Food Research International 116 (2019) 190–199

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Effect of the pre-treatment and the drying process on the phenolic composition of raisins produced with a seedless Brazilian grape cultivar

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ARTICLE INFO

Keywords:
Raisins
BRS Morena
Drying
Phenolic composition
Anthocyanins
Proanthocyanidins
Flavonoids
HPLC-DAD-ESI-MS^n

Chemical compounds:
malvidin 3-glucoside (PubChem CID: 443652)
vitisin A (PubChem CID: 16131430)
vitisin B (PubChem CID: 16138152)
GRP, 2-S-glutationil-*trans*-caftaric (PubChem CID: 71308212)
trans-caftaric acid (PubChem CID: 6440397)
trans-piceid (PubChem CID: 5281718)
myricetin 3-glucoside (PubChem CID: 44259426)
quercetin 3-glucuronide (PubChem CID: 11655911)
(+)-catechin (PubChem CID: 9064)
procyanidin B1(PubChem CID: 11250133)

ABSTRACT

The grape is an important fruit regarding economic and health benefit parameters, because of its large consumption around the world and their bioactive phenolic compounds. The drying process of *BRS Morena* grapes, whether pre-treated or not with olive oil for producing raisins, resulted in qualitative and quantitative changes in their phenolic composition (anthocyanins, flavonols, stilbenes, hydroxycinammic acid derivatives, flavan-3-ols and proanthocyanidins). The raisins with the pre-treatment preserved more anthocyanins and proanthocyanidins than the raisins not pre-treated. Moreover, the total dehydration time accelerated by approximately 40% in the raisins pre-treated. Therefore, the production of raisins of *BRS Morena* grapes pre-treated with olive oil as a natural surfactant constitutes an interesting process from both the industrial and health points of view, because of the remarkable reduction in the processing time and the preservation of high concentrations of flavonoids, which have important claims to health benefits from biological activities.

Abbreviations: acglc, 6″-(acetyl)glucoside; C, (+)-catechin; cfglc, 6″-(caffeoyl)glucoside; CG, (−)-catechin 3-gallate; cmglc, 6″-(*p*-coumaroyl)glucoside; cy, cyanidin; DAD, diode arrange detector; dp, delphinidin; EC, (−)-epicatechin; ECG, (−)-epicatechin 3-gallate; EGC, (−)-epigallocatechin; EGCG, (−)-epigallocatechin 3-gallate; Embrapa, Brazilian Agricultural Research Corporation; ESI-MS^n, electrospray ionization mass spectrometry system; ext., extension unit; g, grams; gal, galactoside; GC, (−)-gallocatechin; GCG, gallocatechin 3-gallate; glc, glucoside; glcU, glucuronide; GRP, 2-*S*-glutationil-*trans*-caftaric; HCAD, hydroxycinnamic acid derivatives; HPLC, high efficiency liquid chromatography; I, isorhamnetin; K, kaempferol; kg, kilograms; L, laricitrin; m, meter; M, myricetin; mDP, mean degree of polymerization; MG, *BRS Morena* fresh grape; MRM, Multiple Reaction Monitoring; mv, malvidin; ND, nondetectable; PA, proanthocyanidin; PC, principal component; PCA, principal component analysis; pH, hydrogen potential; pn, peonidin; PPO, polyphenoloxidase; Q, quercetin; R, raisins without pre-treatment; RO, raisins pre-treated with olive oil; rut, rutinoside (6″-rhamnosylglucoside); s, seconds; S, syringetin; term, terminal unit

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[1] Deceased 23 April 2018.

https://doi.org/10.1016/j.foodres.2018.08.012
Received 23 May 2018; Received in revised form 30 July 2018; Accepted 2 August 2018
Available online 03 August 2018

1. Introduction

The grape is one of the most consumed fruits in its fresh form and it is considered an important raw material economically because of its use in the development of various products, such as wines, juices, jellies and raisins (Georgiev, Ananga, & Tsolova, 2014). Nutritionally, grapes, especially the red ones, are a rich source of phenolic compounds in the diet (Lago-Vanzela, Da-Silva, Gomes, García-Romero, & Hermosín-Gutiérrez, 2011a, 2011b; Rebello et al., 2013; Xia, Deng, Guo, & Li, 2010). Together with other bioactive compounds, such as carotenoids, vitamins E and C, they are known as promoters of human health (Gioxari, Kogiannou, Kalogeropoulos, & Kaliora, 2016; Szajdek & Borowska, 2008).

Among those products derived from grapes, raisins have been prominent in the last decade, especially if they are prepared with seedless grapes (Benlloch-Tinoco, Carranza-Concha, Camacho, & Martínez-Navarrete, 2015). They not just combine market trends, such as "healthiness and wellness" and "convenience and practicality", but also allow the full use of the grape, and can be easily incorporated into other processed foods like yogurts, cakes, *panettone*, ice creams, granola, cereal bars, etc. (Doymaz, 2006).

Hand in hand with the market demand, there has been investment in the development of seedless table grapes with potential to produce raisins (Camargo, Nachtigal, Maia, de Oliveira, and da Protas, 2003). Among these varieties, is the *BRS Morena* grape, developed by the Brazilian Agricultural Research Corporation (Embrapa) by crossing the Marroo seedless and Centennial seedless varieties. This grape has good flavor, firm texture and large berries containing a high concentration of phenolic compounds (Camargo, Nachtigal, Maia, de Oliveira, and Protas, J. F. Da S., 2003; Lago-Vanzela et al., 2011a). Preliminary studies showed that raisins produced with this cultivar had nice flavor, good appearance and softness as well as a characteristic aroma (de Freitas et al., 2013).

In the process of drying grapes to produce raisins, attention has being given to the operations and process efficiency because they are directly influenced by the intrinsic characteristics of the grapes (berry size, volume, sugar concentration, and presence of pruine - waxy membrane which covers the berries cuticular epidermis) (Doymaz, 2006; Esmaiili, Sotudeh-Gharebagh, Cronin, Mousavi, & Rezazadeh, 2007). This waxy coating promotes an effective barrier against water loss, resulting in a slow rate of water removal during the dehydration process of the grape (Adiletta, Russo, Senadeera, & Di Matteo, 2016; Jairaj, Singh, & Srikant, 2009). Therefore, the phenolic compounds present in grapes may be exposed for long periods of time at different temperatures and oxygen levels, leading to numerous chemical and biochemical degradative processes that can lead to the formation of new compounds with different colour and biological activity than those originally present in the fruit (Carranza-Concha, Benlloch, Camacho, & Martínez-Navarrete, 2012; Karadeniz, Durst, & Wrolstad, 2000).

In order to accelerate the drying process to produce raisins, various chemical and physical pre-treatments have been investigated and applied (Adiletta et al., 2016; Jairaj et al., 2009). In the chemical pre-treatments, often, an alkaline solution such as potassium carbonate is combined with other components such as olive oil (Telis, Lourençon, Gabas, & Telis-Romero, 2006) or ethyl oleate (Bingol, Roberts, Balaban, & Devres, 2012; Gabas, Menegalli, & Telis-Romero, 1999). However, the presence of residues of additives in the raisins can lead to human health problems (Adiletta et al., 2016). Studies aiming the understanding and improvement of technical alternatives in the grape drying process, such as pre-treatments using natural products or compounds such as olive oil, along with the preservation of most of the phenolic compounds present in these products are required to increase the nutritional quality of interesting products like raisins.

Therefore, this study aimed to determine the qualitative and quantitative changes of phenolic compounds of raisins produced with the seedless red grape cultivar BRS Morena, pre-treated or not with olive oil as a natural surfactant. The raisins were obtained by conventional convective drying and the profiles of phenolic compounds of fresh and processed grapes (raisins) were determined by high-performance liquid chromatography with a diode array detection coupled with an electrospray ionization mass spectrometry (HPLC-DAD-ESI-MS^n).

2. Material and methods

2.1. Chemicals

All solvents were of chromatographic grade (> 99%); all chemical standards were of analytical grade (> 95%); ultrapure water (Milli-Q system) was used. Chemical standards malvidin 3-glucoside (mv-3-glc), caffeic acid, *p*-coumaric acid, *trans*-caftaric acid, *trans*-piceid, (−)-epigallocatechin (EGC), (−)-gallocatechin (GC), and procyanidin B1 (PB1) were from Phytolab (Vestenbergsgreuth, Germany). The other chemical standards, cyanidin-3-glucoside (cy-3-glc), procyanidin B2 (PB2), quercetin (Q), kaempferol (K), isorhamnetin (I), myricetin (M), syringetin (S), the 3-glucosides of Q, K, I and S, 3-galactosides of Q, K and I, (−)-catechin 3-gallate (CG), (−)-epicatechin 3-gallate (ECG), and (−)-epigallocatechin 3-gallate (EGCG) were from Extrasynthese (Genay, France); gallic acid, *trans*-resveratrol, (+)-catechin (C), (−)-epicatechin (EC), and (−)-gallocatechin 3-gallate (GCG) were from Merck (Kenilworth, USA). Flavonols not commercially available (M-3-glc and quercetin 3-glucuronide (Q-3-glcU)) were previously isolated from Petit Verdot grape skins (Castillo-Muñoz et al., 2009) and the procyanidin B4 (PB4) was kindly supplied by Prof. Fernando Zamora (Departament of Biochemistry and Biotechnology, Universitat Rovira i Virgili, Spain). The vitisins A and B were obtained by reaction of mv-3-glc with pyruvic acid and acetaldehyde (Schwarz, Quast, Von Baer, & Winterhalter, 2003) and the *trans* isomers of resveratrol and its 3-glucosides (piceid) were converted into their respective *cis* isomers by UV irradiation (366 nm light for 5 min in quartz vials) of *trans* isomer solutions in 25% methanol.

2.2. BRS Morena grapes

BRS Morena grapes (MG), vintage of 2014, were harvested in the city of Jales (São Paulo, Brazil) located at 20°15′08" S and 50°33′29″ E, and 500 m above sea level (referred to WGS84 datum (World Geodetic System 1984), at their expected maturity level and in good sanitary conditions.

2.3. Raisins preparation

The MG were selected and sanitized with chlorinated water. Then, half of the selected grape berries were pre-treated with extra virgin olive oil by manual homogenization in the proportion of 1.42 ml of olive oil for each 700 g of grape berries. This pre-treatment was applied aiming to help the breaking of the wax present on the surface of the grape skins and to facilitate the exit of water during the drying process, since the extra virgin olive oil is considered a natural surfactant (maximum acidity ≤0.50%; peroxide index ≤20.00 meq $O_2 \cdot kg^{-1}$). The second half of the selected grape berries were not submitted to any type of pre-treatment (control). Both groups of grape berries were dehydrated separately in a convective drier with hot air (60 °C; 1 $m \cdot s^{-1}$). The drying process was finished after 75% weight reduction, controlled through the weighing of the trays containing the grape berries. The raisins were put into polyethylene bags and stored under freezing until analysis. The trials were performed in triplicate.

2.4. Physicochemical characteristics

The physicochemical characteristics of the MG, the raisin control (no pre-treatment) (R) and the raisins pre-treated with olive oil (RO)

were determined according to official methods of analysis (AOAC, 2005): moisture content; soluble solids (only for the fresh grapes - °Brix at 25 °C); hydrogen potential (pH); titratable acidity content (as tartaric acid g·100 g^{-1}); total sugar content (as glucose g·100 g^{-1}), water activity (for both the raisin samples only). In addition, the weight (g), the length (L) and width (W) (cm) of the grapes and the raisins were determined.

2.5. Identification and quantification of phenolic compounds using HPLC-DAD-ESI-MS^n

The separation, identification and quantification of the phenolic compounds (PC) (anthocyanins, flavonols, hydroxycinnamic acid derivatives (HCAD), stilbenes, flavan-3-ols (monomers and dimers) and proanthocyanidins (PA)) in the 3 samples (MG, R and RO) were performed using previously described methods (Rebello et al., 2013), with minor modifications adapted to the raisin analysis. The compounds of interest of the samples were extracted as described by (Lago-Vanzela et al., 2011a, 2011b), with minor modifications for the raisins samples analyses. The raisins (n = 3; 10 g per sample) were subjected to three repeat extractions with 25 ml of extraction solution (methanol: water: formic acid – 50: 48.5: 1.5) for a complete recovery of phenolic compounds. All procedures were performed in triplicate.

The HPLC separation, identification and quantification of phenolic compounds present in the samples were carried out using an Agilent 1100 Series system (Agilent Technologies, Santa Clara, USA and Macherey-Nagel, Düren, Germany) equipped with a Diode Array Detector (DAD; G1315B) and a LC/MSD Trap VL (G2445C VL) electrospray ionization mass spectrometry (ESI-MS^n) system, coupled to an Agilent ChemStation (version B.01.03) data-processing unit. The mass spectra data was processed using the Agilent LC/MS Trap software (version 5.3).

For the analysis of anthocyanins and their derivatives (vitisins A and B), the aforementioned prepared samples extracts were submitted to filtration using a Chromafil PET 20/25 polyester membrane (0.20 µm), (Macherey-Nagel, Düren, Germany) and injected (10 µl) directly into a Zorbax Eclipse XDB-C18 reversed-phase column (2.1 × 150 mm; 3.5 µm particle size); (Agilent Technologies, Santa Clara, USA), maintained at 40 °C, according to the method described by Rebello et al. (2013). For the analysis of flavonols, HCAD, and stilbenes, aliquots of grape and raisins extracts were subjected to solid-phase extraction using Bond Elut Plexa PCX cartridges (6 cm^3, 500 mg of adsorbent) (Agilent Technologies, Santa Clara, USA) (Castillo-Muñoz, Gómez-Alonso, García-Romero, & Hermosín-Gutiérrez, 2007), filtered through a Chromafil PET 20/25 polyester membrane (0.20 µm, Macherey-Nagel, Düren, Germany) and injected (20 µl) into the same chromatographic system as that used for the anthocyanin analysis.

For PC identification, an ion trap ESI/MS-MS analyser was used in positive (for anthocyanins) and negative (for flavonols, HCAD, and stilbenes) ionization modes, as previously described (Rebello et al., 2013). The identification was mainly based on spectroscopic data (UV–Vis and MS/MS) for authentic standards or data from previous reports (Barcia et al., 2014; Lago-Vanzela et al., 2014; Rebello et al., 2013). For PC quantitation, DAD-chromatograms were extracted at 520 nm (for anthocyanins), 360 nm (for flavonols) and 320 nm (for HCAD).

The analysis of the flavan-3-ol monomers and procyanidin B-type dimers was carried out using an HPLC Agilent1200 series system equipped with DAD (Agilent Technologies, Santa Clara, USA) and coupled to an AB Sciex 3200 TRAP (Applied Biosystems, Foster City, USA) with triple quadrupole, turbo spray ionization (electrospray assisted by a thermonebulization) mass spectroscopy system (ESI-MS/MS). The chromatographic system was managed by an Agilent ChemStation (version B.01.03) data-processing unit, and the mass spectra data were processed using the Analyst MSD software, version 1.5 (Applied Biosystems, Foster City, USA) according to (Rebello et al., 2013).

Structural information concerning the PA was obtained using the pyrogallol-induced acid-catalyzed depolymerization method (Bordiga, Coisson, Piana, Travaglia, & Arlorio, 2009; Rebello et al., 2013). The diluted samples before and after the acid-catalyzed depolymerization method were injected (10 µl) into an Ascentis C18 reversed-phase column (150 mm × 4.6 mm with 2.7 µm particle size, Merck, Kenilworth, USA), maintained at 16 °C. The solvents and gradients used for this analysis, the two types of MS scan used (Enhanced MS – EMS and Multiple Reaction Monitoring – MRM), as well as all the mass transitions (m/z ratios) for identification and quantitation were those previously described (Rebello et al., 2013).

All the standards were used for identification and quantitation through calibration curves covering the expected concentration ranges. For non-available standards, the quantitation was done using the calibration curve of the most similar compound: mv-3-glc for the anthocyanins, Q-3-glc for the flavonol 3-glycosides and their free aglycones, caftaric acid for the HCAD, (+)-catechin (C) for polymeric flavan-3-ols (total PA), individual flavan-3-ol monomers and dimers by their corresponding standards, considering their total sum as mg of C equivalents.

2.6. PA quantification by methylcellulose precipitation

The total content of PA was also determined by the methylcellulose precipitation method (Sarneckis et al., 2006). The PC contained in the grape and raisin extracts were measured at 280 nm before and after the PA precipitation with the methylcellulose. The absorbance differences were correlated with the PA concentration, expressed as mg (−)-epicatechin (EC) equivalentes.

2.7. Data analysis

All the data were treated using a one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls post-hoc test (when P value < 0.05). The Principal Component Analysis (PCA) was performed for each class of compounds and samples in order to detected tendencies ("loadings" at "Rotated Component Matrix" > 0.80). All statistical analyzes were applied at a significance level of 0.05 using version 20 of the IBM SPSS Statistics (SPSS Inc., IBM).

3. Results and discussion

3.1. Raisins production

MG used as raw material for R production had an average berry size (length x width), 2.25 ± 0.09 × 1.55 ± 0.13 cm and weight of 4.79 ± 0.06 g, as well as the following physicochemical characteristics: moisture content, 79.48 ± 0.03%; soluble solids, 18.03 ± 0.06°Brix; total sugar content, 16.13 ± 0.02 g glucose·100 g^{-1} grape; titratable acidity content, 0.66 ± 0.03 g tartaric acid·100 g^{-1} grape; pH, 3.61 ± 0.01.

The grapes dried without olive oil pre-treatment (R) was completed after a time of 41 h, whereas the grapes previously treated with olive oil (RO) had a drying time of 22.5 h, resulting in a 45.13% reduction of the time required for the raisins production. This result was close to that reported in a study carried out by Bingol et al. (2012), where a 50% drying time reduction at 60 °C was achieved after applying a pre-treatment of K_2CO_3 (5%) and ethyl oleate (2%) to Thompson seedless grapes. In a study with *Rubi* grapes, using an immersion solution containing olive oil (0.5%) and potassium carbonate (6%) at 50 °C, drying time was also reduced when compared to grapes that were pretreated with only 2.5% olive oil (Telis et al., 2006). These results show that the use of olive oil, in the pre-treatment of grapes, gives greater efficiency in dehydrating to get raisins. A possible explanation for this positive effect of olive oil can be attributed to its tensioactive action on fatty

acids in the cuticular wax, which results in the formation of micro pores on the surface of the peel and/or collapse of the cells and intermolecular bonds of the tissue, thus improving the internal diffusion of water and consequently drying rates, like the effect observed on pretreatment with potassium carbonate (Grncarevic, 1963; Vazquez, Chenlo, Moreira, & Cruz, 1997).

The corresponding average values of the physicochemical properties obtained for the R samples were: average size of the raisins (length x width), 1.58 ± 0.35 × 0.75 ± 0.17 cm, weight, 1.23 ± 0.13 g, moisture, 18.32 ± 0.92%; total sugars, 87.09 ± 0.01 g glucose·100 g^{-1} raisin; water activity at 25 °C, 0.425 ± 0.006; titratable acidity, 3.09 ± 0.12 g tartaric acid·100 g^{-1} raisin and pH, 3.48 ± 0.05. For RO samples the results were: average size of raisins (length x width), 1.52 ± 0.33 × 0.72 ± 0.16 cm, weight, 1.17 ± 0.12 g, moisture, 18.90 ± 0.34%; total sugars, 90.17 ± 0.01 g glucose·100 g^{-1} raisin; water activity at 25 °C, 0.440 ± 0.007; titratable acidity, 2.69 ± 0.12 g tartaric acid·100 g^{-1} raisin and pH, 3.35 ± 0.21. Comparing the results of the chemical characterization of grapes *in natura* with the raisins, as expected, shows that the drying process has a concentrating effect on the non-volatile constituents present in grapes resulting from the removal of water, making the raisins a highly energetic food.

3.2. Determination of the qualitative and quantitative profiles of PC in MG, R, and RO by HPLC-DAD-ESI-MS^n

3.2.1. Anthocyanins

Using the extracted ion chromatogram (EIC) obtained with the *m/z* values corresponding to each of the different anthocyanidins (aglycones), 19 anthocyanins were detected in the fresh grapes and the raisins (Table 1).

For all three types of BRS Morena samples (grape, R, and RO) a full range of non-acylated 3-glucosylated anthocyanins (delphinidin (dp), cyanidin (cy), malvidin (mv), peonidin (pn), and petunidin (pt)), commonly found in grapes *V. vinifera* (Castillo-Muñoz, Fernández-González, Gómez-Alonso, García-Romero, & Hermosín-Gutiérrez, 2009), their acetyl (ac) derivatives, the complete *trans* series and some *cis* isomers of their *p*-coumaroyl (cm) derivatives were detected, together with the caffeoyl (cf) derivative of mv. There was no chromatographic evidence (using authentic standards), or mass spectral evidence either, of the occurrence of anthocyanins derived from pelargonidin (*m/z* 271) in the samples. A similar result for the BRS Morena grape was reported by Lago-Vanzela et al. (2011a).

In raisins, vitisins A and B were also identified, these compounds being formed from the reaction of pyruvic acid and acetaldehyde, respectively, with mv-3-glc. This presence may be explained by the initial stress undergone by the grapes when submitted to the drying process. Under these drying conditions, the process of respiration of the grapes changes from aerobic to anaerobic and, consequently, elevates the indices of pyruvic acid and acetaldehyde, influencing the formation of vitisins A and B, respectively (Marquez, Dueñas, Serratosa, & Merida, 2012; Vivar-Quintana, Santos-Buelga, Francia-Aricha, & Rivas-Gonzalo, 1999). Marquez, Serratosa, et al. (2012) also reported the synthesis of vitisins A and B during partial dehydration of Merlot and Syrah grapes for producing wine, mainly vitisin B for these both varieties. The authors also pointed out that the synthesis of vitisins occurs to a greater degree for grapes dehydrated off the vine. This is because, in these cases, there is a greater disruption of the grape skin layers which favors the diffusion of the anthocyanins from the skin to their pulp and leads to an increase in the formation of these compounds.

The anthocyanin with the highest molar percentage in all samples was mv-3-glc, however, after dehydration of the grapes, there was a significant increase of this percentage in raisins to the detriment of the percentages of other anthocyanins such as dp-3-glc and their acyl derivatives. This is because mv (trisubstituted methoxylated anthocyanin) is more resistant to thermal degradation than dp (trisubstituted non-

Table 1
Anthocyanins in BRS Morena grape (MG) and raisins with (RO) and without (R) olive oil pre-treatment by HPLC-DAD-ESI-MS/MS (positive ionization mode). Mass spectrum data, molar profiles (percentage of each individual anthocyanins regarding the total content), and total concentration (as equivalents of malvidin 3-glucoside (mv-3-glc)). Given as mean values ± standard deviations (n = 3).

Deprotonated molecule and product ions (*m/z*)	Anthocyanin	molar ratios (%)		
		MG	R	RO
465; 303	dp-3-glc	11.87a ± 1.65	7.54b ± 0.75	8.14b ± 0.71
449; 287	cy-3-glc	3.72ab ± 1.12	4.93a ± 0.43	2.95b ± 0.27
479; 317	pt-3-glc	9.58a ± 0.63	8.96a ± 0.55	8.94a ± 0.57
463; 301	pn-3-glc	11.13b ± 1.41	14.53a ± 0.71	9.61b ± 0.57
493; 331	mv-3-glc	39.19b ± 2.85	43.80a ± 1.45	45.66a ± 0.45
507; 303	dp-3-acglc	0.74a ± 0.05	0.49b ± 0.06	0.84a ± 0.03
491; 287	cy-3-acglc	0.23a ± 0.04	0.26b ± 0.00	0.18a ± 0.02
521; 317	pt-3-acglc	0.77a ± 0.02	0.67b ± 0.03	0.75a ± 0.02
505; 301	pn-3-acglc	0.62a ± 0.01	0.63a ± 0.07	0.54a ± 0.01
535; 331	mv-3-acglc	3.13b ± 0.47	3.01b ± 0.21	3.82a ± 0.22
611; 303	dp-3-cmglc	2.14a ± 0.06	1.30c ± 0.11	1.53b ± 0.06
595; 287	cy-3-cmglc	0.69a ± 0.10	0.76a ± 0.08	0.57a ± 0.02
625; 317	pt-3-cmglc	1.86a ± 0.12	1.57b ± 0.10	1.77a ± 0.04
625; 317	pn-3-cmglc	2.78a ± 0.04	2.71a ± 0.17	2.37b ± 0.05
639; 331	mv-3-cmglc	10.75a ± 1.58	7.86a ± 0.98	11.08a ± 1.23
625; 317	pt-3-*cis*-cmglc	0.09a ± 0.00	0.12a ± 0.02	0.10a ± 0.01
609; 301	pn-3-*cis*-cmglc	0.14b ± 0.01	0.20a ± 0.03	0.14b ± 0.01
639; 331	mv-3-*cis*-cmglc	0.49a ± 0.03	0.30b ± 0.08	0.39ab ± 0.05
655; 331	mv-3-cfglc	0.09c ± 0.01	0.36b ± 0.08	0.62a ± 0.19
Total (mg mv-3-glc/kg of grape FW)		498.17a ± 29.06	55.28c ± 12.27	122.49b ± 16.99
Total (mg mv-3-glc/kg of product FW)		498.17 ± 29.06	251.00 ± 55.72	556.12 ± 77.15
Vitisin A (mg/ kg of grape FW)		ND	1.04a ± 0.15	1.17a ± 0.12
Vitisin A (mg/ kg of product FW)			4.73 ± 0.68	5.30 ± 0.53
Vitisin B (mg/ kg of grape FW)		ND	0.61a ± 0.10	0.48b ± 0.02
Vitisin B (mg/ kg of product FW)			2.77 ± 0.47	2.19 ± 0.10

"a", "b" and "c" in the same line indicate significant differences (Student-Newman-Keuls (SNK) test, α = 0.05).
Abbreviations: dp, delphinidin; cy, cyanidin; pn, peonidin; mv, malvidin; glc, glucoside; acglc, 6″-(acetyl)glucoside; cmglc, 6″-(*p*-coumaroyl)glucoside; cfglc, 6″-(*p*-caffeoyl)glucoside; FW, Fresh Weight; Total, Total anthocyanins content; *trans* configuration if not indicate; ND, nondetectable.

methoxylated anthocyanin). In a study by Marquez, Serratosa, and Merida (2014), evaluating partial drying at 40 °C of three grape varieties (Merlot, Tempranillo and Syrah), changes were observed in the composition of the anthocyanins in the process. The authors reported that when comparing the individual proportions of anthocyanins in fresh grapes with their dried grapes, the dp-3-glc showed a slight decrease, which was similar to that found in this work.

When comparing the total concentrations of anthocyanins from the raisin samples (with and without olive oil) and those of grape, expressed in mg mv-3-glc·kg^{-1} of grape (Table 1), the amount was significantly lower for the raisins, and between the raisins produced the amount of anthocyanins was significantly higher in the RO. These results demonstrate the degradation reactions experienced by anthocyanins, since these are thermolabile and unstable to exposure to high temperatures and oxygen during processing. According to Figueiredo-González, Cancho-Grande, and Simal-Gándara (2013b) and de Torres, Díaz-Maroto, Hermosín-Gutiérrez, and Pérez-Coello (2010), anthocyanins are mainly present in the skin of the grapes and are directly exposed to warm air during the drying of the grapes. In this sense, these compounds are more vulnerable than other compounds (which predominate in the pulp or seeds, for example) to thermal degradation processes. In the present study, the results suggest that, in association with oxidative reactions, raisins without pre-treatment had greater degradation of molecules through breaking of the heterocyclic C-ring of their structure due to the prolonged exposure time of the grapes to the heat treatment.

In Fig. 1a, the principal component analysis (PCA) clearly showed the differentiation between grapes (MG) and raisins (R and RO) according to the variables most associated to PC2 (36.57% of total explained variance). In grapes, the main markers were the total concentration of anthocyanins (Fig. 1a) and the molar ratio of dp-3-cmglc, which presented significantly higher values when compared to raisins (Table 1), while for the raisins, the concentrations of vitisins A and B, as well as the molar ratio of mv-3-glc were the markers (Fig. 1a), these compounds having higher values in raisins (Table 1). The variables most related to PC1 (37.61% of total explained variance) (Fig. 1a) allowed the differentiation between the two types of raisin (R and RO) samples by the molar ratios of mv-3-cmglc and, mainly by mv-3-acglc, in raisins RO (Table 1). In contrast, mainly the molar ratios of the non-acylated anthocyanins derived from cy and pn and the acetyl and *p*-coumaryl anthocyanins derived from cy were markers for the raisins R, having higher concentrations compared to raisins RO.

3.2.2. HCAD and stilbenes

The HCAD commonly found in *V. vinifera* grapes (*trans*-caftaric acid the *cis-and trans*- coutaric acids and *trans*-fertaric acid) were also detected in the MG samples (Table 2). This profile of HCAD was similar to that reported by Lago-Vanzela et al. (2011a) when studying the same cultivar from a different harvest. Therefore, the R and RO samples showed, in addition to the compounds already mentioned for MG, another caftaric acid derivative, the 2-*S*-glutathionyl-*trans*-caftaric acid (GRP). Caftaric acid is the main substrate for grape polyphenoloxidase (PPO) and, in the presence of glutathione leads to the formation of GRP after its oxidation.

The *trans*-caftaric acid was the HCAD predominating in all samples (grapes and raisins with and without olive oil), which is similar to the result reported in a study of raisins produced from the Thompson seedless variety of grape (Williamson & Carughi, 2010). Also, a significant reduction in the molar proportions of the *trans*-coutaric and total HCAD concentrations (mg·kg^{-1} of grapes) occurred for both raisin samples when compared to the values obtained for *BRS Morena* grape. The R samples had a higher amount of HACD (36.01) than the RO samples, which had the lower amount of HACD (13.04). Reductions in HCAD concentration in raisins probably occurred due to oxidation reactions catalyzed by PPO during the first few hours of drying (Da-Silva, Lago-Vanzela, & Baffi, 2015; Figueiredo-González et al., 2013b). The breakdown of the physical integrity of the grape berry, due to the exit of water during drying, promotes the decompartmentalization of the organelles of the vegetal tissue cells, and the release of HCAD, especially caftaric acid, present in the vacuole, together with the oxidative enzymes. In addition, the pretreatment of the grape made it more permeable to both the water, which has been partially evaporated, and the oxygen from the outside air that can diffuse through the interior of the berry favoring oxidative reactions of enzymatic origin. The high molar ratio of GRP in RO samples reinforces the hypothesis that the caftaric acid present in these grapes was intensely oxidized, which reflected negatively on the retention of HCAD in these raisins. Karadeniz et al. (2000), after determining the phenolic composition of Thompson seedless raisins, dehydrated in dehydration tunnel at 71 °C, reported loss of caftaric and coutaric acids in the order of approximately 90%. Peinado, de Lerma, Moreno, & Peinado (2009) and Serratosa, Lopez-Toledano, Merida, & Medina (2008), studying the dehydration of Pedro Ximenez grapes also reported degradation of caftaric, coutaric and fertaric acids.

Stilbenes were found in all three samples, namely the *cis*- and *trans*-forms of the compound piceid, the 3-gluside of resveratrol (Table 2). In MG samples, the *trans*-piceid was the main compound. It is found naturally in grapes, although it can isomerize under ultraviolet light to its corresponding *cis* isomer (Da-Silva et al., 2015). During the drying process, the concentration of the acids occurs. According to Zupancic, Lavric, & Kristl (2015), studying the stability of these compounds, the *trans*-resveratrol is more stable in acidic conditions and under such conditions, the temperature has little effect on its degradation. However, the authors emphasize that, in alkaline conditions, the increasing temperature speeds up the degradation processes involving this compound. Additionally, Callemien, Jerkovic, Rozenberg, & Collin (2005) reported that the presence of oxygen can trigger oxidation reactions that lead to degradation of *trans*-resveratrol. Thus, the effect of degradation due to long exposure to oxygen and temperature during the drying process of BRS Morena grapes, lead to a reduction in the stilbenes concentration in raisins compared to the grapes.

By PCA analysis (Fig. 1b), PC1 (61.84% of the total explained variance) differentiated between grape samples (MG) and raisins samples (R and RO), particularly in grapes for *trans*-coutaric acid, *trans*- and *cis*-piceid and total concentration of HCAD. According to Table 2, the content of *trans*-coutaric acid and the total concentration of HCAD were significantly higher in grapes compared to raisins. PC2 (47.28% of the total explained variance) differentiated raisins with and without olive oil (R and RO), except for one sample of raisins with olive oil (RO-3). The raisins without olive oil had more *trans*-caftaric acid and *cis*–coutaric acid while the raisins with olive oil stood out the fertaric acid and GRP.

3.2.3. Flavonols

The samples studied only had 3-glycosylated flavonols and some of the corresponding free aglycones, with a total of 19 flavonols in the grape and 21 flavonols in the raisins produced both without and with olive oil (Table 3). The profile observed for BRS Morena grapes was similar to that reported by Lago-Vanzela et al. (2011a), however, in the present study, kaempferol 3-rutinoside (K-3-rut) and the free aglycones M, Q, I and S were also detected. The occurrence of these aglycones is probably due to extraction in acidic conditions (Hermosín-Gutiérrez, Castillo-Muñoz, Gómez-Alonso, & García-Romero, 2011). The degree of hydrolysis of a flavonol may depend on its structure and also on the type of sugar attached to the aglycone. For example, in wines, Q derivatives, especially rutin, show a very strong tendency to hydrolyze, generating free Q (Da-Silva et al., 2015).

Among the compounds present in the grape, M-3-glc was the principal flavonol, followed by Q-3-glcU, in agreement with the results described by Lago-Vanzela et al. (2011a), who suggested that similar profiles of flavonols are commonly found in *V. vinifera* grapes. However, R samples had Q-3-glcU as the main compound, followed by Q-3-glc

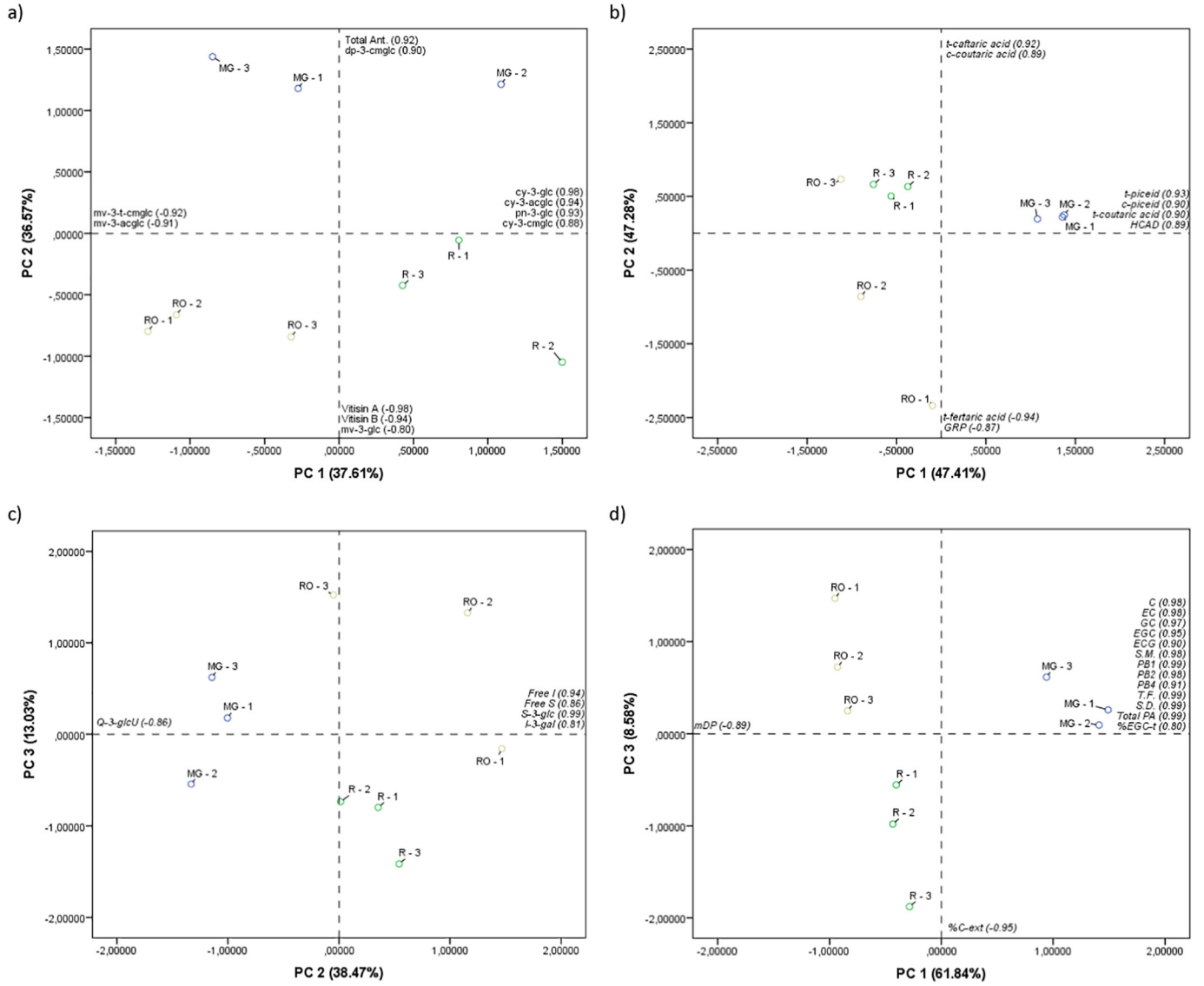

Fig. 1. Principal Component Analysis performed on BRS Morena fresh grape (MG-1, 2, 3), raisins without olive oil (R-1, 2, 3) and raisins with olive oil (RO-1, 2, 3) for the content of (a) anthocyanins (dp, delphinidin; cy, cyanidin; pn, peonidin; mv, malvidin; glc, glucoside; acglc, 6″-(acetyl)glucoside; cmglc, 6″-(*p*-coumaroyl) glucoside Total Ant., Total Anthocyanins; *trans* configuration if not indicated); (b) HCAD and Stilbenes (HCAD, Hydroxycinnamic Acid Derivatives; GRP, Grape Degradation Product; *t, trans; c, cis)*; (c) flavonols (Q-glcU, quercetin-3-glucuronide; free I, free isorhamnetin; free S, free syringetin; I-gal, isorhamnetin-3-galactoside; S-glc, syringetin-3-glucoside); (d) flavan-3-ols and proanthocyanidins (Total PA, Total Proanthocyanidins; T.F., Total Flavan-3-ols; C, (+)-catechin, EC, (−)-epicatechin; ECG, (−)-epicatechin-3-gallate; EGC, (−)-epigallocatechin; GC, (−)-gallocatechin; PB1, procyanidin B1; PB2, procyanidin B2; PB4, procyanidin B4; −t, terminal unit; −ext, extension unit; mDP, mean degree of polymerization; S.D., sum dimers; S.M., sum monomers).

and M-3-glc. The opposite was found for the RO samples, i.e., the main flavonol was M-3-glc, followed by Q-3-glc and Q-3-glcU. On the composition of the raisins, all six principal aglycones (K, Q, I, L, M and S) were found in their free form, even in small percentages, with free Q being the most present in the raisins. Figueiredo-Gonzalez, Cancho-Grande, & Simal-Gandara (2013a) and Marquez, Dueñas, et al. (2012) also reported the emergence of free aglycones after the drying process of grapes (Alicante and Merlot, and Syrah, respectively).

With respect to the concentration of total flavonols, expressed in mg of Q-3-glc equivalent·kg^{-1},when analyzing the data for MG, R and RO separately, comparing the result for each dehydration process (R and RO) with the result obtained for the MG samples it's possible to observe that the pre-treatment with olive oil affect more negatively the amount of flavonols, because the total content found for RO was significantly lower than MG samples, while there was not significantly difference between R and MG results.

A possible explanation for this fact is that, because it is more permeable, the skins of the pretreated grapes might exposed the flavonols more intensely to oxidation processes. In addition, as the dehydration proceeded, the acidity increased and, thus, the glycosylated flavonols underwent hydrolysis in the raisins with olive oil. The resulting free aglycones are very insoluble and unstable, especially against oxidative processes. However, when comparing the total amount of flavonols between the raisins there is no significantly difference in the results founded, so it's possible to conclude that both dehydration process affected the flavonols amount in the same way.

PCA for the flavonols (Fig. 1c) showed that PC2 (38.47% of total explained variance) differentiated between the grape samples (MG) and the raisin samples (R and RO), except for one repetition of raisins with olive oil (RO-3). Q-3-glcU is a marker for the grapes, having one of the highest molar ratio in the grapes, as well as having the lowest molar ratio in raisins with olive oil. PC3 (13.03% of total explained variance) differentiated between raisins without (R) and with olive oil (RO). S-3-glc, I-3-gal and the free aglycones of I and S, were markers for the RO

Table 2
Hydroxycinnamic acid derivatives (HCAD) and stilbenes (resveratrol and its 3-glucoside, piceid) BRS Morena grape (MG) and raisins with (RO) and without (R) olive oil pre-treatment by HPLC-ESI-MS/MS (negative ionization mode). Mass spectrum data, molar profiles (percentage of each individual HCAD regarding the total content), and total concentrations of HCAD, and Stilbene. Given as mean values ± standard deviations (n = 3).

Deprotonated molecule and product ions (m/z)	HCAD[1]	MG	R	RO
		molar ratios (%)		
618;543;489;264	GRP	ND	2.82ab ± 0.45	8.70a ± 5.20
311;179,149,135	*trans*-caftaric acid	72.38a ± 0.45	67.70a ± 0.23	58.27a ± 14.66
295;163,149,119	*trans*-coutaric acid	17.52a ± 0.69	13.57b ± 1.01	9.97c ± 1.52
295;163,149,120	*cis*-coutaric acid	5.67a ± 0.36	8.89a ± 0.23	4.48a ± 4.11
325; 193, 149	*trans*-fertaric acid	4.42a ± 0.67	7.02a ± 0.69	18.59a ± 11.83
Total HACD (mg/kg of grape FW)		88.52a ± 10.14	36.01b ± 11.49	13.04c ± 9.55
Total HACD (mg/kg of product FW)		88.52 ± 10.14	163.49 ± 52.17	59.18 ± 43.37
Pseudomolecular and product ions (m/z)	Stilbene	MG	R	RO
		mg/kg of grape; (mg/kg of product)		
389; 227	*trans*-piceid	3.06a ± 0.51	1.08b ± 0.17; (4.91 ± 0.75)	0.40c ± 0.04 (1.81 ± 0.18)
389; 227	*cis*-piceid	1.47a ± 0.08	0.30b ± 0.15; (1.37 ± 0.67)	0.64b ± 0.64 (2.92 ± 2.92)

"a", "b" and "c" in the same line indicate significant differences (Student-Newman-Keuls (SNK) test, α = 0.05).
Abbreviations: GRP, 2-S-glutationil-*trans*-caftaric; FW, Fresh Weight.

samples showed as markers, their molar ratios being higher than those determined in R samples.

3.2.4. Flavan-3-ol monomers, dimers and proanthocyanidins

Five flavan-3-ol monomers were detected in the MG, R and RO samples (Table 4) being C the principal monomer, followed by very similar concentrations of GC and EC for grapes, R and RO samples, taking into account the standard deviations. In all three samples, three flavan-3-ol dimers (PB1, PB2 and PB4) were also detected, with PB1 being the main one for all samples. These results are similar to those reported by Figueiredo-Gonzalez et al. (2013a), Peinado et al. (2009), and Serratosa et al. (2008) for partially dehydrated Pedro Ximenez grapes.

The total sum of the monomers and the dimers determined in the MG samples was significantly higher than in the R and RO samples, the two latter not presenting a significant difference between their determined values. The combined sum of monomers and dimers of flavan-3-ols for the BRS Morena grapes was approximately 32.74 mg of (+)–catechin equivalent/kg of grapes, this amount lower than the usual values found for *V. vinifera* cultivars. However, such values can be justified due to the absence of seeds in the variety studied, since flavan-3-ol are compounds found in higher concentration in the seeds and pomace (Da-Silva et al., 2015). However, this value is higher than the value reported by Lago-Vanzela et al. (2011a) for the same cultivar, due to the inherent differences in the harvest of the grapes used in the respective studies. When comparing the value of this total sum with those determined for raisins, they were higher in MG samples, and between the R and RO samples there was no significant difference.

In addition to the identification and quantification of the flavan-3-ols, a PA depolymerization reaction was also carried out for

Table 3
Flavonols in BRS Morena grape (MG) and raisins with (RO) and without (R) olive oil pre-treatment by HPLC-DAD-ESI-MS/MS (negative ionization mode). Mass spectrum data, molar profiles (percentage of each individual flavonol regarding the total content), and total concentration (as equivalents of quercetin 3-glucoside (Q-3-glc)). Given as mean values ± standard deviations (n = 3).

Deprotonated molecule and product ions (m/z)	Flavonol	molar ratios (%)		
		MG	R	RO
493; 317	M-3-glcU	3.61a ± 0.20	2.04b ± 0.24	1.25c ± 0.54
479; 317	M-3-gal	1.20a ± 0.18	0.55b ± 0.05	0.50b ± 0.12
479; 317	M-3-glc	30.92a ± 2.28	15.75b ± 1.13	24.29ab ± 9.14
463; 301	Q-3-gal	1.41a ± 0.19	2.23a ± 0.19	1.24a ± 0.70
477; 301	Q-3-glcU	22.41a ± 1.31	27.36a ± 2.22	17.05a ± 9.68
463; 301	Q-3-glc	14.89b ± 1.94	23.11a ± 1.30	17.81b ± 2.66
609; 301	Q-3-rut	1.04a ± 0.10	1.68a ± 0.27	1.58a ± 0.41
493; 331	L-3-glc	9.88a ± 1.17	5.31b ± 0.43	9.03a ± 2.21
447; 285	K-3-gal	0.49ab ± 0.37	0.84a ± 0.11	0.16b ± 0.11
461; 285	K-3-glcU	0.89a ± 0.05	1.41a ± 0.19	1.20a ± 0.61
447;285	K-3-glc	1.76a ± 0.09	3.49a ± 0.38	2.91a ± 2.41
593; 285	K-3-rut	0.52a ± 0.24	0.83a ± 0.18	0.87a ± 0.35
477; 315	I-3-gal	0.28b ± 0.10	0.29b ± 0.06	0.64a ± 0.14
477; 315	I-3-glc	2.98b ± 0.09	4.57a ± 0.56	4.58a ± 0.43
507; 345	S-3-glc	2.91a ± 0.47	3.31a ± 0.45	6.30a ± 2.61
317; 317	free M	2.13a ± 0.47	0.72b ± 0.17	1.72a ± 0.66
301	free Q	0.36b ± 0.10	4.25a ± 0.65	4.47a ± 0.17
331	free L	ND	0.25b ± 0.10	0.57a ± 0.07
285	free K	ND	0.20a ± 0.07	0.15a ± 0.02
315	free I	0.46a ± 0.15	0.65a ± 0.03	0.97a ± 0.33
345	free S	1.86a ± 0.78	1.15a ± 0.29	2.69a ± 1.37
Total (mg Q-3-glc/ Kg fresh grape FW)		52.21a ± 7.29	31.73ab ± 7.17	24.53b ± 15.47
Total (mg Q-3-glc/ Kg product FW)			144.04 ± 32.54	111.39 ± 70.25

"a", "b" and "c" in the same line indicate significant differences (Student-Newman-Keuls (SNK) test, α = 0.05).
Abbreviations: M, myricetin; Q, quercetin; L, laricitrin; K, kaempferol; I, isorhamnetin; S, syringetin; glcU, glucuronide; gal, galactoside; glc, glucoside; rut, rutinoside (6″-rhamnosylglucoside); FW, Fresh Weight; ND, non detectable.

Table 4
Flavan-3-ols monomers and dimers (B-type procyanidins) content in BRS Morena grape (MG) and raisins with (RO) and without (R) olive oil pre-treatment. Given as mean values ± standard deviations (n = 3).

Flavan-3-ol monomers	*m/z* pairs[1]	catechin equivalents - mg/kg of grape		
		MG	R	RO
(+)-catechin	289/137; 289/164	13.26a ± 1.19	5.21b ± 1.07	5.69b ± 0.66
(−)-epicatechin	289/137; 289/164	2.39a ± 0.40	0.57b ± 0.07	0.83b ± 0.18
(−)-gallocatechin	305/109; 305/137	3.62a ± 0.28	0.59b ± 0.08	0.72b ± 0.40
(−)-epigallocatechin	305/109; 305/137	0.86a ± 0.29	0.21b ± 0.07	0.24b ± 0.03
(−)-epicatechin-3-gallate	441/245; 441/289	0.56a ± 0.09	0.11b ± 0.02	0.14b ± 0.04
Total monomers (mg/kg grape, catechin equivalents FW[1])		20.27a ± 2.07	6.61b ± 1.00	7.53b ± 1.11
Flavan-3-ol dimers	*m/z* pairs[1]	MG	R	RO
Procyanidin B1	577/425; 577/407	19.51a ± 2.29	6.43b ± 1.18	9.44b ± 1.39
Procyanidin B2	577/425; 577/407	3.46a ± 0.38	0.97b ± 0.22	1.46b ± 0.13
Procyanidin B4	577/425; 577/407	1.89a ± 0.29	0.60b ± 0.21	0.81b ± 0.31
Total dimers (mg/kg grape, catechin equivalents FW)		12.47a ± 1.26	4.01b ± 0.60	5.88b ± 0.83
Total (mg/kg grape, catechin equivalents FW)		32.74a ± 3.27	10.62b ± 1.59	13.41b ± 1.94

"a" and "b" in the same line indicate significant differences (Student-Newman-Keuls (SNK) test, α = 0.05).
Abbreviations: FW, Fresh Weight.
[1] Mass transition pairs data for MRM scan used in the identification of the flavan-3-ols.

Table 5
Structural characterization of proanthocyanidins (PA) (MDP, mean degree of polymerization; % galloylation, % of 3-galate units; % prodelphinidins, % of epigallocatechin; and % of each flavan-3-ol monomer in extension and terminal units), total concentration of PA (as (+)-catechin equivalents) in *BRS Morena* grape (MG) and raisins with (RO) and without (R) olive oil pre-treatment. Given as mean values ± standard deviations (n = 3).

Proanthocyanidin	MG	R	RO
mDP	5.42b ± 0.24	6.86a ± 0.28	6.42a ± 0.17
% galloylation	2.72a ± 0.16	2.38a ± 0.10	2.55a ± 0.19
% prodelphinidin	18.08a ± 3.39	17.77a ± 0.52	17.54a ± 1.12
% terminal-C	68.77b ± 1.54	73.42a ± 1.17	75.38a ± 2.43
% terminal-EC	10.81a ± 0.78	10.59a ± 1.33	9.28a ± 0.46
% terminal-GC	14.44a ± 1.05	11.72a ± 1.34	11.43a ± 1.55
% terminal-EGC	2.83a ± 0.35	1.78b ± 0.35	1.51b ± 0.56
% terminal-ECG	3.15a ± 0.49	2.49a ± 0.15	2.41a ± 0.30
% extension-C	1.84ab ± 0.05	1.75b ± 0.09	2.10a ± 0.21
% extension-EC	78.35a ± 3.97	78.06a ± 0.98	77.51a ± 1.25
% extension-EGC	17.27a ± 3.88	17.89a ± 0.78	17.86a ± 1.27
% extension-CG	2.54a ± 0.10	2.30a ± 0.11	2.52a ± 0.18
Total PA (mg/kg grape FW)[1]	555.90a ± 96.98	149.95b ± 5.10	210.62b ± 2.35
Condensed tannins (mg/kg grape FW)[2]	1186.90a ± 224.30	767.07a ± 162.03	785.80a ± 108.61

"a" and "b" in the same line indicate significant differences (Student-Newman-Keuls (SNK) test, α = 0.05).
Abbreviations: mDP, mean degree of polymerization; C, (+)-catechin; EC, (−)- epicatechin; GC, (−)-gallocatechin; EGC, (−)-epigallocatechin; ECG, (−)- epicatechin 3-gallate; FW, Fresh Weight.
[1] Total PA: calculated by the total sum of the concentrations of extension and terminal units.
[2] PA: condensed tannins determined by the methylcellulose precipitation method, as (−)-epicatechin equivalents.

characterization of main structural features of PA, namely the flavan-3-ols which are not monomers, i.e., dimers, trimers, and oligomers to polymers (Table 5). The results showed that, in relation to the total PA concentration, the R and RO samples had significantly lower amount than the MG, although there was not significantly differences between the both raisins produced. Karadeniz et al. (2000) indicated that the most labile polyphenols during the dehydration process were procyanidins and flavan-3-ols, since they had been completely degraded in all raisin samples as well as in frozen grapes. Additionally, it is worth mentioning that the method used to determine PAs has some limitations, the most important being that can only evaluate the structural characteristics of PA that have a B-type interflavan bond, which breaks during the depolymerization reaction. A-type ether-bonded PAs do not depolymerize, i.e. the ester bond does not break, thus preventing their analysis by this method.

In order to circumvent this limitation, a second determination of PA was performed as condensed tannins by the methylcellulose precipitation method (Sarneckis et al., 2006). The results from this last method showed significantly higher concentrations when compared to those obtained by HPLC for all samples (grapes and raisins without and with olive oil). After the depolymerization of PA, the release of flavan-3-ol monomers as terminal and extension units (linked to pyrogallol reactant) occurred. For the structural characterization of these compounds, the % of galloylation and % of prodelphinidins were calculated. In this case, only the CG extension units and ECG terminal units accounted for % galloylation, while GC terminal units together with EGC terminal and extension units accounted for % prodelphinidins. Overall, the PA showed typical characteristics of *V. vinifera* grape skin PA, namely, low % of galloylation (2.38–2.72%), high % of prodelphidinis (17.54–18.08%), however the mDP was relatively low (6.42–6.86% in R and RO samples, significantly higher than 5.42% in MG samples) in comparison with *V. vinifera* grapes (Cáceres-Mella, Talaverano, Villalobos-González, Ribalta-Pizarro, & Pastenes, 2017). The latter characteristic suggest that BRS Morena PA should be perceived as poorly astringent and that its reactivity towards proteins should be little relevant (Quijada-Morín, Williams, Rivas-Gonzalo, Doco, & Escribano-Bailón, 2014), which are very interesting for grape juice production, for instance.

In general, the PA structural analysis from the MG samples showed results consistent with previous studies data (Lago-Vanzela et al.,

2011a). The extension units formed in the MG PA were the same as the four (C, EC, EGC and GC) found in the grape skins of the *V. vinifera* grape Merlot (Souquet, Cheynier, Brossaud, & Moutounet, 1996), and in the literature for the same cultivar (Lago-Vanzela et al., 2011a). In this study, EC was the main extension unit (78.35%) followed by EGC (17.27%) for all samples (grapes and raisins without and with olive oil), although these results did not present a significant difference. Regarding the raisins, no data were found in the literature on the structural characteristics of PA, however, the results for the raisins with and without oil differed very little from those of grape. Regarding the terminal units for the three samples, terminal-C was the main one, and the percentages determined in raisins was significantly higher than those determined in grapes, which could be expected due to the concentration of these compounds through dehydration and the probable hydrolysis of larger oligomers into smaller compounds (Figueiredo-Gonzalez et al., 2013a).

PC1 (61.84%) and PC3 (8.58%) accounted for 70.42% of the total explained variance, where PC1 differentiated the grape samples from the raisins by higher contents of flavan-3-ols monomers, dimers, total PA (HPLC analysis) and condensed tannins by methylcellulose precipitation method (Fig.1d; Table 4). In addition, the grape samples were characterized by the percentage of EGC terminal units, being significantly higher than the percentage determined for raisins. The raisins were characterized by their higher degree of polymerization in comparison to the grape samples (Table 5). On the other hand, PC3 in Fig. 1g allowed the differentiation of the samples of raisins without olive oil (R) from raisins with olive oil (RO), and the marker for the RO was the extension unit of C, since it presents a significantly higher percentage than that of the R samples (Table 5).

4. Conclusions

The process of drying the grapes with or without pre-treatment with extra virgin olive oil brought about changes in the qualitative and quantitative profile of phenolic compounds of BRS Morena grapes, which can be easily visualized by principal component analysis (PCA). The raisins produced without any pre-treatment, due to the long drying time, presented lower retention of flavan-3-ols, proanthocyanidins and, mainly, of anthocyanins, when compared to the retentions obtained for the same compounds quantified in the raisins from grapes pretreated with olive oil.

The pre-treatment of the grapes with olive oil and further dehydration to produce raisins speeded up the processing time. On the other hand, it also favored the rupture of the pruine present in the grape skins, making them more permeable to external oxygen, which probably accelerated the oxidative processes. Thus, the flavonols, hydroxycinnamic acid derivatives and stilbenes were more extensively degraded in pre-treated raisins.

Therefore, the addition of olive oil proved to be more efficient from the industrial point of view, since it significantly reduced the total drying time, and at same time preserved a higher concentration of anthocyanins and proanthocyanidins in the raisins produced, making them an interesting product from a health point of view, due to their important biological activity claims.

Funding

This work was supported by CAPES (master scholarship - grant number 1409615) and FAPESP (grant numbers 2014/11514-8 and 2017/16482-5). The sponsors had no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Acknowledgments

We thank Prof. Dr. Isidro Hermosín-Gutiérrez (in memoriam), who had a huge contribution to make this research and paper possible and unfortunately deceased (April 23th, 2018) before it's publishment. We also thank to EMBRAPA Grape and Wine (Jales, Brazil) for supplying the grape samples used in this work.

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