isabel Precoce + BRS Violeta (known as GJA and GJB), one Isabel Precoce + BRS Violeta organic (GJC), one Isabel Precoce + BRS Cora (GJD) and an Isabel Precoce + BRS Magna (GJE). Two commercial labels of wine Isabel Precoce + BRS Cora (denominated WF and WG) were acquired in the local market. Seven grape nectars labels (*V. labrusca*) were purchased from supermarkets in Petrolina PE, Brazil, named NH, NI, NJ, NL, NM, NN and NO.

To obtain analytical characteristics of the samples, classical pH analyzes (potentiometer pH Analyzer - Tecnal (Brazil)), soluble solids (°Brix) (digital refractometer HI 96801 Hanna, USA) and titratable acidity (TA) were determined, following the methodologies described in Organisation Internationale de la Vigne et du Vin (2011). The color intensity (CI) was determined by obtaining the indices at 420 nm, 520 nm and 620 nm with a UV-Vis UV 2000A spectrophotometer, Instrutherm (Brazil), using glass cuvettes with a 0.2 cm path length (Glories, 1984). (See Table 1).

2.3. Method of extraction of aromatic compounds

The extraction of the aromatic compounds was by the liquid-liquid method (LLE), using ethyl acetate as organic solvent, following the methodology described by Guedes de Pinho and Bertrand (1995). To

obtain the extract, 5 g of anhydrous sodium sulfate, 1 mL of the internal standard (2-octanone at 44.8 mg L^{-1}) were added to 50 mL of the sample. Three successive extractions were carried out with 8, 2 and 2 mL of ethyl acetate, respectively, in vortex for 5 min. The organic phases of the three extractions were separated after 3 min of rest, mixed and centrifuged at 3000 turns (Educat EEQ-9004/B centrifuge (Paraná, Brazil).) The supernatant was filtered on Nylon membrane $0.22 \mu\text{m}$ porosity (Millex Millipore, Barueri, SP, Brazil).

2.4. Instrumentation and chromatographic conditions

The analyzes were performed using a Gas Chromatograph, Agilent Technologies model 7820A GC system coupled to a mass selective detector (MSD), Agilent Technologies model 5977E MSD. The data acquisition and processing were via MassHunter Agilent Technologies™ Software (Santa Clara, CA, USA). The chromatographic conditions used were adapted from that described by Guedes de Pinho and Bertrand (1995) to decrease the running time. The split mode was used with a ratio of 30:1 and a flow rate of 15 mL/min at 200 °C. The column used was the capillary column CP-WAX 52 CB (Varian, Lake Forest, CA, USA), with a flow rate of 0.5 mL/min ($30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$). The oven temperature was 60 °C (1 min), heating at 10 °C/min up to 200 °C (5 min) and maximum oven temperature at 220 °C (heating rate at 10 °C/min), with total run time of 20 min. The temperature of the detector was monitored at 200 °C and the mobile phase was helium gas with purity 5.0. The runs were performed in Selected Ion Monitoring (SIM) modes, where the specific ions for the search and quantification of MA, 2-AAP and furaneol. Identification of the molecules was done through the external standards. 1 μL of the extract was injected, and the organic compounds MA, 2-AAP and furaneol were identified by the retention time of the pure substance and confirmed by the mass spectrum. The specific ions sought for 2-octanone were (m/z) 43, 58 and 71, furaneol (m/z) 43, 57 and 128, 2-AAP (m/z) 92, 120, 135, 136 and m/z of 92, 119 and 151 for MA. The retention time of 2-octanone was 5.56 min, furaneol 14.63 min and 2-AAP and MA 16.67 and 16.91 min, respectively. The chromatogram obtained from the separation of the patterns in the SIM mode is shown in Fig. 1.

2.5. Model validation

The validation parameters, according to the guide for validation and analytical quality control published of the Ministry of Agriculture of Brazil (Brasil, 2011), using standard solutions, white samples, synthetic wines and juices, and fortified samples. The validation parameters used were linearity, specificity, precision, recovery and limits of detection and quantification.

2.5.1. Linearity

Linearity was obtained by means of the calibration curve of the compounds in five concentrations. The external patterns of furaneol, 2-AAP, MA and internal standard were diluted in synthetic wine and juice and extracted as described in item 2.4, for further injection. The synthetic wine consisted of a solution composed of 12% ethanol, 6 g L^{-1} tartaric acid and 2 g L^{-1} glucose in ultrapure water. The synthetic juice consisted of a solution containing 70 g L^{-1} fructose, 70 g L^{-1} glucose and 6 g L^{-1} tartaric acid in ultrapure water. A concentration versus peak area calibration curve, determined from regression analysis using the least squares method, was constructed.

2.5.2. Specificity

In the evaluation of the specificity of the method, samples of grape juice and red wines were used. The purity of the chromatographic peaks of the samples was made by means of the retention time of the compound and the identification of the specific ions in mass spectrum-MSD.

2.5.3. Precision and recovery

The precision was evaluated by means of the coefficient of variation (CV%) obtained from the results of six injections of replicates of extraction grape juice samples and red wines added of internal standard and fortified with the compounds studied. The recovery was calculated by comparing the values obtained for each fortified compound in relation to the initial value contained in the sample.

2.5.4. Limit of detection (LOD) and limit of quantification (LOQ) of the method

Limit of detection (LOD) and limit of quantification (LOQ) were obtained following the method described by Hubaux and Vos (1970). Three standards of the compounds under study were prepared, with concentrations close to LOD value found in the literature and analyzed in triplicate. An analytical curve was constructed by plotting the values obtained from the analysis of the standards versus the actual values, obtaining the slope of the curve, intercept and coefficient of correlation. The residual standard deviation (RSD) was calculated by comparing the values obtained in the analysis of the actual values. LOD and LOQ were established as 3 and 10 times the RSD, respectively, added with the intercept of the curve.

2.6. Statistical analysis

The results obtained from the analyzes of the studied samples were submitted to analysis of variance (*one-way ANOVA*) and compared by the Tukey test at 5% of error probability, with the aid of the *R-Studio* program (R-Studio Inc., Version 1.0.143, Boston, USA).

3. Results and discussion

3.1. Method validation

Different temperature ramps of separation of the aromatic compounds were evaluated for decreasing the running time. The new separation condition was applied in the validation and characterization study of the grape derivatives.

3.1.1. Linearity and specificity

The results obtained for linearity and specificity are presented in Table 2. The compounds studied showed good linearity, since the values of the determination coefficient (R^2) obtained ranged from 0.9952 to 0.9994. The values of R^2 obtained are in accordance with that established in Brazil (2011) for analytical methods, where a correlation coefficient (R) > 0.99 is required for calibration curves.

The specificity of a method consists in its ability to distinguish the analytes in the presence of other matrix components, which may include impurities, degradation products, among others, being of fundamental importance to guarantee reliability in the quantification of secondary metabolites (ICH, 2005). In the present study the specificity was evaluated using the mass spectrum using the SIM mode, in which the specific ions for each of the three compounds studied were previously selected. For MA, 2-AAP and furaneol the ions obtained confirmed the presence of these compounds, demonstrating that the method studied has adequate specificity for the proposed use.

3.1.2. Precision and recovery

The results obtained for precision (CV%) ranged from 4.53 to 12.9 for furaneol and MA, respectively, in the grape juice matrix. In the wine matrix the CV% ranged from 3.53 to 5.47 for 2-AAP and furaneol, respectively (Table 2). The values of CV% in the three aromatic compounds studied are below the maximum limit established by Brazilian legislation, which is 20% (Brasil, 2011), indicating a good precision of the method.

The values found in the percentage of recovery (%) of the juices were 85.6 for MA, 76.6 for 2-AAP and 97.6 for furaneol. In the wine

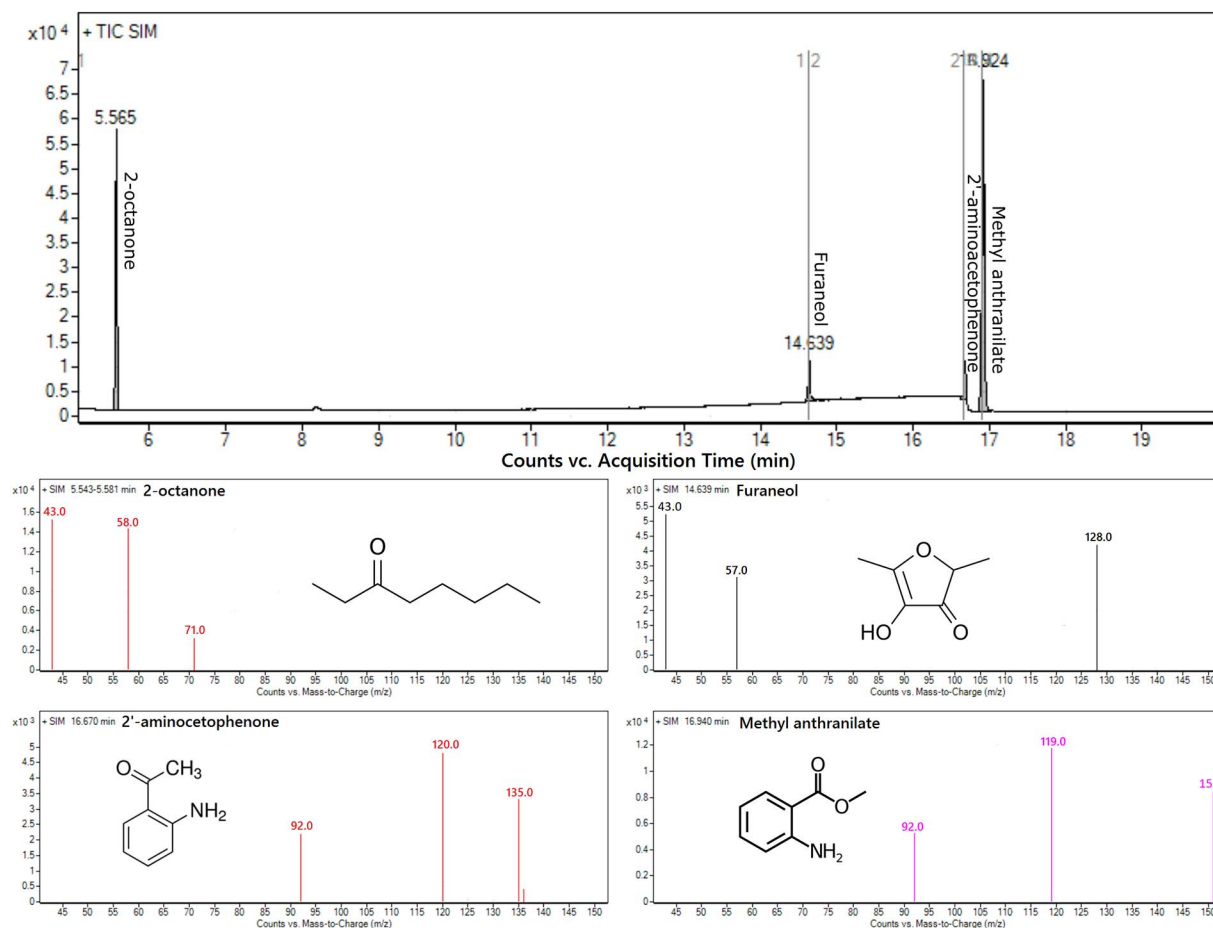


Fig. 1. Chromatogram of separation and mass spectrum of the internal standard 2-octanone (RT = 5.56 min), furaneol (RT = 14.63 min), 2'-aminoacetophenone (RT = 16.67 min) and methyl anthranilate (RT=16.91 min).

matrix, MA recovery was 86.4, 2-AAP 85 and furaneol 106.3. These values are acceptable for using this methodology in scientific research, since the limits established by Brazilian legislation for analytical methods vary from 80% to 107% (Brasil, 2011).

The values obtained for precision and recovery in this study are in agreement with those found in other validation works for the determination of similar compounds in wines and grape juice, *V. labrusca* and hybrids, using LLE, SPE and SPME extraction techniques, and determination by GC-MS in run times ranging from 24 to 60 min (Guedes de Pinho & Bertrand, 1995; Iyer et al., 2012; Panighel et al., 2010; Zulj et al., 2015). These results demonstrate that the present method is a simple and rapid methodology of determination of these compounds in beverages derived from grapes.

3.1.3. Limit of detection (LOD) and limit of quantification (LOQ) of the methods

The LOD and LOQ values demonstrate the ability of a method to detect and quantify low concentrations of a substance with reliability (Eurachem, 2014). The LOD values ranged from 23 to 94 $\mu\text{g L}^{-1}$ to MA and 2-AAP, respectively. LOQ values were 96–277 $\mu\text{g L}^{-1}$ for MA and 2-AAP, respectively. Considering that the limits of sensory perception of the compounds studied in wines and grape juice range from 1 to 300 $\mu\text{g L}^{-1}$ (Fan et al., 2007; Nelson, Acree, Lee, & Butts, 1977; Perry & Hayes, 2016; Rapp, 1996), LOD values were considered acceptable for use of this methodology in analyzes of wines and juices from *V. labrusca*, because these are matrices in which these aromas are considered majorities.

Determination of MA, 2-AAP and furaneol in Brazilian wines, grape juices and nectars

Table 2

Values obtained in the validation parameters of the method of determining the aromatic compounds methyl anthranilate (MA), 2'-aminoacetophenone (2-AAP) and furaneol in grape juice and wines.

Aromatic compounds	Calibration range (mg L^{-1})	Calibration curve ^a	Coefficient of determination (R^2)	Recovery (%)		Precision (CV%)		RSD	LOD ($\mu\text{g L}^{-1}$)	LOQ ($\mu\text{g L}^{-1}$)
				Juices	Wines	Juices	Wines			
MA	0.5–10	$A = 49914 \times C \left(\frac{\text{mg}}{\text{L}}\right) + 1506$	0.9994	85.6	86.4	12.9	3.77	0.041	23	96
2-AAP	0.25–5	$A = 146152 \times C \left(\frac{\text{mg}}{\text{L}}\right) - 14834$	0.9952	76.6	85	10.56	3.53	0.069	94	277
Furaneol	0.5–10	$A = 13481 \times C \left(\frac{\text{mg}}{\text{L}}\right) - 1837$	0.9992	97.6	106.3	4.53	5.47	0.048	24	159

^a A = peak area and C = mass concentration.

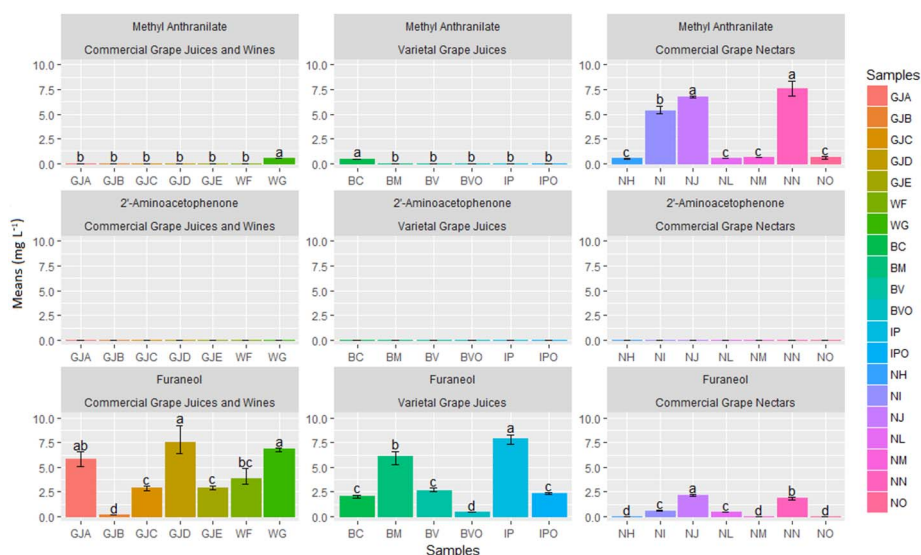


Fig. 2. Values of furaneol, 2'-aminoacetophenone and methyl anthranilate in grape juices and wines produced with the new grape varieties (*V. labrusca* and hybrids) cultivated in Northeast Brazil, and Brazilian grape nectars. Means in the bars followed by equal letters between the samples do not differ among themselves by the Tukey test at 5% of error probability.

The results obtained in the analyses of MA, 2-AAP and furaneol in grape juices, nectars and wines, are presented in Fig. 2. The presence of 2-AAP was not detected in the grape, wine and nectar juice evaluated. The methyl anthranilate was detected only in grape juice BC (BRS Cora) and wine WG in values of 460 and 500 $\mu\text{g L}^{-1}$, respectively. We highlight that the WG wine has the BRS Cora grape in its composition, suggesting that the presence of the MA can be a typical characteristic of the aroma of this hybrid variety. In a study by Panighel et al. (2010), values of 18–19 and 7–13 $\mu\text{g L}^{-1}$ for MA and 2-AAP, respectively, were reported for grape juice of the hybrid varieties (*V. labrusca* \times *V. riparia*) “Clinton” and possible (*V. rupestris* \times *V. lincedumii* \times crossing) “Seibel 19.881”. In grape juice of the “Concord” variety (*V. labrusca*) elaborated at different maturation stages and process conditions, MA values varying from 155 to 6120 $\mu\text{g L}^{-1}$ (Iyer et al., 2012). Gasperin et al. (2015) mention values varying from 12 to 17 $\mu\text{g L}^{-1}$ and 184–249 $\mu\text{g L}^{-1}$ to 2-AAP and MA, respectively, in wines made with the variety “Bordô” (*V. labrusca*) from Rio Grande do Sul, southern region of Brazil.

In relation to grape nectars, MA was present in all analyzed samples, where values ranged from 490 to 7570 $\mu\text{g L}^{-1}$. In the label of all studied nectars the addition of “natural grape aroma” is mentioned, which explains the results obtained since the MA is one of the main flavoring agents used to produce sensorial characteristics of grapes in foods and beverages (Wang & De Luca, 2005).

Furaneol was present in all samples of grape juice and wine, ranging from 150 $\mu\text{g L}^{-1}$ to 7930 $\mu\text{g L}^{-1}$. In grape nectars this compound was detected in four samples (NI, NJ, NL and NN) with values ranging from 450 $\mu\text{g L}^{-1}$ to 2082 $\mu\text{g L}^{-1}$. In varietal juice, furaneol was present in higher concentrations in the Isabel Precoce and BRS Magna grapes, in values higher than 6003 $\mu\text{g L}^{-1}$. Several authors mention the presence of this aromatic compound in *Vitis labrusca* or hybrid grapes, with values similar to those found in this study, suggesting that furaneol may be one of the main compounds responsible for the typical aroma of these varieties (Darici, Cabaroglu, Ferreira, & Lopez, 2014; Gasperin et al., 2015; Guedes de Pinho & Bertrand, 1995; Sasaki, Takase, Kobayashi, Matsuo, & Takata, 2015). According to Rapp (1996) the sensory perception threshold of furaneol is 300 $\mu\text{g L}^{-1}$, and in the great majority of the studied juices and wines its concentration was higher than 2000 $\mu\text{g L}^{-1}$, which shows that this compound possesses a considerable relevance in the aroma of products derived from *V. labrusca* hybrid grapes grown in the Northeast of Brazil. The lower values of furaneol in the nectars can be explained by the fact that this beverage is

normally produced by the dilution of concentrated juice of grape in water, in approximate values of 50% of the original grape juice in the formulated beverage. According to Kobayashi et al. (2013) furaneol is a component responsible for the characteristic aromas of strawberry, pineapple and mango.

4. Conclusions

The validated and applied methodology allowed the quantification of the aromatic compounds MA, 2-AAP and furaneol in only 20 min of running, being considered a simple and rapid method for the determination of these compounds in grape derivatives drinks. The values obtained in the validation parameters: linearity (R^2), specificity, precision ($CV\%$), recovery (%), limit of detection (LOD) and limit of quantification (LOQ) were considered satisfactory for the purpose. In the characterization of grape juice samples and SFV wines the main quantified compound was furaneol. In the characterization of the Brazilian commercial nectars the compound that stood out was the MA, being its presence attributed to the use of flavorants in these products. The present work evidences a significant contribution of furaneol in the characteristic aroma of grape juice and wines elaborated with the new Brazilian grape varieties (*V. labrusca* and hybrids) in Northeast Brazil.

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References

- Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Guia de Validação e Controle de Qualidade Analítica: Fármacos em Produtos para Alimentação Animal e Medicamentos Veterinários. Secretaria de Defesa Agropecuária, Brasília, 2011. (2011). Retrieved from http://www.agricultura.gov.br/arq_editor/file/Laboratorio/Guia-de-validacao-controle-de-qualidade-analitica.pdf (in portuguese).
- Coelho, E. M., da Silva Padilha, C. V., Miskinis, G. A., de Sá, A. G. B., Pereira, G. E., de Azevedo, L. C., & dos Santos Lima, M. (2018). Simultaneous analysis of sugars and organic acids in wine and grape juices by HPLC: Method validation and characterization of products from Northeast Brazil. *Journal of Food Composition and Analysis*, 66, 160–167.
- Darici, M., Cabaroglu, T., Ferreira, V., & Lopez, R. (2014). Chemical and sensory

- characterisation of the aroma of Çalkarası rosé wine. *Australian Journal of Grape and Wine Research*, 20, 340–346.
- Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics** (2nd ed.). Retrieved from <http://www.eurachem.org>.
- Fan, W., Tsai, I., & Qian, M. (2007). Analysis of 2-aminoacetophenone by direct-immersion solid phase microextraction and gas chromatography-mass spectrometry and its sensory impact in Chardonnay and Pinot gris wines. *Food Chemistry*, 105, 1144–1150.
- Gasperin, A., Echeverrigarai, S., Bonomi, J. C., Dutra, S. V., Spinelli, F. R., & Vanderlinde, R. (2015). A levedura e o carácter foxado do vinho tinto de mesa da variedade Bordô. *Revista Brasileira de Viticultura e Enologia*, 7, 62–67. Retrieved from <https://www.enologia.org.br/revista/downloads/1>.
- Glories, Y. (1984). La couler des vins rouges, 2^e Partie Mesure, Origine et Interpretation. *Conn. Vigne Vin*, 18, 253–271.
- Guedes de Pinho, P., & Bertrand, A. (1995). Analytical determination of furaneol (2,5-dimethyl-4-hydroxy-3 (2H)-furanone). Application to differentiation of white wines from hybrid and various *Vitis vinifera* cultivars. *American Journal of Enology and Viticulture*, 46, 181–186.
- Hadi, M. A. M. E., Zhang, F. J., Wu, F. F., Zhou, C. H., & Tao, J. (2013). Advances in fruit aroma volatile research. *Molecules*, 18, 8200–8229.
- Hubaux, A., & Vos, G. (1970). Decision and detection limits for linear calibration curves. *Analytical Chemistry*, 42, 849–855.
- ICH. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (2005). In: *Q2B (R1): Guideline on validation of analytical procedure-methodology*. Brussels: ICH Steering Committee.
- Iyer, M. M., Sacks, G. L., & Padilla-Zakour, O. I. (2012). Assessment of the validity of maturity metrics for predicting the volatile composition of concord grape juice. *Journal of Food Science*, 77, 19–25.
- Kobayashi, H., Sasaki, K., Tanzawa, F., Matsuyama, S., Suzuki, S., Takata, R., & Saito, H. (2013). Impact of harvest timing on 4-hydroxy-2,5-dimethyl-3(2H)-furanone concentration in 'Muscat Bailey A' grape berries. *Vitis*, 52, 9–11.
- Lima, M. S., Silani, I. S. V., Toaldo, I. M., Correa, L. C., Biasoto, A. C. T., Pereira, G. E., ... Ninow, J. L. (2014). Phenolic compounds, organic acids and antioxidant activity of grape juices produced from new Brazilian varieties planted in the Northeast Region of Brazil. *Food Chemistry*, 161, 94–103.
- Marinelli, P. S., Otoboni, A. M., Barbalho, S. M., Oshiiwa, M., Semeghini, A., Oliva, E., ... Nicolau, C. C. T. (2016). Physicochemical characteristics of orange and grape nectars marketed in Brazil. *International Journal of Food and Nutritional Sciences*, 5, 6–14.
- Meyers, J. M., Sacks, G. L., & Vanden Heuvel, J. E. (2013). Glycosylated aroma compound responses in 'riesling' wine grapes to cluster exposure and vine yield. *HortTechnology*, 23, 581–588.
- Morris, J. R., & Striegler, K. R. (2005). *Processing fruits: Science and Technology* (2nd ed.). Boca Raton, FL: CRC Press.
- Natividade, M. M. P., Correa, L. C., Souza, S. V. C., Pereira, G. E., & Lima, L. C. D. O. (2013). Simultaneous analysis of 25 phenolic compounds in grape juice for HPLC: Method validation and characterization of São Francisco Valley samples. *Microchemical Journal*, 110, 665–674.
- Nelson, R. R., Acree, T. E., Lee, C. Y., & Butts, R. M. (1977). Methyl Anthranilate as an aroma constituent of American wine. *Journal of Food Science*, 42, 57–59.
- OIV – Organisation Internationale de la Vigne et du Vin (2011). Recueil des methods internationales d'analyse des vins et des mouts. *8th Assemblée Générale, 21 June 2010, Paris* (edition 2011).
- Padilha, C. V. S., Biasoto, A. C. T., Corrêa, L. C., Lima, M. D. S., & Pereira, G. E. (2017). Phenolic compounds profile and antioxidant activity of commercial tropical red wines (*Vitis vinifera* L.) from São Francisco Valley, Brazil. *Journal of Food Biochemistry*, 41, 1–9.
- Padilha, C. V. S., Miskinis, G. A., De Souza, M. E. A. O., Pereira, G. E., Oliveira, D., Bordignon-Luiz, M. T., & Lima, M. S. (2017). Rapid determination of flavonoids and phenolic acids in grape juices and wines by RP-HPLC/DAD: Method validation and characterization of commercial products of the new Brazilian varieties of grape. *Food Chemistry*, 228, 106–115.
- Panighel, A., Vedova, A. D., De Rosso, M., Gardiman, M., & Flamini, R. (2010). A solid-phase microextraction gas chromatography/ion trap tandem mass spectrometry method for simultaneous determination of 'foxy smelling compounds' and 3-alkyl-2-methoxy-pyrazines in grape juice. *Rapid Communication Mass Spectrometry*, 24, 2023–2029.
- Perry, D. M., & Hayes, J. E. (2016). Effects of matrix composition on detection threshold estimates for methyl anthranilate and 2-aminoacetophenone. *Food*, 5(35), 1–10.
- Rapp, A. (1996). Volatile compounds in wines of new fungus resistant grapevine varieties compared to wines of *Vitis vinifera* cultivars. *OIV Oenologie, 76e Assemblée générale de l'OIV, Paris*.
- Sasaki, K., Takase, H., Kobayashi, H., Matsuo, H., & Takata, R. (2015). Molecular cloning and characterization of UDP-glucose: Furanol glucosyltransferase gene from grapevine cultivar Muscat Bailey A (*Vitis labrusca* × *V. vinifera*). *Journal of Experimental Botany*, 66, 6167–6174.
- Schmarr, H. G., Keiser, J., & Krautwald, S. (2016). An improved method for the analysis of 2-aminoacetophenone in wine based on headspace solid-phase microextraction and heart-cut multidimensional gas chromatography with selective detection by tandem mass spectrometry. *Journal of Chromatography A*, 1477, 64–69.
- Silva, J. K., Cazarin, C. B. B., Correa, L. C., Batista, A. G., Furlan, C. P. B., Biasoto, A. C. T., & Marostica-Junior, M. R. (2015). Bioactive compounds of juices from two Brazilian grape cultivars. *Journal of the Science of Food and Agriculture*, 96, 1990–1996.
- Slegers, A., Angers, P., Ouellet, E., Truchon, T., & Pedneault, K. (2015). Volatile compounds from grape skin, juice and wine from five interspecific hybrid grape cultivars Grownin Québec (Canada) for wine production. *Molecules*, 20, 10980–11016.
- Toscano, L. T., Tavares, R. L., Toscano, L. T., da Silva, C. S. O., de Almeida, A. E. M., Biasoto, A. C. T., ... Silva, A. S. (2015). Potential ergogenic activity of grape juice in runners. *Appl. Physiol. Nutr. Metab.*, 40, 899–906.
- Wang, J., & De Luca, V. (2005). Grape fruit esters, including 'foxy' methyl anthranilate. *The Plant Journal*, 44, 606–619.
- Zulj, M. M., Maslov, L., Tomaz, I., & Jeromel, A. (2015). Determination of 2-aminoacetophenone in white wines using ultrasound assisted SPME coupled with GC-MS. *Journal of Analytical Chemistry*, 70, 814–818.