gallic acid); TFA, total flavanols (mg/L catechin); OD, ortho-diphenols (mg/L catechin); [a]Browning index (absorbance at 420 nm); [b]Antioxidant activity expressed as Trolox equivalents (mmol TEAC L^{-1}). T3, 3 months; T6, 6 months; T9, 9 months; T15, 15 months aging on lees.

PHENOLIC COMPOSITION OF SKINS AND PULPS OF GRAPES FOR TRADITIONAL SPARKLING WINES IN THE NORTHEAST OF BRAZIL

COMPOSITION PHÉNOLIQUE DE PELLICULES ET PULPES DE RAISINS DESTINÉS À L'ELABORATION DE VINS MOUSSEUX AU NORD-EST DU BRÉSIL

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Abstract

Among the compounds contributing to the grape and wine qualities, phenolics play an important role. Therefore, the objective of this study was to determine the phenolic compounds of skins and pulps of white and red grapes at harvest, destined to traditional sparkling wines, from two winegrowing regions producing tropical wines in the Northeast of Brazil. Chenin Blanc and Syrah were harvested in June 2015 in the São Francisco Valley, at 350 m of altitude, while Chardonnay and Pinot Noir grapes were harvested in September 2015 in the Chapada Diamantina-Bahia State, at 1,100 m of altitude. Twenty phenolics were determined by HPLC in skins and pulps separated manually and extracted using ethanol, each sample composed by 50 berries in triplicate. In the pulps of the white grapes, quercetin-3-glucoside (flavonol) was the most concentrated compound in Chardonnay (2.73 mg Kg^{-1}), while in Chenin Blanc was the galic acid (0.25 mg Kg^{-1}). In the red cultivars, the most concentrated compound determined in the pulps of Pinot Noir was quercetin-3-glucoside (1.89 mg Kg^{-1}), while in Syrah was epigallocatechin gallate (flavanol) (0.31 mg Kg^{-1}). In Chenin Blanc and Chardonnay skins, the most concentrated compound was quercetin-3-glucoside (9.20 and 129.30 mg Kg^{-1}, respectively). In the red grapes, the most concentrated compound was also the same for Pinot Noir and Syrah, the malvidin-3-glucoside anthocyanin (254.13 and 649.13 mg Kg^{-1}, respectively). It is interesting to highlight that not only cultivar effect collaborated to the phenolic profiles, but also the geographic localization of the winegrowing areas.

Keywords: *Vitis vinifera* L., grape, phenolic compounds, HPLC

Résumé

Parmi les composés qui contribuent pour la qualité de raisins et de vins, les phénoliques jouent un important rôle. Ainsi, l'objectif de cette étude a été determiner les composés phénoliques de pellicules et de pulpes de raisins blancs et rouges, destinés à l'élaboration de vins mousseux traditionnels, de deux régions de production de vins tropicaux au Nord-Est du Brésil. Chenin Blanc et Syrah ont été récoltés en Juin 2015 dans la Vallée du São Francisco, à 350 m d'altitude, tandis que Chardonnay et Pinot Noir ont été récoltés en septembre 2015 dans la Chapada Diamantina, l'État de Bahia, à 1.100 m d'altitude. Vingt phénoliques ont été déterminés par HPLC en pellicules et pulpes separées manuellements et extraites avec de l'éthanol, étant chaque échantillon constitué par 50 baies en triplicate. Dans les pulpes des raisins blancs, la quercetine-3-glucoside (flavonol) a été la plus concentrée en Chardonnay (2,73 mg Kg^{-1}), tandis qu'en Chenin Blanc a été l'acide gallique (0,25 mg Kg^{-1}). Dans les cépages noirs, le composé phénolique le plus concentré déterminée dans les pulpes de Pinot Noir a été aussi la quercetine-3-glucoside (1,89 mg Kg^{-1}), tandis que dans les pulpes de Syrah a été l'epigallocatechine gallate (flavonol) (0,31 mg Kg^{-1}). Dans les pellicules de Chenin Blanc et Chardonnay, le composé le plus concentré a été la quercetine-3-glucoside (9,20 et 129,30 mg Kg^{-1}

respectivement). Dans les cépages rouges, le composé le plus concentré a été le même pour Pinot Noir et Syrah, l'anthocyanine malvidine-3-glucoside (254,13 et 649.13 mg Kg^{-1}, respectivement). C'est interessant de souligner que ce n'est pas que le cépage qui a joué un rôle sur la composition phénolique, mais aussi la localizaion géographique des aires vitivinicoles.

Mots-clés: Vitis vinifera L., raisin, composes phénoliques, HPLC

Introduction

Phenolics are composed basically by non-flavonoid (hydroxybenzoic and hydroxycynamic acids and stilbenes) and flavonoid compounds (anthocyanins, flavonols and flavanols). Grape has the highest concentrations of these compounds as compared to other fruits (Obreque-Slier et al. 2013; Perestrelo et al. 2012; Pinilla et al., 2012; Burin et al., 2011; Pereira et al., 2005).

Differences between phenolic compound concentrations are strongly influenced by grape cultivar and also dependent of the vegetal tissue, between skins and pulps (Pantelic´ et al. 2016; Teixeira et al. 2013; Lorrain et al. 2013; Pereira et al., 2005). Normally, flavanols and some phenolic acids are located in the seeds, while hydroxycynamic acids in the pulp, and flavonols, some phenolic acids and anthocyanins in the grape skins (Ćurko et al., 2014; Zhang et al., 2014). For white grapes, phenolics are less importante and undesirable, as compared to red grapes, and few studies are carried out in this way (Montealegre et al. 2006). Few studies describing phenolic compounds for grapes destined to sparkling wines are available. In this contexte, the objective of this study was to determine phenolic composition of skins and pulps of White and red cultivars (Vitis vinifera L.) destined to white and rosé traditional sparkling wines in a new winegrowing region located in the Northeast of Brazil, the Chapada Diamantina, in Bahia State.

Material and Methods

Chemicals

Ethanol was purchased from Merck (Darmstadt, Germany). Methanol, acetonitrile and phosphoric acid were obtained from Vetec Química Fina Ltda. (Rio de Janeiro, Brazil), J. T. Baker (Phillipsburg, NJ, USA) and Fluka (Switzerland), respectively. Malvidin-3-glucoside, cyanidin-3-glucoside, peonidin-3-glucoside, delphinidin-3-glucoside and pelargonidin-3-glucoside, kaempferol-3-glucoside, myricetin-3-glucoside, quercitin-3-glucoside, isorhamnetin-3-glucoside, (-)-epicatequin gallate, (-)-epigallocatequin, syringic acid, and t-resveratrol were purchased from Extrasynthese (Genay, France). Galic, cynamic and caffeic acids were obtained from Chem Service (West Chester, USA). P-cumaric and chlorogenic acids were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultra-pure water was obtained from Milli-Q® (Millipore, Bedford, MA, USA).

Grape samples

Grapes of Chardonnay and Pinot noir varieties (Vitis vinífera L.) were harvested in September 2015 from an Observation Unity (UO) located in Morro do Chapéu, in Bahia State, Chapada Diamantina-CD (11° 33' 11'' S and 41° 09' 27'' W, at 1,100 m of elevation), 95 days after pruning. Both varieties are grafted onto Paulsen 1103 rootstock. Grapes of Chenin Blanc and Syrah varieties were also harvested in September 2015, in Casa Nova, Bahia State, São Francisco Valley-SFV (9° 16' S and 40° 52' W, at 413.5 m of elevation), 100 days after pruning. Both varieties are grafted onto IAC-766 (Riparia do Traviú x Vitis Caribaea) rootstock. The main difference between these two winegrowing areas, is that in Chapada Diamantina, vines are pruned two times and obtained one only harvest per year, while in São Francisco Valley vines are pruned two times and get two harvests per year (Pereira et al., 2016). Both regions are located in the Northeast region of Brazil, SFV producing commercial tropical there are 30 years ago, while CD did not yet have commercial wines, the first ones will be in the market in 2019, the tests and evaluations started in 2010.

Extraction of skin and pulp

The skins and pulp were separated manually from 50 berries in triplicate, to achieve two separate extractions. The skins were ground using a Waring blender for 2 min and then extracted with 96% ethanol (80 mL of EtOH per 40 skins) for 1 hour. The pulp was extracted on ice with 96% ethanol (80 mL of EtOH per 40 pulps) for 1 hour with agitation, than centrifuged at 7,500 rpm for 7 min (Pereira te al., 2005). Then, 1.5 mL of the supernatant were placed in eppendorf tubes, evaporated at vacuum and recupered with 1.5 mL with the mobile phase, and filtered using a 0.45 µm membrane filter and placed in an amber vial.

Phenolics were determined by high performance liquid chromatography-HPLC using a Waters equipment (model Aliance e2695) equipped with DAD, according to the methodology developed by Natividade et al. (2013).

All analyses were carried out in triplicate and results were expressed as means and standard deviation.

Statistical analysis

The statistical analysis was performed using variance analysis (ANOVA), by SPSS Inc. 17.0 software (Chicago, IL, USA).

Results and Discussion

Results of the classical analyses of the grape musts of Chardonnay, Chenin Blanc, Pinot noir and Syrah are showed in the Table 1.

Musts from São Francisco Valley-SFV, of Chenin Blanc and Syrah grapes presented the highest values of soluble sugars (20.40 and 20.1ºBrix, respectively), while Pinot noir presented the lowest value (19.03 ºBrix). For acidity, musts from Chapada Diamantina-CD, of Chardonnay and Pinot noir, presented the highest values as compared to musts of Chenin Blanc and Syrah. These results were expected because São Francisco Valley is a warmer region than Chapada Diamantina (climatic data not shown) (Pereira et al., 2016). In both cases, high acidity was important to elaborate traditional sparkling wines.

Table 2 shows results obtained of the HPLC analyses of the phenolic compounds determined in skins and pulps of grapes harvested from four cultivars installed in two regions of the Northeast of Brazil, São Francisco Valley-SFV and Chapada Diamantina-CD, in Bahia State. It was possible to identify and quantify 19 phenolic compounds. Significant differences were found according to the vegetal tissue, between skins and pulps, and also according to the varieties. None anthocyanin was detected in the pulps of all grapes. Pulps of Chardonnay Chardonnay presented the highest concentrations of kaempferol-3-glucoside, quercetin-3-glucoside, rutin-3-glucoside and syringic acid as compared to the others. Pulps of Pinot noir presented the highest values of Isorhamnetin-3-glucoside, (-)-epigallocatechin and cinnamic acid than then others. Pulps of Syrah presented higher concentrations of caffeic acid as compared to the other pulps. The results of the sum of all phenolics determined is interesting, because white grapes presented the lowest values, that was expected (Chardonnay higher then Chenin Blanc), and in the red grapes pulps of Pinot Noir presented higher concentrations as compared to pulps of Syrah.

Results obtained from skins also presented significant results. As expected, none anthocyanin was found in the white grapes. Pulps of Chardonnay presented the highest values of kaempferol-3-glucoside and quercetin-3-glucoside. It is interesting because the highest concentration of quercetin-3-glucoside was found in both tissues of Chardonnay, skins and pulps, as compared to the other cultivars. Skins of Pinot noir presented the highest concentrations of (-)-epicatechin gallate, (-)-epigallocatechin and p-coumaric acid. Skins of Syrah presented the highest values of all anthocyanins (malvidin-3-glucoside, cyanidin-3-glucoside, peonidin-3-glucoside, delphinidin-3-glucoside and pelargonidin-3-glucoside), isorhamnetin-3-glucoside, myricetin-3-glicoside, caffeic acid and t-resveratrol as compared to the other grape skins. The sum of all phenolics in the skins, the white grapes presented the lowest values (Chardonnay was higher than Chenin Blanc), and the highest values for the red grapes (Syrah higher than Pinot Noir). The values found in this study for the

anthocyanin malvidin-3-gluciside were higher than those obtained by Torres et al. (2010). The flavonol quercetin-3-glucoside was the most concentrated found in the grapes, in pulps and skins, and Chardonnay presented the highest values of this compound in both tissues. The values determined of quercetin-3-glucoside in skins were higher than those found by Jin et al. (2009) in eight varieties studied in China. The highest concentration of flavonols in this study was determined in the skins, the same result obtained by Pantelic' et al. (2016) studying thirteen Vitis vinifera L. varieties in Serbia. Flavanols were determined in low concentrations for all tissues of the varieties, because the focus of the harvest was to elaborate traditional sparkling wines. These compounds are related to bitterness and body for red wines (Monagas et al. 2005). Phenolic acids were determined in some tissues and varieties (Table 2), and syringic was determined in highest concentration in pulps of Chardonnay, while p-coumaric acid was found in highest values in skins of Pinot Noir. Resveratrol was not detected in both tissues of Chardonnay and Pinot Noir, cultivated in Chapada Diamantina, at 1,100 m of elevation. Pantelic' et al. (2016) also did not find this compound in Pinot Noir cultivated in Serbia.

Differences of the phenolics found in this study are related to the cultivar but also could be influenced by climatic and pedologic factors, in each locality. Altitude can play an important role on phenolics.

Conclusion

Skins and pulps of four grape varieties from different winegrowing regions of Brazil presented different results of phenolic compounds. Chardonnay presented quercetin-3-glucoside in highest concentrations in both tissues. Pinot Noir presented higher sum of all phenolics in the pulps than others. Skins of Syrah presented the highest values of all anthocyanins and the sum of all phenolics.

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Table 1. Physicochemical analyses of grape musts at harvest destined to traditional sparkling wines in the Northeast of Brazil, 2015 vintage.

Grape variety	pH	TSS (°Brix)	TA (g L^{-1})
Chardonnay-CD	3.22 ±0.03	19.40 ±0.8	11.28 ±0.92
Chenin Blanc-SFV	3.04 ±0.20	20.40 ±0.37	10.20 ±0.52
Pinot Noir-CD	3.42 ±0.32	19.03 ±0.12	11.05 ±0.35
Syrah-SFV	3.20 ±0.40	20.11 ±0.74	8.10 ±0.61

Results are expressed as means ± standard deviation of three replicates. TSS: Total Soluble Solids; TA: titratable acidity in grams per liter of tartaric acid. CD: Chapada Diamantina; SFV: São Francisco Valley.**Table 2.** Phenolic compounds determined in pulps and skins of grapes from four varieties harvested in 2015, in the Northeast of Bra

Phenolic compounds (mg kg^{-1})	Pulp				Skin			
	Chardonnay	Chenin Blanc	Pinot Noir	Syrah	Chardonnay	Chenin Blanc	Pinot Noir	Syrah
Anthocyanins								
Cyanidin-3-glucoside	nd	nd	nd	nd	nd	nd	28.07 ±6.99b	216.13 ±2.13a
Pelargonidin-3-glucoside	nd	nd	nd	nd	nd	nd	8.27 ±2.32b	39.10 ±7.59a
Delphinidin-3-glucoside	nd	nd	nd	nd	nd	nd	16.73 ±0.31b	133.60 ±1.38a
Malvidin-3-glucoside	nd	nd	nd	nd	nd	nd	254.13 ±4.62b	649.13 ±5.61a
Peonidin-3-glucoside	nd	nd	nd	nd	nd	nd	60.07 ±2.00b	130.15 ±1.07a
Flavonols								
Kaempferol-3-glucoside	1.17 ±0.42a	0.09 ±0.06c	0.45 ±0.16b	0.13 ±0.06c	15.40 ±2.27a	3.83 ±2.11c	6.67 ±1.63bc	9.78 ±1.10b
Isorhamnetin-3-glucoside	0.13 ±0.09b	0.03 ±0.02b	0.43 ±0.13a	0.07 ±0.06b	0.67 ±0.12c	0.30 ±0.01d	6.73 ±1.86b	27.47 ±5.03a
Myricetin-3-glicoside	0.01 ±0.02b	nd	0.03 ±0.05ab	0.07 ±0.02a	nd	nd	0.47 ±0.12b	6.73 ±0.40a
Quercetin-3-glucoside	2.73 ±0.61a	0.17 ±0.10c	1.89 ±0.37b	0.29 ±0.17c	129.30 ±1.21a	9.20 ±3.39d	114.31 ±2.47b	51.43 ±5.75c
Rutin-3-glucoside	0.33 ±0.17a	0.07 ±0.05bc	0.13 ±0.05b	0.04 ±0.04c	3.80 ±0.72a	2.13 ±0.55a	2.13 ±0.99a	2.37 ±0.21a
Flavanols								
(-)-Epicatechin gallate	0.17 ±0.08a	0.23 ±0.02a	0.18 ±0.13a	0.31 ±0.08a	0.60 ±0.20b	1.23 ±0.35b	2.00 ±0.20a	1.00 ±0.20b
(-)-Epigallocatechin	0.85 ±0.31b	0.07 ±0.02c	1.39 ±0.05a	0.17 ±0.02c	0.40 ±0.03b	0.17 ±0.15b	1.80 ±0.40a	0.23 ±0.06b
Phenolic acids								
Caffeic acid	nd	0.13 ±0.05b	nd	0.17 ±0.02a	nd	0.13 ±0.058b	nd	0.90 ±0.10a
Cinnamic acid	nd	nd	0.12 ±0.03a	0.04 ±0.05b	nd	0.10 ±0.01a	nd	0.10 ±0.04a
Gallic acid	0.18 ±0.02a	0.25 ±0.10a	0.21 ±0.08a	0.15 ±0.10a	nd	0.23 ±0.15a	nd	0.37 ±0.21a
Syringic acid	0.73 ±0.26a	nd	0.37 ±0.12b	nd	1.07 ±0.46a	nd	0.80 ±0.69a	nd
o- Coumaric acid	0.21 ±0.10a	nd	0.19 ±0.02a	nd	0.87 ±1.03a	nd	0.67 ±0.31a	nd
p-Coumaric acid	nd	0.23 ±0.05a	0.25 ±0.06a	0.23 ±0.08a	0.07 ±1.12b	0.20 ±0.03b	3.33 ±0.81a	0.30 ±0.32b
Stilbene								
t-Resveratrol	nd	0.08 ±0.07a	nd	0.08 ±0.05a	nd	0.53 ±0.21b	nd	0.93 ±0.12a

Sum of the phenolics	**6.51**	**1.35**	**23.46**	**15.74**	**152.18**	**18.05**	**506.18**	**1,269.72**

Different etters in the same row represent significant differences among varieties according to Tukey test, 0.05. nd: No detected.