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MILENE TEIXEIRA BARCIA

STUDY OF PHENOLIC COMPOUNDS AND ANTIOXIDANT CAPACITY OF BY-PRODUCTS FROM WINEMAKING PROCESS

ESTUDO DOS COMPOSTOS FENÓLICOS E CAPACIDADE ANTIOXIDANTE DE SUBPRODUTOS DO PROCESSO DE VINIFICAÇÃO

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Faculdade de Engenharia de Alimentos

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ABSTRACT

Grape marc (skins and seeds) and lees (sediment solids) are the most abundant by-products of the winemaking process, because the first represents approximately 5% of the total grapes processed and the second, 4% of the total volume of wine produced. Some studies have shown that winery residues contain phenolic compounds, potent antioxidants that were not fully transferred to the wine during the winemaking process, thus with economic and functional interest. Thus, the objective of this work was characterize the phenolic compounds of industrials residues generated by the vinification and correlate their levels with the antioxidant capacity. The phenolic content of grapes in natura, grape marc (skins and seeds), and lees, in two consecutive years (2011 and 2012), of varieties BRS Violeta, BRS Lorena, Cabernet sauvignon and Cabernet franc, was determined by SPE and HPLC-DAD-ESI MS/MS. The effect of three drying techniques applied to vinification by-products (grape marc and lees) was also evaluated: dried at 50 °C, drying by spray-drying and freeze-drying, as well as the stability of by-products dried submited at 25°C in 0, 30 and 90 days. The antioxidant capacity was evaluated by ORAC, FRAP, ABTS and β-caroteno/linoleic acid, and their correlations with total anthocyanins and phenolics. Were identified 25 anthocyanins and 9 piranoathocyanins in the samples analyzed. The composition of anthocyanins BRS Violeta was mainly derived by diglucosides. However, in samples of Cabernet sauvignon and Cabernet franc antocynanins 3-glucosides were majority. The pyranoanthocyanins were found only in fermented skins of the cultivars Cabernet sauvignon and Cabernet franc and the lees of all cultivars. Flavonols were found mainly quercetin, isorhamnetin, myricetin, laricitrin, and syringetin. In grape glycosides predominated, and residues were obtained higher proportions of free aglycones, especially the lees of wine (85%). In BRS Lorena, type flavonol quercetin was the majority in the skin (91%) and the lees (95%). Already in all samples BRS Violeta, derivatives of caffeic acid were predominant. The caftaric acids (53%) and coutaric acid (17%) were high in the samples of the cultivars Cabernet sauvignon and Cabernet franc. In all samples of grapes and lees were detected the presence of trans-resveratrol, trans-piceid and cis-piceid (except BRS Violeta). In the residue dried at 50 °C and spray-drying showed a 50% reduction in the total phenolic content of the residues of four different varieties of grape at time zero when compared to the lyophilized. In all methods of antioxidant capacity, BRS Violeta samples showed higher values when compared to BRS Lorena, Cabernet sauvignon and Cabernet franc. The byproducts of winemaking had elevated total phenolics, average values ranged from 810 to 8557 mg/kg for fermented skins; 1515 to 9520 mg/kg for seeds, and 1148 to 4261 mg/kg for lees. In grapes used in the processing of wine the phenolic content was found of 530 to 4330 mg/kg dried grape. Thus, the by-products have a high content of antioxidant compounds and therefore have the potential for industrial reuse.

Keywords: by-products, winemaking, phenolic, antioxidants.

RESUMO

Bagaço (cascas e sementes) e borra (sedimentos sólidos) são os mais abundantes subprodutos do processo de vinificação, pois o primeiro representa aproximadamente 5% do total de uva processada e, o segundo, 4% do volume total de vinho produzido. Alguns estudos demonstraram que os resíduos vinícolas contêm importantes compostos fenólicos, potentes antioxidantes, que não foram totalmente transferidos ao vinho durante o processo de vinificação, assim despertando interesse econômico e funcional. Sendo assim, o objetivo deste trabalho foi caracterizar os compostos fenólicos de resíduos vinícolas, e correlacionar os seus teores com a capacidade antioxidante. Foi determinado o teor de compostos fenólicos das uvas *in natura*, do bagaço (casca e semente) e borras, obtidas em duas safras (2011 e 2012), das variedades BRS Violeta, BRS Lorena, Cabernet sauvignon e Cabernet franc, por SPE e HPLC-DAD-ESI MS/MS. Também foi avaliado o efeito de três técnicas de secagem aplicadas aos subprodutos da vinificação (bagaço e borra): secagem em estufa a 50 °C (D50), secagem por spray-drying (SP), e secagem por liofilização (FD), assim como a estabilidade dos subprodutos secos D50 e SP, submetidos a 25 °C durante 0, 30 e 90 dias. A capacidade antioxidante foi avaliada por ORAC, FRAP, ABTS e β-caroteno/ácido linoleico, e suas correlações com antocianinas e fenólicos totais. Foram identificadas 25 antocianinas e 9 piranoatocianinas nas diferentes amostras analisadas. A composição de antocianinas de BRS Violeta foi principalmente pelos derivados diglicosídeos. No entanto, nas amostras de Cabernet sauvignon e Cabernet franc as antocinaninas majoritárias foram 3-glicosídeos. As piranoantocianinas foram encontradas somente em cascas fermentadas das cultivares Cabernet sauvignon e Cabernet franc e nas borras de todas as cultivares. Os flavonois encontrados foram principalmente, quercetina, isoramnetina, miricetina, laricitrina e siringetina. Nas uvas predominaram os glicosilados, e nos resíduos obtiveram maiores proporções de agliconas livres, especialmente nas borras de vinho (85%). Na BRS Lorena, quercetina foi o flavonol majoritário nas cascas (91%) e nas borras (95%). Já em todas as amostras de BRS Violeta, os derivados do ácido caféico foram predominantes. Os ácidos caftárico (53%) e cutárico (17%) foram superiores nas amostras das cultivares Cabernet sauvignon e Cabernet franc. Em todas as amostras de uvas e borras detectou-se a presença de *trans*-resveratrol, *trans*-piceid e *cis*-piceid (com exceção da BRS Violeta). Nos resíduos secos em estufa a 50°C e spray-drying observou-se uma redução de até 50% no teor de compostos fenólicos totais dos diferentes resíduos das quatro variedades de uva no tempo zero, quando comparado com o liofilizado. Em todos os métodos de capacidade antioxidante, as amostras BRS Violeta demonstraram valores superiores quando comparadas a BRS Lorena, Cabernet sauvignon e Cabernet franc. Os subprodutos da vinificação secos apresentaram concentração elevada de compostos fenólicos totais, em média os valores oscilaram de 810 à 8557 mg/kg para casca fermentada; de 1515 à 9520 mg/kg para semente; e 1148 à 4261 mg/kg para borra, nas cultivares estudadas. Nas uvas inteiras secas utilizadas no processamento do vinho o teor de compostos fenólicos encontrados foi de 530 à 4330 mg/kg. Sendo assim, os subprodutos analisados possuem elevado teor de compostos antioxidantes e, portanto, com potencial para reaproveitamento industrial.

Palavras-chave: subprodutos, vinificação, fenólicos, antioxidantes.

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"Talvez não tenha conseguido fazer o melhor, mas lutei para que o melhor fosse feito. Não sou o que deveria ser, mas Graças a Deus, não sou o que era antes"
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INTRODUÇÃO GERAL

A vitivinicultura brasileira tem demonstrado um crescimento significativo nos últimos anos. O Brasil ocupou a colocação de 13° país com a maior produção de vinhos, produzindo em média 261 milhões de litros de vinhos ao ano, o que mostra um panorama favorável ao setor para os próximos anos (Mello, 2012).

Em vista da sua alta atividade agrícola, a geração de resíduos pelo setor vitivinícola tem crescido, pois cerca de 60% das uvas plantadas no país são destinadas à produção de vinhos, o que gera, em resíduo, aproximadamente 5% na forma de bagaço, em relação às uvas produzidas, e 4% na forma de borra, em relação ao vinho produzido.

O bagaço (casca e semente) é o mais abundante resíduo vinícola, o qual é gerado após a maceração concomitante da fermentação alcoólica e prensagem das uvas na produção de vinho tinto ou logo após a prensagem das uvas previamente esmagadas, na produção de vinho branco. Já a borra, constituída por finas partículas de resíduo de uva e leveduras mortas, é obtida após a fermentação do mosto, pelo processo de decantação. (Maragkoudakis et al., 2013, Paradelo, Moldes, & Barral, 2010, Cortes, Rodríguez, Salgado, & Domínguez, 2011).

Até o presente momento, dados da indústria vinífera demonstram que esses resíduos estão sendo utilizados como ração animal, fertilizantes e na destilação de álcool. No entanto, possuem o sério inconveniente da presença de etanol, o que pode causar danos ao meio ambiente e aos animais. Esta situação explica o interesse crescente em explorar os subprodutos da vinificação para uso de forma mais nobre, como extração de compostos fenólicos (Torres et al., 2002, Lafka, Sinanoglou, & Lazos, 2007, Llobera & Cañellas, 2007, Rockenbach, Silva, Rodrigues, Kuskoski, & Fett, 2008).

Os subprodutos da vinificação têm atraído a atenção de pesquisadores devido a elevada quantidade de metabólitos secundários, incluindo os ácidos fenólicos, flavan-3-óis e antocianinas presentes. Parte desses compostos é transferida ao vinho, no entanto a maior parte permanece nos resíduos, pois esses compostos estão presentes nas partes sólidas da uva e sua extração depende principalmente das condições tecnológicas utilizadas durante a vinificação como: tempo de maceração, temperatura, intensidade e duração da pressão, uso de enzimas, tipo de levedura e concentração SO_2. Dessa forma torna-se possível a extração destes compostos naturais e a avaliação de sua capacidade antioxidante (Makris, Boskou & Andrikopoulos, 2007, Gallego, García-Carpintero, Sánchez-Palomo, Viñas, & Hermosín-Gutiérrez, 2012).

Na literatura, não se encontram dados científicos que apresentem informações quanto à composição química da borra vinícola. Sendo assim, a realização de estudos com esse tipo de resíduo pode ser uma opção promissora para a verificação da presença de compostos fenólicos, tendo em vista sua importância para a saúde. Em relação ao bagaço alguns estudos tem sido focado em resíduos de variedades *Vitis vinifera*, no entanto pouco se sabe sobre o potencial dos compostos fenólicos das variedades de uvas não-viníferas (Sant'Anna, Brandelli, Marczak, & Tessaro, 2012).

Os principais compostos fenólicos presentes no bagaço derivado da indústria vinícola são as antocianinas, presentes na casca de uva tinta (Rockenbach et al., 2008), as catequinas e epicatequinas, presentes em maior quantidade na semente (Torres et al., 2002, Yilmaz & Toledo, 2004, Karvela, Makris, Kalogeropoulos, Karathanos, & Kefalas, 2009), e os estilbenos e ácidos fenólicos, presentes na casca e semente (Cataneo, Caliari, Gonzaga, Kuskoski, & Fett 2008, Lafka, Sinanoglou, & Lazos, 2007, Karvela et al., 2009).

Estudos sobre bagaço vinícola demonstram grandes variações no teor de compostos fenólicos encontrados, abrangendo uma faixa de 0,1 a 7,0 g equivalente de ácido gálico/100 g de bagaço seco. Essa oscilação deve-se, principalmente, as diferentes variedades de uvas utilizadas, das técnicas de vinificação diversificadas, e dos diferentes métodos de análises para esse tipo de resíduo (Wollgast, & Anklan, 2000, Negro, Tommasi, & Miceli, 2003, Llobera, & Cañellas, 2007, Rockenbach et al., 2008, Cataneo et al., 2008). Estudos realizados com as sementes das uvas demonstram a presença de 5 a 8% de compostos fenólicos (Shrikhande, 2000), sendo que, desse total, 63 a 70% são taninos (Amendola, Faveri, & Spigno, 2010).

O mecanismo múltiplo da capacidade antioxidante dos compostos fenólicos são expressos pela habilidade de sequestrar radicais livres, quelar metais e pelo sinergismo com outros antioxidantes. Estudos demonstram que o bagaço, composto por casca e semente, apresenta alto teor de compostos fenólicos e considerável capacidade antioxidante (Mielnik, Olsen, Vogt, Adeline, & Skrede, 2006, Rockenbach et al., 2008, Rockenbach, Gonzaga, Rizelio, Gonçalves, Genovese, & Fett, 2011).

Devido a isso, a recuperação de compostos fenólicos a partir de resíduos industriais está ganhando atenção, especialmente atribuído às propriedades antioxidantes que estes compostos exercem, além de outras atividades, como, anti-inflamatória, anticancerígena e antimutagênica (Cheng, Bekhit, McConnell, Mros, & Zhao, 2012, Rubilar, Pinelo, Shene, Sineiro, & Nuñez, 2007, Shrikhande, 2000).

A caracterização química dos compostos fenólicos presentes em subprodutos da vinificação (bagaços e borras) constitui a informação básica para a avaliação da viabilidade de seu uso em indústrias de alimentos, químicas e farmacêuticas, sendo favorável ao meio

ambiente, além de possuir baixo custo e alta eficiência (Babbar, Oberoi, Uppal, & Patil, 2011).

REFERÊNCIAS BIBLIOGRÁFICAS

Amendola, D., Faveri, D.M., & Spigno, G. (2010). Grape marc phenolics: Extraction kinetics, quality and stability of extracts. *Journal of Food Engineering, 97*, 384–392.

Babbar, N., Oberoi, H. S., Uppal, D. S. & Patil, R. T. (2011). Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. *Food Research International*, *44,* 391–396.

Cataneo, C. B., Caliari, V.,Gonzaga, L. V., Kuskoski, E. M., & Fett, R. (2008). Atividade antioxidante e conteúdo fenólico do resíduo agroindustrial da produção de vinho. *Semina: Ciências Agrárias, Londrina, 29,* 1, 93-102.

Cheng, V. J., Bekhit, A. E. A., McConnell, M., Mros, S. & Zhao, J. (2012). Effect of extraction solvent, waste fraction and grape variety on the antimicrobial and antioxidant activities of extracts from wine residue from cool climate. *Food Chemistry, 134,* 474–482.

Cortés, S., Rodríguez, R., Salgado, J. M. & Domínguez, J. M. (2011). Comparative study between Italian and Spanish grape marc spirits in terms of major volatile compounds. *Food Control, 22*, 673-680.

Gallego, M. A. G., García-Carpintero, E. G., Sánchez-Palomo, E., Viñas, M. A. G., & Hermosín-Gutiérrez, I. (2012). Oenological potential, phenolic composition, chromatic characteristics and antioxidant activity of red single-cultivar wines from Castilla-La Mancha. *Food Research International, 48*, 7–15,

Karvela, E., Makris, D. P., Kalogeropoulos, N., Karathanos, V. T., & Kefalas, P. (2009). Factorial design optimisation of grape (Vitis vinifera) seed polyphenol extraction. *European Food Research Technology, 229*, 731–742.

Lafka, T.,Sinanoglou, V., & Lazos, E. S. (2007). On the extraction and antioxidant activity of phenolic compounds from winery wastes. *Food Chemistry, 104*, 1206–1214.

Llobera, A. & Cañellas, J. (2007). Dietary fibre content and antioxidant activity of Manto Negro red grape (Vitis vinifera): pomace and stem. *Food Chemistry, 101*, 659–666.

Makris, D.P., Boskou, G., & Andrikopoulos, N.K. (2007). Polyphenolic content and in vitro antioxidant characteristics of wine industry and other agri-food solid waste extracts. *Journal of Food Composition and Analysis, 20,*125–132.

Maragkoudakis, P.A., Nardi, T., Bovo, B., D'Andrea, M., Howell, K. S., Giacomini, A., & Corich, V. (2013). Biodiversity, dynamics and ecology of bacterial community during grape marc storage for the production of grappa. International Journal of Food Microbiology, 162, 143-151.

Mello, L. M. R. (2012). Vitivinicultura mundial: principais países e posição do Brasi**l**. Comunicado técnico*, ISSN 1808-6802, 1-8.*

Mielnik, M.B., Olsen, E., Vogt, G., Adeline, D., & Skrede, G. (2006). Grape seed extract as antioxidant in cooked, cold stored turkey meat. *Food Science and Technology, 39,* 3, 191-198.

Negro, C., Tommasi, L., & Miceli, A. (2003). Phenolic compounds and antioxidant activity from red grape marc extracts. *Bioresource Technology, 87*, 41–44.

Paradelo, R., Moldes, A. B., & Barral, M. T. (2010). Utilization of a factorial design to study the composting of hydrolyzed grape marc and vinification lees. *Journal of Agricultural and Food Chemistry*, *58,* 3085–3092.

Rockenbach, I. I., Silva, G. L., Rodrigues, E., Kuskoski, E. M., & Fett, R. (2008) Influência do solvente no conteúdo total de polifenóis, antocianinas e atividade antioxidante de extratos de bagaço de uva (Vitis vinifera) variedades Tannat e Ancelota. *Ciência Tecnologia de Alimentos, Campinas, 28*(Supl.), 238-244.

Rockenbach, I. I., Gonzaga, L. V., Rizelio, V. M., Gonçalves, A. E. S. S., Genovese, M. I., & Fett, R. (2011). Phenolic compounds and antioxidant activity of seed and skin extracts of red grape (Vitis vinifera and Vitis labrusca) pomace from Brazilian winemaking. *Food Research International, 44,* 897–901.

Rubilar, M., Pinelo, M., Shene, C., Sineiro, J. & Nuñez, M. J. (2007). Separation and HPLC-MS Identification of Phenolic Antioxidants from Agricultural Residues: Almond Hulls and Grape Pomace. *Journal of Agricultural and Food Chemistry*, *55,* 10101–10109.

Sant'Anna, V., Brandelli, A., Marczak, L. D. F., & Tessaro, I. C. (2012). Kinetic modeling of total polyphenol extraction from grape marc and characterization of the extracts. *Separation and Purification Technology*, *100*, 82–87.

Shrikhande, A. J. (2000). Wine by-products with health benefits. *Food Research International, 33*, 469-474.

Torres, J. L., Varela, B., Garcia, M. T., Carilla, J., Matito, C., Centelles, J. J., Cascante, M., Sort, X., & Bobe, R. (2002). Valorization of Grape (Vitis vinifera) Byproducts. Antioxidant and Biological Properties of Polyphenolic Fractions Differing in Procyanidin Composition and Flavonol Content. *Journal of Agricultural and Food Chemistry, 50*, 7548−7555.

Wollgast, J. & Anklan, E. (2000). Polyphenols in chocolate: is there a contribution to human health?. *Food Research International, 33*, 449-459.

Yilmaz, Y. & Toledo, R. T. (2004). Major Flavonoids in Grape Seeds and Skins: Antioxidant Capacity of Catechin, Epicatechin, and Gallic Acid. *Journal of Agricultural and Food Chemistry, 52,* 255−260.

OBJETIVOS

Objetivo Geral

Estudar os compostos fenólicos presentes em resíduos de vinificação, tais como bagaço (casca e semente) e borra, oriundos de uma vinícola localizada em São Roque-SP e a utilização de métodos de secagem para a obtenção de subprodutos estáveis, correlacionando esses compostos com a capacidade antioxidante.

Objetivos Específicos

1) Determinar o teor de compostos fenólicos e antocianinas totais das uvas *in natura* e nos diferentes resíduos da vinificação em variedades híbridas (BRS Violeta e BRS Lorena) e *Vitis vinifera* (Cabernet sauvignon e Cabernet franc) por duas safras consecutivas.

2) Comparar a eficiência dos métodos antioxidantes (ORAC, FRAP, ABTS e β-caroteno/ácido linoleico) na estimativa da capacidade antioxidante e correlacionar com o teor de compostos fenólicos e antocianinas totais contidos nos subprodutos.

3) Avaliar a estabilidade dos resíduos de vinificação secos (cascas fermentadas, sementes e borras) em 0, 30 e 90 dias de armazenamento, quanto ao teor total de compostos fenólicos e antocianinas totais e da capacidade antioxidante.

4) Identificar e quantificar os compostos fenólicos e antocianinas presentes nas uvas, e nos subprodutos, utilizando a combinação das metodologias analíticas SPE e HPLC-DAD-ESI MS/MS (do inglês, Extração em fase sólida e Cromatografia líquida de alta eficiência acoplada a espectrômetro de massas), por duas safras consecutivas (2011 e 2012).

5) Avaliar o efeito de três técnicas de secagem aplicadas aos subprodutos da vinificação no teor e perfil de compostos fenólicos e antocianinas.

REVISÃO BIBLIOGRÁFICA

Revisão Bibliográfica

1 Uva e Vinho no Brasil

1.1 Produção e Mercado

A vitivinicultura brasileira tem apresentado crescimento significativo nos últimos anos, decorrente da vigorosa expansão na área cultivada e na tecnologia de produção de uvas e de elaboração de vinhos. Em 2012 a produção de uvas no Brasil foi de aproximadamente 1 milhão de toneladas, sendo 57% destas uvas destinada a produção de vinhos e sucos. Por não se dispor de estatísticas sobre a produção nacional de vinhos e sucos de uvas, e pelo fato do estado do Rio Grande do Sul ser o responsável por cerca de 90% da produção nacional desses produtos, utiliza-se esse estado para ter uma boa aproximação do desempenho da agroindústria vinícola do país (Mello, 2013).

Do total de produtos industrializados a partir de uva, 60% são vinhos e 40% são sucos e outros derivados da uva e do vinho. Em 2012 a produção brasileira foi, em média, de 261 milhões de litros de vinho, sendo 81% vinho de mesa e 19% vinho fino no Rio Grande do Sul. O alto consumo de vinhos de mesa pode ser explicado pelo poder aquisitivo da população brasileira, pois esses vinhos são comercializados por preços relativamente acessíveis, assim como pela preferência cultural e aspecto sensorial (Mello, 2011, Mello, 2013, Sobrinho, 2013).

A partir desses dados pode-se observar uma característica incomum quanto ao setor vinícola no Brasil. Pois, no mercado existem tanto produtos de variedades americanas e híbridas (*Vitis labrusca e Vitis bouquirna),* quanto produtos de variedades de uvas finas (*Vitis vinifera*), diferentemente do que ocorre em outros países, onde somente é permitido produtos oriundos das variedades de uvas finas. Conforme dados da Embrapa, em 2010 o

Brasil mostrava-se como 14° maior produtor mundial de uvas, 20° em área cultivada com videiras e 13° colocado em produção de vinhos no mundo, tendo aumentado sua produção em 20,73% quando comparada ao ano de 2000 (Mello, 2012).

Segundo dados da produção de uvas no ano de 2012 fornecido pela Embrapa Uva e Vinho, o estado de São Paulo foi o terceiro maior produtor de uvas (177 mil toneladas) do país (Mello, 2013). Os cultivos estão concentrados principalmente em dois pólos vitícolas: um na região leste e outro na região noroeste. Na região leste, a área de vinhedos é da ordem de 7.870 hectares. Essa região foi dividida em 3 grupos, no primeiro grupo estão as cidades de Jundiaí, Vinhedo, Indaiatuba, Valinhos e Campinas, destacando-se pela produção de uva americana para vinhos de mesa, representando cerca de 67% da área cultivada, no segundo grupo, centrado no município de São Roque, estão as uvas destinadas à elaboração de vinho, representando aproximadamente 4% da área cultivada e finalmente, no terceiro grupo, que tem por município pólo São Miguel Arcanjo, estão as uvas de mesa. Na Região Noroeste de São Paulo, centrada na cidade de Jales, a viticultura ocupa cerca de 1.212 hectares e está em fase de expansão. O nível tecnológico é alto, proporcionando colheitas da ordem de 40 toneladas/hectare (Mello, 2011).

Existem, no mundo, milhares de variedades de uva. A maioria delas pertence à espécie *Vitis vinifera,* de origem europeia, utilizadas para produção de vinhos finos, enquanto outras, classificadas como *Vitis labrusca* e *Vitis bourquina*, são uvas americanas, difundidas para o consumo *in natura*, ou utilizadas como matéria-prima para a elaboração de vinhos de mesa. A viticultura brasileira apresenta grande diversidade, atualmente, são mais de 120 cultivares de *Vitis vinifera* e mais de 40 cultivares de uvas americanas (*Vitis labrusca* e *Vitis bourquina)* e híbridas interespecíficas. Algumas se consagraram pela ampla

capacidade de adaptação e pelas características dos vinhos que originam; outras, de adaptação mais restrita, permaneceram em suas regiões de origem, proporcionando a elaboração de produtos típicos e exclusivos. No Brasil, a vitivinicultura começou com base em uvas não viniferas, variedades das espécies *Vitis labrusca* e *Vitis bourquina*, usadas para a elaboração de vinhos de mesa. O sul do país consolidou-se, predominantemente, com a cultivar Isabel, seguida de outras uvas americanas, como Herbemont, Seibel 2, Niágara Branca, Niágara Rosada e Jacquez. Já no Estado de São Paulo, inicialmente a cultivar Isabel foi plantada, seguida pelas cultivares Seibel 2 e Máximo (IAC 138-22) (Camargo, Tonietto, & Hoffmann, 2011).

A partir de meados do século XX começaram a ser elaborados vinhos finos, com uvas de variedades de *Vitis vinifera*, onde os primeiros vinhos varietais deram-se a partir de Cabernet Franc, Merlot e Riesling Itálico. Após alguns anos foram implantadas uvas de origem francesa, como Cabernet Sauvignon, Tannat, Sémillon e Chardonnay e, mais recentemente, Pinot Noir, Tempranillo, Sauvignon Blanc, Moscato Giallo, Viognier, Syrah, Alicante Bouschet, Chenin Blanc e Moscato Canelli (Guerra, Mandelli, Tonietto, Zanus, & Camargo, 2009, Camargo et al., 2011).

Com relação ao desenvolvimento e a difusão das uvas americanas e híbridas, atualmente o Brasil tem mostrado perspectivas de grande expansão, pois já se tem volume significativo de produção, destacando-se as varietais brancas Moscato, Embrapa e BRS Lorena, e as tintas BRS Rúbea, BRS Cora, BRS Violeta, BRS Carmem, Isabel Precoce e Concord Clone 30, usadas para produção de sucos e vinhos.

1.2 Composição das Uvas

De uma perspectiva de vinificação, a baga da uva tem três principais tipos de tecido

(**Figura 1**): polpa, casca e sementes. Estes tecidos variam consideravelmente na sua composição e, portanto, por extensão contribuindo de forma diferente para a composição global do vinho (Kennedy, 2002).

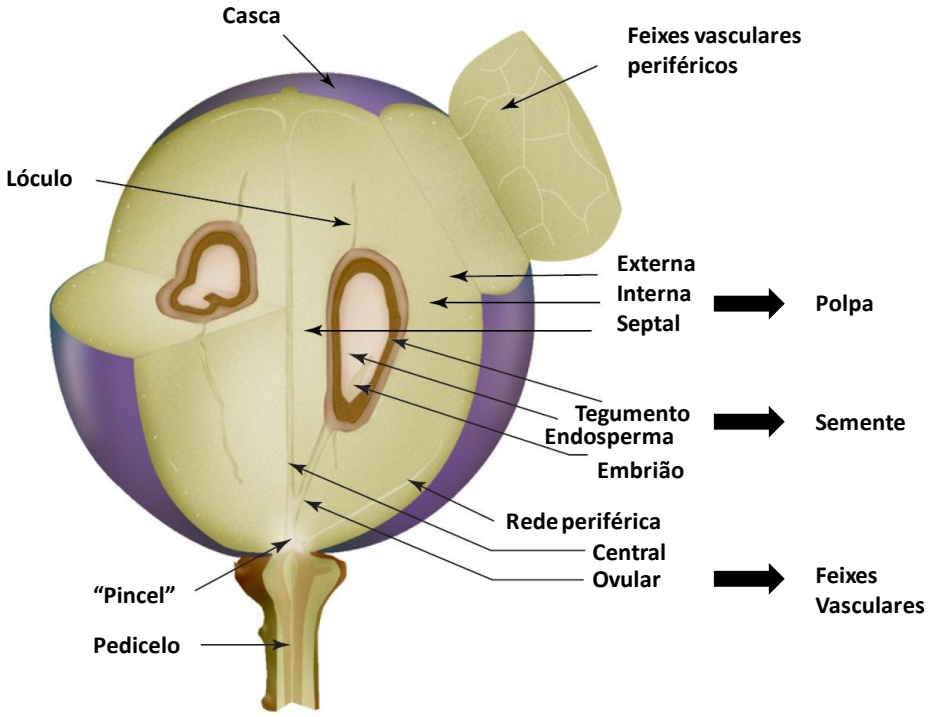


Figura 1. Esquema anatômico da baga de uva (Kennedy, 2002).

De maneira geral, a casca da uva representa de 5 a 10% do peso fresco da uva, e age como barreira hidrofóbica contra a contaminação fúngica, além de proteger a fruta da desidratação, raios ultravioletas e injúrias físicas. Adicionalmente, contém substâncias responsáveis pelo sabor, aroma e pigmentação. Esta última característica é devida a presença de pigmentos chamados antocianinas, as quais são responsáveis pela cor dos vinhos, pois são compostos extraíveis através da maceração da fruta durante a vinificação. A concentração desses compostos varia de acordo com a variedade, estação e condições ambientais (Kennedy, 2002).

A polpa, representa cerca de 78% do peso da fruta e é constituída de açúcares, ácidos orgânicos, cátions minerais, compostos nitrogenados, substâncias pécticas e compostos fenólicos não flavonoides. No entanto, algumas bagas contém polpa pigmentada, contribuindo assim para a coloração dos vinhos, como por exemplo aBRS Violeta (Kennedy, 2002, Rebello et al., 2013).

Por último, as sementes da uva apresentam um papel importante na qualidade final do vinho, pois contribuem significativamente com a concentração de flavan-3-óis e procianidinas (responsáveis pela adstringência e amargor). Representam cerca de 4% do peso da uva e aproximadamente 60% da concentração fenólica total da uva (Falcão, 2007).

Durante a maturação das uvas, vários processos bioquímicos ocorrem em diferentes taxas, e são específicos para cada estágio, podendo citar o aumento do número e do tamanho das sementes, mudança no tamanho da baga, e acúmulo de açúcares e antocianinas (variedades tintas). No entanto, a maturação das uvas é influenciada pela cultivar, topografia, condições climáticas sazonais e vinhedos. O conhecimento da maturação da uva tem importância na qualidade final do vinho, assim algumas técnicas foram desenvolvidas para o seu monitoramento e decisão da data de colheita para fins de vinificação. A variável tradicionalmente utilizada é a concentração de sólidos solúveis totais (°Brix), que juntamente com a acidez titulável e/ou pH (ácido málico), caracterizam a maturação comercial, pois servem de base para o cálculo do teor alcoólico do vinho, onde cerca de 90% dos sólidos são açúcares fermentáveis (Falcão, 2007, Bindon et al., 2013).

A legislação brasileira vigente define que o vinho é uma bebida obtida da fermentação alcoólica completa ou parcial da uva fresca, esmagada ou não, ou do mosto de uvas sãs, frescas e maduras. Diferentemente do que ocorre em quase todos os países

produtores, que utilizam apenas videiras européias (*Vitis vinifera*), ao setor vitivinícola brasileiro é permitido elaborar também vinhos de mesa de uvas americanas. Portanto, os vinhos brasileiros podem ser elaborados com uvas classificadas como não viniferas (*Vitis labrusca* e *Vitis bouquina*) e/ou híbridas, podendo conter vinhos de variedades *Vitis vinifera* (Brasil, 1988, Brasil, 2004, Sobrinho, 2013).

1.3 Processamento do Vinho

A conversão das uvas em vinho envolve uma série de transformações complexas, onde o sabor e o aroma são derivados de diferentes componentes do processo, e o conteúdo de açúcares, pigmentos, aroma, sabor e outros componentes químicos são oriundos das uvas e das alterações impostas pelo processamento, tais como extração, fermentação, sulfitagem, trasfegas, clarificação, estabilização e envelhecimento do vinho (Jackon, 2008).

Inicialmente, após a colheita e o transporte, a separação do engaço das bagas é efetuada com a finalidade de limitar a adstringência, o amargor e o gosto desagradável de herbáceo. As uvas, brancas ou tintas, são esmagadas e prensadas, dando origem ao mosto. Diferentemente do vinho tinto, onde as cascas entram em contato direto com o mosto para que ocorra a extração dos pigmentos e dos taninos durante a maceração, o processamento do vinho branco se faz sem a presença das cascas das uvas, etapa chamada de desmontagem (separação do mosto das partes sólidas das uvas após o esmagamento dando origem ao bagaço) (Filho, 2010).

A etapa de prensagem é importante para liberação do suco da fruta, aumentando assim o rendimento devido ao alto teor de açúcares, o qual é importante na fermentação. Nesta etapa é adicionado o dióxido de enxofre (SO_2) com a função de higienizar o mosto, impedindo que as leveduras selvagens fermentem os açúcares, produzindo assim

componentes de odor e sabor desagradáveis, além de impedir a ação das polifenoloxidases (Filho, 2010).

Na moderna enologia, não se concebe a elaboração de vinho sem o uso de leveduras selecionadas. A levedura *Saccharomyces cerevisiae* é a que tem melhor capacidade de transformar totalmente os açúcares da uva em álcool etílico e outros compostos. Quando a uva não contém teor necessário de açúcar, pode-se realizar a chaptalização, processo de adição de açúcar na concentração estabelecida pela legislação (Filho, 2010).

O mosto é submetido ao processo de fermentação, que passa por duas etapas, uma fermentação alcoólica seguida de uma fermentação malolática. A primeira é a etapa mais importante, apresentando duração de aproximadamente 5 a 25 dias em temperatura de 25-30°C, tempo necessário para que o vinho alcance a densidade (1010 e 1015 g/L) e teor de açúcar ideais (3-4 g/L). A temperatura de fermentação, a relação bagaço/mosto e a frequência das remontagens (cerca de 3 vezes ao dia) também constituem-se em agentes determinantes de variação do teor de fenóis totais nos vinhos, além de homogeneizar a massa em fermentação, controlar a temperatura e evitar o desenvolvimento de micro-organismos indesejáveis (Filho, 2010).

Após a fermentação alcoólica ocorre a descuba, visando a separação do líquido (vinho tinto em elaboração) das partes sólidas, sólido este chamado de bagaço (cascas e sementes). O líquido separado é submetido a fermentação malolática por 2 a 4 semanas, nesta etapa ocorre formação de ácido lático e CO_2 a partir do ácido málico (mais comum em vinho tinto), proporcionando maior estabilidade biológica e complexidade de aroma e sabor aos vinhos. Com o fim da fermentação malolática, é realizada a trasfega, que consiste em separar o vinho da borra (partículas sólidas insolúveis que se depositaram naturalmente

no período em que transcorre a fermentação). Por fim, realiza-se a clarificação e a estabilização, com o objetivo de remover partículas em suspensão no vinho e evitar a turvação, e a filtração, para eliminar a presença de micro-organismos e partículas em suspensão. Neste momento o mosto foi definitivamente transformado em vinho, o qual pode ser engarrafado, ou envelhecido nos barris, e envasado (Campos, 2005, Guerra, 2002, Guerra et al., 2009, Góes, 2005, Filho, 2010).

O vinho é um fluido complexo, que contém água, açúcares, ácidos, alcoóis e uma gama de compostos fenólicos. Estes são derivados das uvas e da madeira utilizada no envelhecimento do vinho. A concentração dos compostos fenólicos no vinho é dependente dos fatores como tempo de maceração, temperatura e tempo de fermentação, dentre outros. Sendo assim, parte desses compostos permanecem nos sólidos da uva (bagaço) e parte é passada para o vinho.

2 Resíduos Vinícolas

O Brasil, por ser um país de grande atividade agrícola, é um dos que mais produzem resíduos agroindustriais. Diante deste problema, a busca de alternativas para utilização da matéria orgânica gerada vem crescendo dentro de vários centros de pesquisa (Cataeno, Caliari, Gonzaga, Kuskoski, & Fett, 2008).

Nos últimos anos, a produção e mercado de vinho tem se destacado na economia brasileira e pesquisas mostram que o cenário é favorável para o setor vitivinícola nos próximos anos. Assim, dentre os diversos resíduos gerados no país, destacam-se os vinícolas, como o bagaço e borra. Pois cerca de 60% da colheita de uva

é utilizada para vinificação, gerando um resíduo de bagaço, em torno de 5% do peso das uvas processadas, e da borra, em torno de 4% do vinho total produzido.

O bagaço (**Figura 2A e 2B**) é o mais abundante dos resíduos, sendo produzido após a prensagem das uvas, durante a produção de vinho branco, ou após a fase de maceração/fermentação alcoólica durante a produção de vinho tinto. Já a borra, constituída de finas partículas de resíduo de uvas e leveduras mortas, é obtida por processo de decantação após a fermentação do mosto (Maragkoudakis et al., 2013, Paradelo, Moldes, & Barral, 2010, Cortés, Rodríguez, Salgado, & Domínguez, 2011).

Figura 2. Bagaço de resíduo vinícola. A) BRS Lorena e B) Cabernet franc.

Borra do vinho (**Figura 3**) é considerada como o resíduo que fica depositado nos recipientes que contém vinho após a fermentação, quando da armazenagem, ou após tratamento autorizado, bem como o resíduo obtido pela filtração e/ou pela centrifugação deste produto. Este resíduo é composto principalmente por micro-organismos (leveduras), ácido tartárico, matéria inorgânica e compostos fenólicos (Pérez-Serradilla & Luque de Castro, 2008).

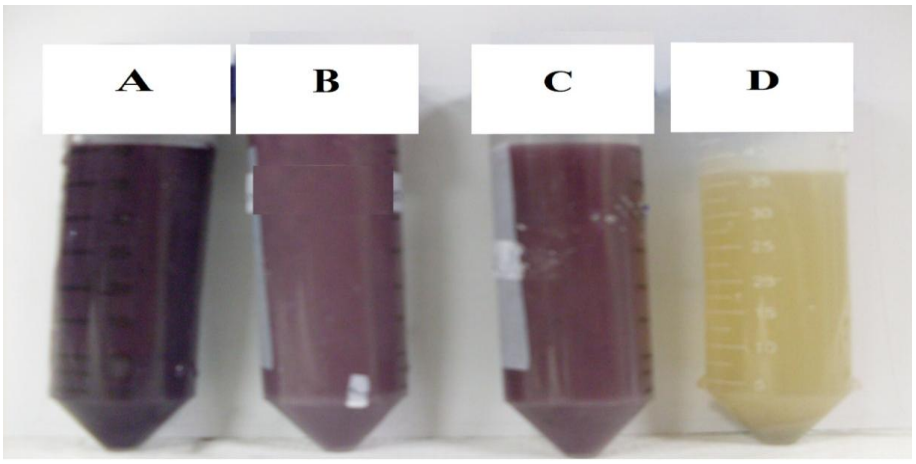


Figura 3. Borra de BRS Violeta (A), Cabernet sauvignon (B), Cabernet franc (C) e BRS Lorena (D).

As quantidades de borras obtidas dependem de vários fatores, nomeadamente os inerentes à própria constituição das uvas, estado de maturação e de higiene das bagas, fatores climáticos e às técnicas de vinificação adotadas, não permitindo estabelecer um valor preciso de borra gerada (Silva, 2003).

Segundo dados da indústria, os resíduos gerados na produção de vinho estão sendo utilizados como ração animal, mesmo apresentando o inconveniente da presença de álcool, e como fertilizantes de vinhedos, sendo o bagaço, ainda, utilizado na destilação de álcool pelas vinícolas. Entretanto, a maior parte desses resíduos ainda é descartada sem tratamento, causando danos ao meio ambiente, como a contaminação de águas superficiais e subterrâneas (Cabras, Angioni, Garau, Minelli, Melis, & Pirisi,1997, Diaz, Madejón, López, López, & Cabrera, 2002, Campos, 2005, Torres et al., 2002, Lafka, Sinanoglou, & Lazos, 2007, Llobera & Cañellas, 2007, Rockenbach, Silva, Rodrigues, Kuskoski, & Fett, 2008).

A utilização dos resíduos da indústria vinífera representa um avanço significativo na

manutenção do equilíbrio do meio ambiente. Pois, nas grandes quantidades de resíduos produzidos geram sérios problemas de armazenagem, de transformação, ou de eliminação, em termos ecológicos e econômicos (Alonso, Guillen, Barroso, Puertas, & Garcia, 2002, Arvanitoyannis, Ladas, & Mavromatis, 2006, Lafka et al., 2007, Cataneo, et. al., 2008).

Na literatura são poucos os trabalhos que exploram esses resíduos. O destino dado a estes, tal como é feito, causa um déficit econômico na cadeia produtiva, uma vez que muitos deles são ricos em compostos bioativos, ou seja, compostos com potentes poderes antioxidantes e, por isso, com elevado valor comercial, despertando assim o interesse científico e econômico. Dentre os compostos bioativos, os resíduos gerados pelas vinícolas são fontes ricas em compostos fenólicos, já que apresentam uma expressiva quantidade resultante do processamento. A soma de bagaço (cascas e sementes), engaço e borra representam, em média, cerca de 30% do volume de uvas utilizadas para a produção vinícola, o que os torna uma fonte promissora de substâncias bioativas naturais com alto valor agregado (Melo et al., 2011). Esta situação explica o interesse crescente em explorar os compostos fenólicos presentes nos resíduos vinícolas em virtude da sua funcionalidade, como é o caso dos antioxidantes.

Estudos já realizados com o bagaço demonstraram que o mesmo contém compostos que não foram totalmente extraídos durante o processo de fabricação do vinho, pois possui uma elevada quantidade de metabolitos secundários, incluindo os ácidos fenólicos, flavan-3-óis e antocianinas (Makris Boskou & Andrikopoulos, 2007). Muitos estudos têm sido focados em subprodutos da vinificação de variedades *Vitis vinifera*, entretanto pouco se sabe sobre o potencial de polifenóis das variedades de uvas não-viníferas (Sant'Anna, Brandelli, Marczak, & Tessaro, 2012).

Segundo Ascheri, Ascheri e Carvalho (2006), o bagaço vitivinícola pode ser reaproveitado em ingredientes que possam substituir parte das calorias de alimentos ricos em carboidratos, além de influenciar em vários aspectos a digestão, a absorção e o metabolismo.

A literatura sobre borra relata que a atividade da enzima β-glucosidase da levedura pode influenciar na concentração de antocianinas e outros compostos fenólicos, como flavonoides ou ácidos hidroxicinâmicos. No entanto o mecanismo pelo qual esse fenômeno ocorre não está bem esclarecido (Morata, Gómez-Cordovés, Colomo, & Suárez, 2005, Pérez-Serradilla & Luque de Castro, 2008). Adicionalmente, não foram encontrados dados científicos que apresentem informações quanto à composição química da borra vinícola. Sendo assim, a realização de estudos com este tipo de resíduo pode ser uma opção promissora para a verificação da presença de compostos fenólicos, tendo em vista sua importância para a saúde.

Dos trabalhos encontrados na literatura, a grande maioria se conceitua no estudo dos compostos fenólicos residuais. Amico, Napoli, Renda, Ruberto, Spatafora e Tringali (2004), Arvanitoyannins et al. (2006) e Amendola, Faveri e Spigno (2010) apresentaram um estudo relatando as propriedades funcionais dos resíduos vinícolas, especificamente o bagaço analisados, onde os principais compostos encontrados foram compostos fenólicos, antocianinas, catequinas, ácido tartárico, ácido málico, açúcares, ácidos graxos e minerais. Krammerer, Claus, Carle e Schieber (2004) caraterizaram os compostos fenólicos de bagaços produzidos na Germânia, e identificaram 13 antocianinas, 11 ácidos fenólicos, catequinas e estilbenos. Karvela, Makris, Kalogeropoulos, Karathanos e Kefalas (2009) encontraram, em sementes de resíduos vinícolas da Grécia, os seguintes compostos: ácido

gálico, ácido tânico, catequina e dímeros de flavanois.

Deste modo, é importante explorar os subprodutos de vinícolas, como o bagaço e borra, que apresentam constituintes funcionais importantes para as indústrias de alimentos, farmacêuticas e químicas, permitindo assim agregar valor a estes resíduos industriais, dando-lhes um destino mais nobre (Arvanitoyannins et al., 2006).

3 Compostos Fenólicos de Uvas e Vinhos

A videira (*Vitis spp*.), dentre tantos outros vegetais, destaca-se em função dos altos teores de compostos fenólicos que estão presentes nos tecidos dos frutos, folhas e sementes, bem como pela variabilidade de estruturas químicas encontradas. Atualmente, um número crescente de trabalhos têm estudado os aspectos quali/quantitativos destes compostos em biomassas de diversas espécies e variedades de videiras e de seus produtos, como em vinhos e sucos e outros subprodutos (Maraschin, 2003, Cadot, Chevalier, & Barbeau, 2011, Lago-Vanzela et al., 2013, Bindon et al., 2013).

Quimicamente, os compostos fenólicos pertencem a uma classe de moléculas com uma grande diversidade estrutural, caracterizando-se por apresentar um anel aromático, possuindo um ou mais grupos hidroxila diretamente associados com a estrutura de anel, podendo variar desde moléculas fenólicas simples a compostos altamente polimerizados (Zhu, Zhang, & Lu, 2012).

Os compostos fenólicos são metabólitos secundários que não participam de vias metabólicas responsáveis pelo crescimento e reprodução, sendo assim, sua natureza e concentração variam enormemente.

Podem ser classificados em dois grupos: não flavonoides e flavonoides. Os flavonóides são os que apresentam a estrutura química descrita como C_6-C_3-C_6 consistindo de dois anéis fenólicos (A e B), ligados por um centro pirano (contendo oxigênio) anel (C). Já os não flavonoides são compostos por ácidos fenólicos, taninos hidrolisáveis e estilbenos (C_6-C_2-C_6) (Melo & Guerra, 2002, Angelo & Jorge, 2007, Cataeno, et al., 2008, Del Rio et al., 2013).

Variações na substituição do anel C resultam em importantes classes de flavonoides, como flavonóis, flavonas, flavanonas, flavanois (ou catequinas), isoflavonas e antocianidinas. Já as substituições dos anéis A e B originam diferentes compostos dentro de cada classe de flavonoides (Peterson & Dwyer, 1998, Angelo et al., 2007, Del Rio et al., 2013).

Os flavonoides são largamente sintetizados no retículo endoplasmático celular, antes de serem translocados e armazenados no vacúolo central da célula produtora. Sua suposta função nas uvas (e outros plantas) é como uma linha de defesa contra patógenos microbianos, pragas e herbívoros e contra variações no conteúdo de água, luz, radiações UV e deficiência de minerais (Terrier, Poncet-Legrand, & Cheynier, 2009, He et al., 2012, Jackon, 2008).

Os flavonoides são formados da combinação de derivados sintetizados da fenilalanina (via metabólica do ácido chiquímico) e do ácido acético. O grupo dos flavonóides é também conhecido como polifenólicos e, geralmente, ocorrem em plantas na forma de glucosídeos, sendo uma das classes de substâncias responsáveis pela atribuição do perfil sensorial. A distribuição dos flavonoides nos vegetais depende de diversos fatores, podendo variar de acordo com a ordem e família do vegetal, bem como com a variação das

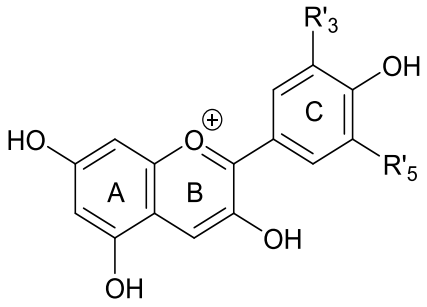
espécies. Como exemplo, pode-se citar as uvas de variedades tintas que caracterizam-se por apresentar teores mais elevados de compostos fenólicos nos tecidos da casca, dos frutos e sementes, que as variedades brancas e rosadas (Aherne & O'brien, 2002, Bobbio & Bobbio, 1989, Cordenunsi, Nascimento, Genovese, & Lajolo, 2002, Fennema, 1993, Sluis, Dekker, Jager, & Jongen, 2001).

Entre os flavonoides, sua ocorrência se dá principalmente nas cascas de uvas tintas, e em menor proporção na polpa das castas tintureiras, sendo responsáveis pelas cores azuis, violeta, vermelha e púrpura. Além da possibilidade de serem utilizadas como substitutos de corantes sintéticos em alimentos, é também estudada a relação entre sua capacidade antioxidante e possíveis efeitos benéficos à saúde (Jordão, Silva, & Laureano, 1998, Hou, 2003, Duthie, 2007, Zafra-Stone, Yasmin, Bagchi, Chatterjee, Vinson, & Bagchi, 2007, Hafeez et al., 2008, Castillo-Muñoz, Gomez-Alonso, Garcia-Romero, Gomes, Velders, & Hermosín-Gutiérrez, 2009).

As antocianinas estão incluídas no grupo de pigmentos de ocorrência natural (**Figura 4**). Estruturalmente, as antocianinas são derivados glicosilados do cátion 2-fenil benzopirilium, também denominado de cátion flavílico. A substituição na posição 3 do anel C, e na posição 5 e 7 do anel A, por uma ou mais unidades de açúcar na antocianidina (aglicona) resulta numa antocianina. As variações estruturais das antocianinas denotam de diferentes açúcares ligados, da polimerização e nos modos ou posições de hidroxilação e metilação. Em alguns casos, os açúcares apresentam-se acilados pelos ácidos *p*-cumárico, cafeíco, fenílico e vanílico, dentre outros ácidos (Terci & Rossi, 2002, Okumura, Soares, & Cavalheiro, 2002).

Na literatura já foram mencionadas 23 antocianidinas nas uvas, que diferem entre si

pelo número e posição dos grupos hidroxilas/metoxilas, embora haja predominância de 5 antocianidinas: cianidina, delfinidina, peonidina, petunidina e malvidina, presentes tanto nas uvas viníferas quanto nas não viníferas, também foram encontradas pequenas quantidades de perlagonidina nas uvas viníferas (**Figura 4**). As suas quantidades relativas variam com a variedade, entretanto a malvidina é sempre majoritária. É característico das *Vitis vinifera* encontrar uma molécula de glicose ligada na posição 3 (3-glicosídeo), uma vez que outras espécies do gênero *Vitis* contém diglicosídeos nas posições 3 e 5 (3,5-diglicosídeos), como em *Vitis labrusca* (por exemplo, Concord) e V*itis rupestris*, *Viti riparia*, e as espécies asiáticas *Vitis amurensis* contém ambos os 3-glicosídeos e 3,5-diglicosídeos, já as uvas *Vitis rotundifolia* (Muscadine) contém apenas 3,5-diglicosídeos (Jordão et al.,1998, Blouin & Guimberteau, 2002, Flanzy, 2003, Maraschin, 2003, Kong, Chia, Goh, Chia, & Brouillard, 2003, Andersen & Markham, 2006, Castillo-Muñoz et al., 2009).



R'3	R'5	Nome da Aglicona
OH	H	Cianidina
OCH3	H	Peonidina
OH	OH	Delfinidina
OH	OCH3	Petunidina
OCH3	OCH3	Malvidina

Figura 4. Estrutura das antocianinas encontradas em uvas e derivados (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2006).

Diferentemente das uvas, a composição fenólica de vinhos, demonstram que cerca de 25% de antocianinas podem ter sido polimerizadas com flavonoides ou outros compostos fenólicos durante a fermentação. Este nível pode subir para mais de 40% dentro

de um ano, podendo chegar até o nível de 100% depois de vários anos (Jackon, 2008).

No vinho tinto feito a partir de cultivares de uva *Vitis vinifera,* as piranoantocianinas são formadas nos primeiros passos da fermentação, principalmente derivado da reação de ácido pirúvico com antocianinas (antocianidina-3-glicosídeos) e acetaldeído, dois metabolitos intermediários de levedura (**Figura 5**) (Nixford & Hermosín-Gutiérrez, 2010, Blanco-Vega, López-Bellido, Alia-Robledo, & Hermosín-Gutiérrez, 2011). Portanto, não é surpreendente encontrar uma espécie de piranoantocianinas em subprodutos da vinificação, como foi relatado para bagaço de uva da cultivar siciliana Nerello Mascalese (Amico et al., 2004).

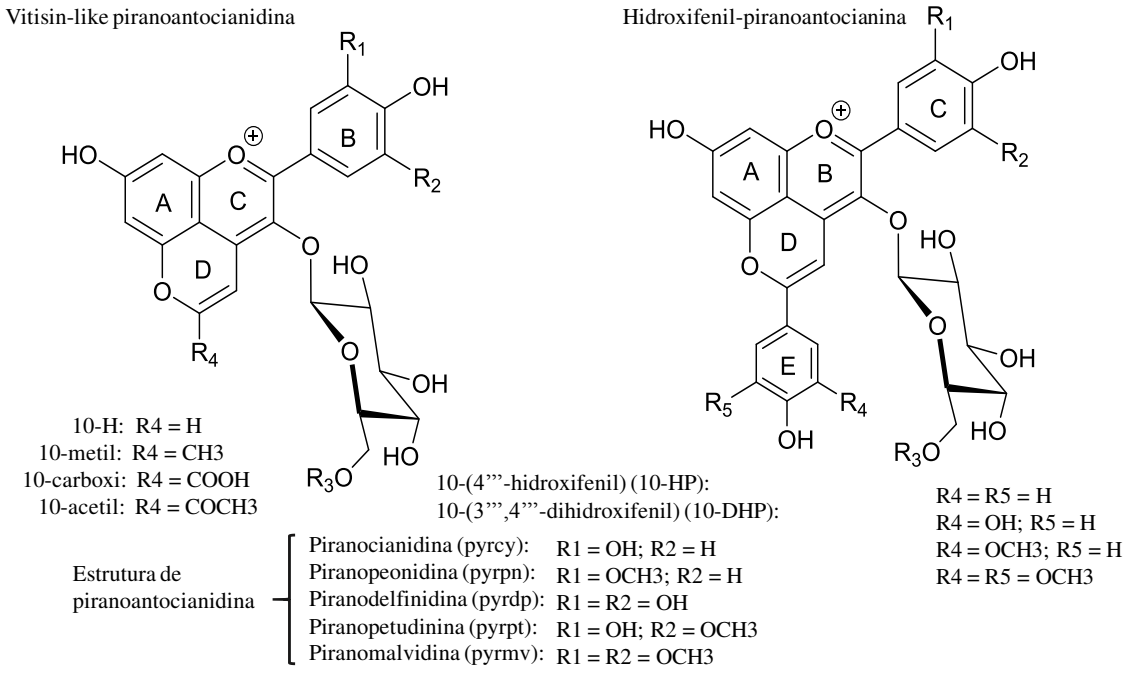


Figura 5. Estrutura química das piranoantocianinas (Rentzsch, Schwarz, & Winterhalter, 2007).

Diferentemente dos vinhos tintos, pouco se sabe sobre o desenvolvimento e natureza química da cor do vinho branco. A pequena quantidade de material fenólico

encontrado nos vinhos brancos consiste de não flavonóides (hidroxicinâmicos), tais como caftárico, ácido *p*-cumárico e ferúlico (Jackson, 2008).

Castillo-Muñoz, Gómez-Alonso, García-Romero e Hermosín-Gutiérrez (2007) relatam que a ocorrência de flavonóis nas cultivares de castas tintas é representada por seis estruturas: campferol, quercetina, isoramnetina, miricetina, laricitrina e siringetina (**Figura 6**). Alguns trabalhos descrevem a presença dos flavonois miricetina e isoramnetina também em uvas brancas. Além disso, os flavonois predominantes em *Vitis vinifera* são quercetina e miricetina, enquanto que nas uvas *Vitis labrusca* é a quercetina (Castillo-Muñoz, Gómez-Alonso, García-Romero, & Hermosín-Gutiérrez, 2010).

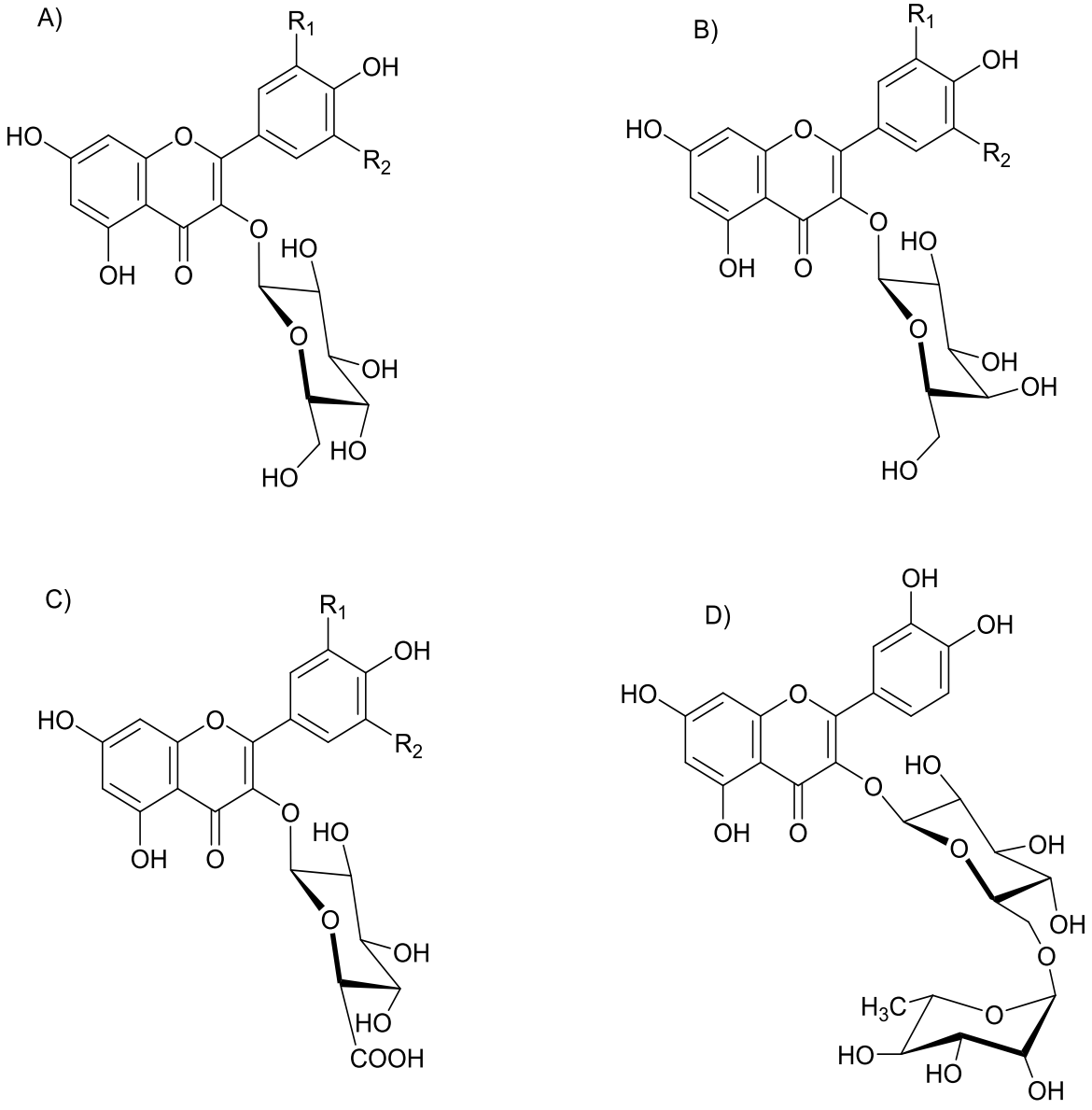


Figura 6. Estruturas dos flavonols identificados em uvas: kaempferol (R1=R2=H); quercetina (R1=OH, R2=H); isoramnetina (R1=OCH3, R2=H); miricetina (R1=R2=OH); laricitrina (R1=OCH3, R2=OH); siringetina (R1=R2=OCH3). a) flavonol-3-O-glicosídeo; b) flavonol-3-O-galactosídeo; c) flavonol-3-O-glicuronídeo; d) rutina (quercetina-3-O-(6"-ramnosil)-glicosídeo) (Hermosín-Gutiérrez, Castillo-Muñoz, Gómez-Alonso, & García-Romero, 2011).

Os conjugados de flavonol são principalmente 3-O-glicosídeos, e a presença de açúcares em outras posições do esqueleto de flavonóis nunca foi relatada. Para

isoramnetina em uvas, apenas derivados de glicose tem sido identificados, mas miricetina, quercetina também podem ocorrer como galactosídeo e glicuronídeos (Rebello et al., 2013).

Os flavan-3-óis são compostos encontrados em maior quantidade nas sementes de uvas (**Figura 7**). A associação de várias unidades monoméricas de catequinas e epicatequinas são denominadas proantocianidinas ou taninos condensados, estes não são facilmente hidrolisáveis. No caso das variedades de *Vitis vinifera*, os principais elementos são formas monoméricas de (+)- catequina e (-)-epicatequina e seus oligômeros e polímeros (Blouin, et al., 2002, Maraschin, 2003, Terrier et al., 2009).

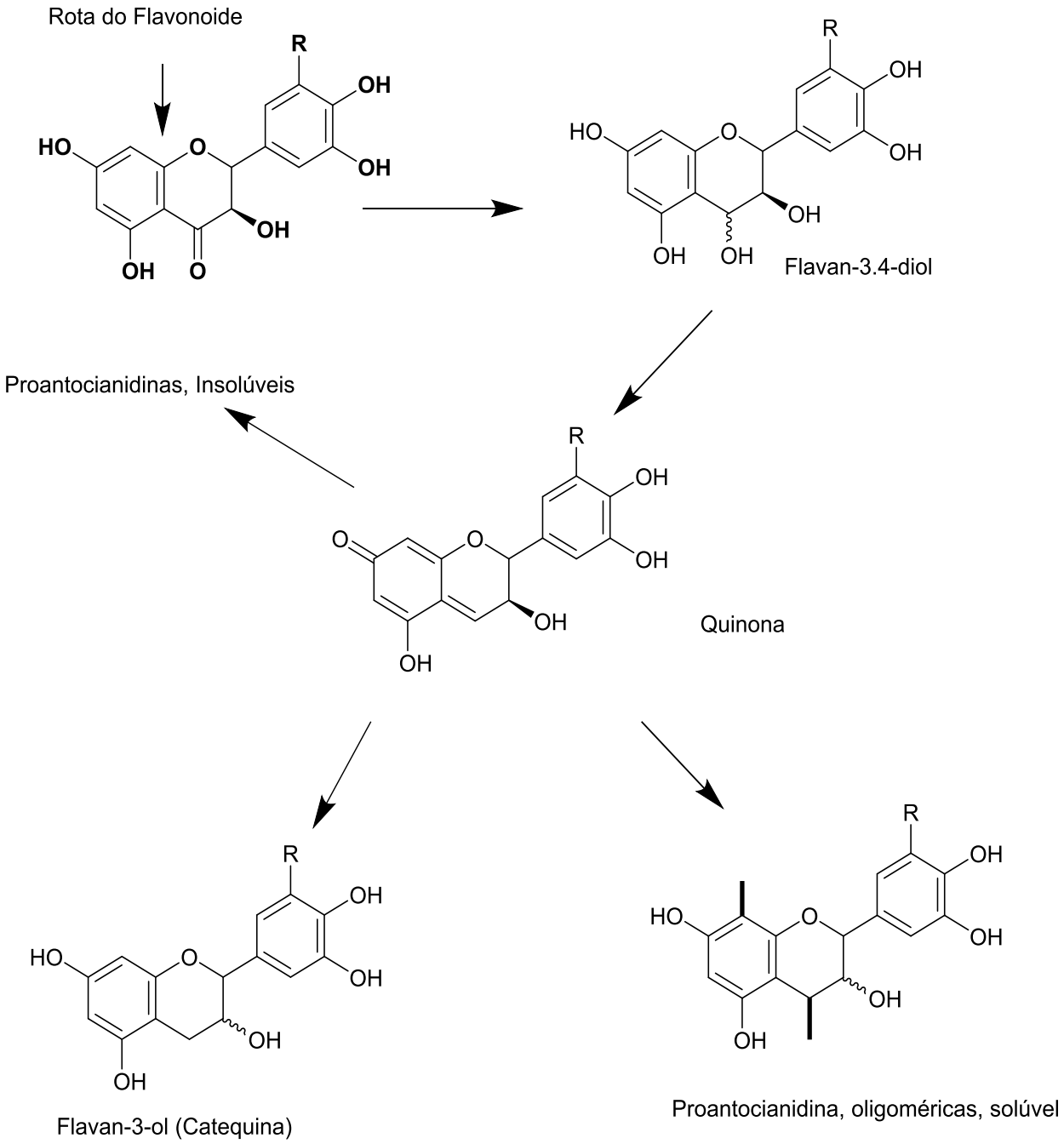


Figura 7. Metabolismo das proantocianidinas (Haslam, 2007).

Dentre os compostos fenólicos, os taninos de alto peso molecular têm a capacidade de se combinarem com proteínas e outros polímeros, como os polissacarídeos, provocando a sensação de adstringência. Já os taninos de baixo peso molecular tendem a proporcionar o

sabor amargo (Abe, Mota, Lajolo, & Genovese, 2007).

Os taninos hidrolisáveis resultam da ligação de um açúcar, geralmente a glicose, a um composto fenólico, principalmente o ácido gálico ou o ácido elágico, assim formando galotaninos ou elagitaninos, respectivamente. Esses compostos não contêm moléculas de flavonoides e não são encontrados naturalmente nas uvas. No entanto, estão presentes na madeira utilizadas no armazenamento ou envelhecimento de vinhos e, portanto, podem ser transferidas ao vinho durante esses processos.

Os ácidos hidroxibenzóicos incluem os ácidos gálico, *p*-hidroxibenzóico, protocatecuico, vanílico e siríngico, os quais apresentam a estrutura comum C_6–C_1; enquanto os ácidos hidroxicinâmicos apresentam uma cadeia lateral com três carbonos (C_6–C_3), como os ácidos caféico, ferúlico, *p*-cumárico e sinápico (Burns, Gardner, Matthews, Duthie, & Crozier, 2001, Balasundram, Sundram, & Samman, 2006).

Ácidos hidroxicinâmicos (HCA) são os representativos das classes de ácidos fenólicos encontrados em uvas e vinhos. Os principais HCA encontrados em uvas e vinhos são o ácido caftárico, ácido *p*-cutárico e ácido fertárico (ácido feruloiltartárico) (**Figura 8**). Normalmente encontram-se nas polpas das uvas os ácidos caftárico e fertárico na forma *trans*, os quais são liberados na prensagem da uva. Já o ácido *ρ*-cutárico apresenta-se principalmente na forma cis. Esses ácidos estão associados com o processo de escurecimento do vinho e são precursores de compostos fenólicos voláteis. Durante o processo de fermentação do vinho, a hidrólise parcial destes tipos de ésteres origina os ácidos hidroxicinâmicos livres. Esses são transformados em ésteres etílicos, ou seja, etil cafeato e etil cumarato (Beer, Gelderblom, & Manley, 2002, Garrido & Borges, 2011).

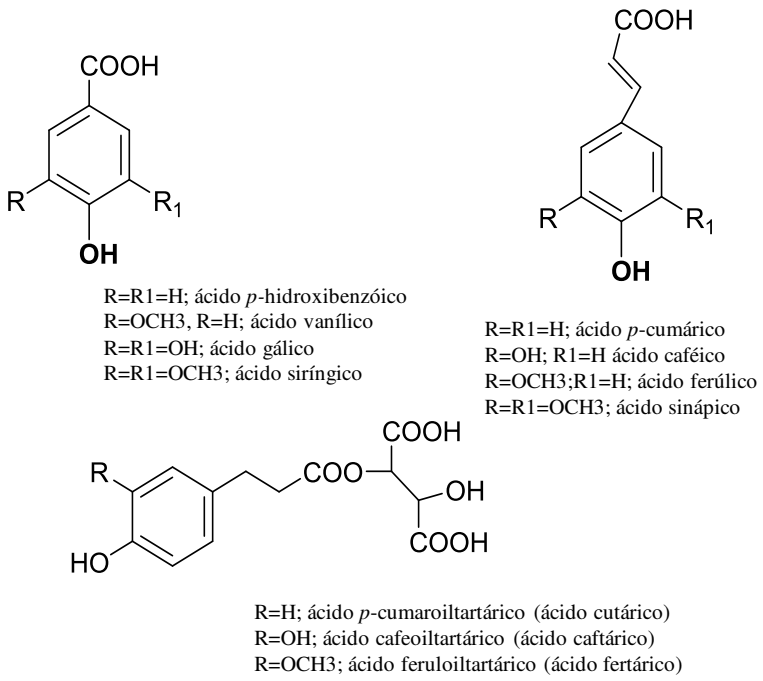
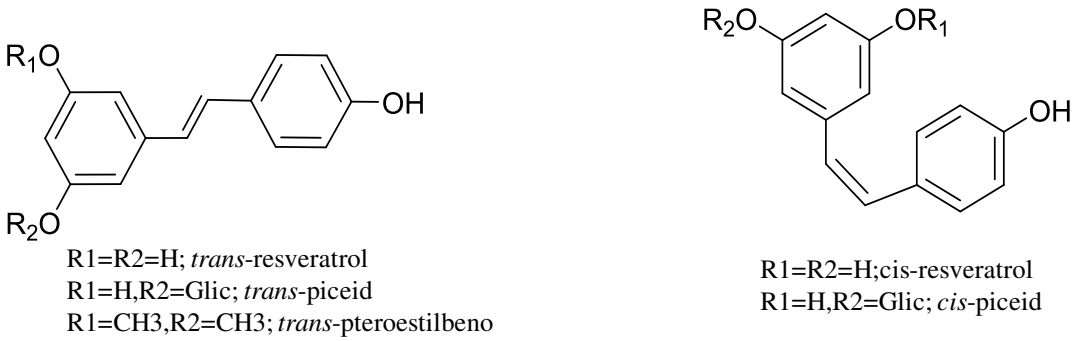


Figura 8. Principais ácidos fenólicos encontrados em uvas e derivados (Garrido et al., 2011).

Além destes compostos presentes na uva, encontra-se também o resveratrol, um polifenol pertencente à classe dos estilbenos. O resveratrol (3,5,4′-trihidroxiestilbeno) é um composto fenólico formado por dois anéis aromáticos ligados por uma ponte de etileno. É sintetizado na planta através de uma enzima, a estilbeno-sintase, que combina uma molécula de hidroxicinnamoil-Coenzima A (CoA) e três moléculas de malonil-CoA, sob duas formas isômeras: *trans*-resveratrol e *cis*-resveratrol (**Figura 9**). O resveratrol foi identificado no vinho, nas folhas de videira e na casca das uvas, e sua concentração diminui significativamente durante a maturação da uva (Garrido et al., 2011).



R1=R2=H; *trans*-resveratrol
R1=H,R2=Glic; *trans*-piceid
R1=CH3,R2=CH3; *trans*-pteroestilbeno

R1=R2=H;cis-resveratrol
R1=H,R2=Glic; *cis*-piceid

Figura 9. Principais formas de resveratrol encontrados em Vitis spp (Garrido et al., 2011).

Modificações no resveratrol, tais como glicosilação, metilação e polimerização, produzem piceid, pterostilbeno e os viniferins. O *trans*-resveratrol tem atraído atenção especial, pois sua conformação espacial pode ativar a absorção de cálcio pelos vasos sanguíneos, desencadeando a vaso-dilatação, o que contribui para a redução da pressão arterial, fato este demonstrado por estudos epidemiológicos, onde declaram correlação inversa entre o consumo moderado de vinho e a incidência de doenças cardiovasculares (Maraschin, 2003, Abe et. al., 2007, Potter, 2009).

4 Capacidade Antioxidante dos Compostos Fenólicos

Atualmente existe um incremento das pesquisas na área de determinação de capacidade antioxidante em decorrência da busca de um estilo de vida mais saudável e da constatação de que certos alimentos possuem substâncias biologicamente ativas que trazem benefícios à saúde ou efeitos fisiológicos desejáveis. Dentre as reações de deterioração que ocorrem nos alimentos, as oxidativas estão entre as mais importantes, pois provocam alterações de sabor, aroma, textura e a produção de radicais livres. Em humanos esses

radicais desempenham um papel importante na degeneração e processos patológicos de diversas doenças graves, como câncer, neurodegenerativas, aterosclerose, dentre outras (Degaspari & Waszczynskyj, 2004, Babbar, Oberoi, Uppal, & Patil, 2011).

Existem diversas maneiras para definir o termo antioxidante, no entanto, estudos descrevem como moléculas simples ou complexas que visam inibir ou eliminar os danos oxidativos de diversos substratos (Gutteridge & Halliwell, 2010, López-Alarcón & Denicola, 2013). Nesse contexto, a propriedade antioxidante se deve, principalmente, a estrutura fenólica. Portanto, o grupo OH da estrutura fenólica, podem doar hidrogênio ou um elétron à radicais livres formados, por exemplo, hidroxil ($HO^{\cdot}$), peroxil ($ROO^{\cdot}$) e peroxinitrito ($ONOO^{-}$), estabilizando-os e transformando em uma molécula relativamente estável. Assim as duas possíveis vias para a ação dos antioxidantes são: reações de transferência de um átomo de hidrogênio (*Hydrogen Atom Transfer*, HAT) ou de transferência de um elétron (*Single Electron Transfer*, SET) (Mamede & Pastore, 2004, Prior, Wu, & Schaich, 2005, Cataeno et al., 2008).

Os compostos fenólicos, juntamente com outros compostos redutores presentes na dieta humana, tais como vitamina C, vitamina E e carotenoides, tem capacidade antioxidante devido ao sequestro de radicais livres, interrompendo a reação em cadeia provocada por estes, além de atuarem também nos processos oxidativos catalisados por metais e da habilidade de complexarem-se com outras macromoléculas tais como proteínas e polissacarídeos, tanto em *vitro* como *vivo*. Essas reações proporcionam a proteção dos tecidos do corpo contra o estresse oxidativo e de patologias associadas (Soares, 2002, Taipong, Boonprakob, Crosby, Cisneros-Zevallos, & Byrne, 2006, Babbar et al., 2011).

As metodologias para a determinação da capacidade antioxidante são numerosas e

podem estar sujeitas a interferências. Por isso, atualmente, preconiza-se a utilização de metodologias diferentes pela multiplicidade de modos de ação antioxidante de polifenóis, já que nenhum ensaio usado isoladamente para determinar a capacidade antioxidante irá refletir exatamente a "capacidade antioxidante total" de uma amostra. Os ensaios de capacidade antioxidante *in vitro* são importantes para verificar se há ou não correlação entre antioxidantes potentes e os níveis de estresse oxidativo (Prior et al., 2005).

A complexidade envolvida na ação *in vivo* dos antioxidantes levou ao desenvolvimento de diferentes metodologias *in vitro* com o objetivo de estimar experimentalmente a capacidade dos antioxidantes. Segundo López-Alarcón e Denicola (2013) os ensaios químicos baseiam-se no consumo de radicais livres, na proteção de uma molécula exposta a esses radicais, na redução dos íons cúpricos ou férricos, assim como, na capacidade de inibir a oxidação da lipoproteína de baixa densidade (LDL).

Atualmente as metodologias mais empregadas na determinação da capacidade antioxidante por espectrofotometria se baseiam na descoloração de radicais estáveis pela ação de antioxidantes, como por exemplo pela descoloração do radical ABTS (2,2´azinobis (3-etilbenzotiazolina-6-ácido sulfônico) e DPPH (2,2-diphenyl-1-picrylhydrazyl), pela capacidade de redução do ferro (FRAP), pela capacidade de absorção do radical oxigênio (ORAC) e pela descoloração (oxidação) do β-caroteno induzida pelos produtos de degradação oxidativa do ácido linoleico (Caroteno-Linoleato). Os resultados obtidos são expressos em relação a um composto padrão com capacidade antioxidante conhecida, como Trolox (Nixdorf et al., 2010, Spigno, Tramelli, & Faveri, 2007, Cataeno et al., 2008), ácido gálico (Amendola et al., 2010) ou vitamina C (Cataeno et al., 2008). Atualmente existem outras técnicas que visam obter resultados quanto a capacidade antioxidante, como as

espécies reativas de oxigênio (ROS) e nitrogênio (RNS). No entanto, com relação à desativação das ROS e de RNS específicas, são encontrados poucos trabalhos na literatura que demonstram a capacidade antioxidante de compostos fenólicos, provavelmente devido a técnica necessitar de equipamento e materiais muito específicos. Geralmente estes dados são expressos em valores de IC50, que é a concentração inibitória *in vitro* que reduz 50% o efeito oxidativo provocado pelas espécies reativas testadas no meio (Chisté, 2011).

Em estudos referentes à capacidade antioxidante de resíduos vinícolas, utilizando essas técnicas citadas anteriormente, é declarado que o extrato etanólico de bagaço apresentou 93% de inibição da descoloração do radical DPPH (2,2-diphenyl-1-picrylhydrazyl) (Lafka et al., 2007) e ao analisar extrato etanólico do bagaço de uva Tannat comprovaram que esta variedade apresenta, 226 µMol TEAC/g e 395 µMol TEAC/g de bagaço (TEAC: capacidade antioxidante equivalente ao Trolox) pelas metodologias de ABTS e FRAP, respectivamente (Rockenbach et al.,2008). Valores similares foram encontrados por Melo et al. (2011) em bagaço de uva Verdejo pelo método de ABTS (aproximadamente 200 µMol Trolox/g de resíduo). O alto potencial antioxidante pelo método ABTS para bagaços de uva também foi encontrado por Ruberto et al. (2007).

Yilmaz e Toledo (2006), ao utilizar em método de ORAC observou para sementes Muscadine, Merlot e Chardonnay teores de 310,8; 344,8 e 637,8 µMol Trolox/g amostra seca, respectivamente, e para cascas de Merlot e Chardonnay, teores de 69,8 e 102,8 µMol Trolox/g amostra seca, respectivamente. Estes valores corroboram com os estudos apresentados para outras frutas, como o demonstrado por Pertuzatti, Barcia, Jacques, Vizzotto, Godoy e Zambiazi (2012), que apresenta valores 552,2-1046,5 µmol Trolox/g para frutos de mirtilos *in natura*. Barcia, Pertuzatti, Jacques, Godoy e Zambiazi (2012)

relatou, para jambolão, uma média de 505,6 μmol TE/g de fruto *in natura* colhidos em diferentes cidades do Rio Grande do Sul.

Quando analisadas uvas inteiras liofilizadas, nota-se que os resíduos vinícolas apresentam-se superiores, já que uvas Niágara Rosada, Folha de Figo, Syrah, Merlot e Moscato Embrapa exibem capacidade antioxidante inferiores (7,6; 19; 10,3; 12,1; 2,7 μmol equivalente de Trolox.g^{-1}, respectivamente) (Abe et al., 2007).

Diversos trabalhos relatam correlação positiva entre o teor total de fenólicos e capacidade antioxidante em uvas, cascas de uvas, sementes de uvas, resíduos vinícolas e outras frutas (Rockenbach, Gonzaga, Rizelio, Gonçalves, Genovese, & Fett, 2011, Thaipong et al., 2006, Melo et al., 2011, Abe, 2007).

5 Referências Bibliográficas

Abe, L. T., Mota, R. V., Lajolo, F. M., & Genovese, M. I. (2007). Compostos fenólicos e capacidade antioxidante de cultivares de uvas *Vitis labrusca L.* e *Vitis vinifera* L. *Ciência e Tecnologia de Alimentos, 27*, 2, 394-400.

Aherne, S. A., & O'brien, N. M. (2002). Dietery flavonols: chemistry, food content, and, metabolism. *Nutrition, 18,* 1, 75-81.

Alonso, A. M., Guillen, D. A., Barroso, C. G., Puertas, B., & Garcia, A. (2002). Determination of Antioxidant Activity of Wine Byproducts and Its Correlation with Polyphenolic Content. *Journal Agriculture Food Chemistry, 50*, 5832−5836.

Amendola, D., Faveri, D.M., & Spigno, G. (2010). Grape marc phenolics: Extraction kinetics, quality and stability of extracts. *Journal of Food Engineering, 97*, 384–392.

Amico, V., Napoli, E. M., Renda, A., Ruberto, G., Spatafora, C., & Tringali, C. (2004). Constituents of grape pomace from the Sicilian cultivar 'Nerello Mascalese'. *Food Chemistry, 88*, 599–607.

Andersen, O. M., & Markham, K. R. (2006). *Flavonoids: Chemistry, Biochemistry and Applications*. New York: CRC Press Taylor & Francis Group, (Chapter 10).

Angelo, P. M., & Jorge, N. (2007). Compostos fenólicos em alimentos – Uma breve revisão. *Revista Instituto Adolfo Lutz, 66*, 1, 232-240.

Arvanitoyannis, I. S., Ladas, D., & Mavromatis, A. (2006). Potencial uses and aplications of treated wine waste: a review. *International Journal of Food and Technology, 41*, 475-487.

Ascheri, D. P. R., Ascheri, J. L. R., & Carvalho, C. W. P. (2006). Caracterização da farinha de bagaço de jabuticaba e propriedades funcionais dos extrusados. *Ciência Tecnologia Alimentos, Campinas, 26*, 4, 897-905.

Babbar, N., Oberoi, H. S., Uppal, D. S., & Patil, R. T. (2011). Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. *Food Research International*, *44,* 391–396.

Balasundram, N., Sundram, K., & Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry, 99*, 191-203.

Barcia, M. T., Pertuzatti, P. B., Jacques, A. C., Godoy, H. T., & Zambiazi, R. C. (2012). Bioactive Compounds, Antioxidant Activity and Percent Composition of Jambolão Fruits *(Syzygium cumini*). *The Natural Products Journal, 2,* 129-138.

Beer, D., Gelderblom, W. C. A., & Manley, M. (2002). Phenolic compounds: a review of their possible role as in vivo antioxidants of wine. *South African Journal for Enology and Viticulture*, *23*, 48-61.

Bindon, K., Varela, C., Kennedy, J., Holt, H., & Herderich, M. (2013). Relationships between harvest time and wine composition in Vitis vinifera L. cv. Cabernet Sauvignon 1. Grape and wine chemistry. *Food Chemistry*, *138,* 1696-1705.

Blanco-Vega, D., López-Bellido, F. J., Alía-Robledo, J. M., & Hermosín-Gutiérrez, I. (2011). HPLC-DAD-ESI-MS/MS characterization of pyranoanthocyanins pigments formed in model wine. *Journal of Agricultural and Food Chemistry*, *59*, 9523-9531.

Blouin, J., & Guimberteau, G. (2002). Principales componentes de la uva y evolución durante la maduración. In: J. Blouin, & G. Guimberteau (Ed.). *Maduración y madurez de la uva.* (pp.57-100). Madri: Mundi-Prensa.

Bobbio, F. O., & Bobbio, P. A. (1989). Introdução à química de alimentos. (2.ed.). São Paulo: Varela.

BRASIL. Ministério da Agricultura Pecuária e Abastecimento – MAPA. Portaria nº 229 de 25 de outubro de 1988. Aprova norma referente à "complementação dos Padrões de Identidade e Qualidade do Vinho". Diário Oficial da União, Brasília, DF, 31 de outubro de 1988. Disponível em: <http://extranet.agricultura.gov.br/sislegis-consulta>. Acesso em: 10/12/2013.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento - MAPA. Lei nº 10.970 de 12 de novembro de 2004. Altera dispositivos da LEI 7.678 de 8 de novembro de 1988. A produção, circulação e comercialização do vinho e derivados da uva e do vinho. Diário Oficial da União. Brasília, 16 de novembro de 2004. Disponível em: <http://extranet.agricultura.gov.br/sislegis-consulta>. Acesso em: 10/10/2013.

Burns, J., Gardner, P. T., Matthews, D., Duthie, M. E. J. L., & Crozier, A. (2001). Extraction of Phenolics and Changes in Antioxidant Activity of Red Wines during Vinification. *Journal of Agriculture and Food Chemistry, 49,* 5797-5808.

Cabras, P., Angioni, A., Garau, V. L., Minelli, E. V., Melis, M., & Pirisi, F. M. (1997). Pesticides in the distilled spirits of wine and its byproducts. *Journal of Agricultural and Food Chemistry, 45*, 2248–2251.

Cadot, Y., Chevalier, M., & Barbeau, G. (2011). Evolution of the localization and composition of phenolics in grape skin between veraison and maturity in relation to water availability and some climatic conditions. *Journal of the Science of Food and Agriculture,* 91, 1963–1976.

Camargo, U. A., Tonietto, J., & Hoffkmann, A. (2011). Progressos na viticultura brasileira. *Revista Brasileira de Fruticultura, volume especial*, 144-149.

Campos, L. M. A. S. Obtenção de extratos de bagaço de uva Cabernet Sauvingnon (*Vitis vinifera*): Parâmetros de processo e modelagem matemática. Dissertação de mestrado, Universidade Federal de Santa Catarina, Brasil, 2005.

Castillo-Muñoz, N., Gomez-Alonso, S., Garcia-Romero, E., Gomes, M. A., Velders, A. H., & Hermosín-Gutiérrez, I. (2009). Flavonol 3-0- Glycosides Series of Vitis vinifera Cv. Petit Verdot Red Wine Grapes. *Journal Agriculture Food Chemistry, 57,* 209-219.

Castillo-Muñoz, N., Gómez-Alonso, S., García-Romero, E., & Hermosín-Gutiérrez, I. (2007). Flavonol profiles of vitis vinifera red grapes and their single-cultivar wines. *Journal Agriculture Food Chemistry, 55,* 992−1002.

Castillo-Muñoz, N., Gómez-Alonso, S., García-Romero, E., & Hermosín-Gutiérrez, I. (2010) Flavonol profiles of Vitis vinifera white grape cultivars. *Journal of Food Composition and Analysis, 23,* 699–705.

Cataneo, C. B., Caliari, V., Gonzaga, L. V., Kuskoski, E. M., & Fett, R. (2008). Atividade antioxidante e conteúdo fenólico do resíduo agroindustrial da produção de vinho. *Semina: Ciências Agrárias, Londrina, 29,* 1, 93-102.

Chisté, R. C. Avaliação da extração de compostos bioativos com propriedades antioxidantes e corantes presentes em urucum e piquiá. Tese de doutorado, Universidade Estadual de Campinas, Brasil, 2011.

Cordenunsi, B. R., Nascimento, J. R. O., Genovese, M. I., & Lajolo, F. M. (2002). Influence of cultivar on quality parameters and chemical composition of strawberry fruits grown in Brazil. *Journal Agriculture Food Chemistry*, *50*, 9, 2581-2586.

Cortés, S., Rodríguez, R., Salgado, J. M., & Domínguez, J. M. (2011). Comparative study between Italian and Spanish grape marc spirits in terms of major volatile compounds. *Food Control, 22*, 673-680.

Degáspari, C. H., & Waszczynskyj, N. (2004). Propriedades antioxidantes de compostos fenólicos. *Visão Acadêmica, 5,* 1, 33-40.

Del Rio, D., Rodriguez-Mateos, A., Spencer, J. P. E., Tognolini, M., Borges,G., & Crozier, A. (2013). Dietary (Poly) phenolics in Human Health: Structures, Bioavailability, and Evidence of Protective Effects Against Chronic Diseases. *Antioxidants & Redox Signaling*, 18, 1818-1892.

Diaz, M. J., Madejón, E., López, F., López, R., & Cabrera, F. (2002). Optimization of the rate vinasse/grape marc for co-composting process. *Process Biochemistry, 37*, 1143–1150.

Duthie, S. J. (2007). Berry phytochemicals, genomic stability and cancer: Evidence for chemoprotection at several stages in the carcinogenic process. *Molecular Nutrition Food Research 51,* 665-674.

Falcão, D. L. Caracterização analítica e sensorial de vinhos Cabernet sauvignon de diferentes altitudes de Santa Catarina. Tese de doutorado, Universidade Federal de Santa Catarina, Brasil, 2007.

Fennema, O. R. (1993). Química de los alimentos. (2.ed.). Zaragoza: Acribia.

Filho, W. G. V. (2010). Bebidas alcoólicas. Ciência e Tecnologia. (Volume 1). São Paulo: Editora Blucher, 461p.

Flanzy, C. (2003). Enología: Fundamentos Científicos y Tecnológicos. (2° Ed.). Madrid: A. Madrid Vicente.

Garrido, J., & Borges, F. (2011). Wine and grape polyphenols. A chemical perspective. *Food Research International*, *44*, 3134-3148.

Góes, F. J. Desenvolvimento e otimização do processo fermentativo para a produção de vinho branco a partir da uva Itália. Dissertação de mestrado, Universidade Federal de São Carlos, Brasil, 2005.

Guerra, C. C., Mandelli, F., Tonietto, J., Zanus, M. C., & Camargo, U. A. *(2009).* Conhecendo o essencial sobre uvas e vinhos. Documento n° 48*, ISSN 1516-8107, 1-69.*

Guerra, C.C. (2002). Maturação da uva e condução da vinificação para a elaboração de vinhos finos. In: Viticultura e Enologia - Atualizando Conceitos. *Anais do Viticultura e Enologia - atualizando conceitos, 1*, 179-192.

Gutteridge, J. M.C., & Halliwell, B. (2010). Antioxidants: Molecules, medicines, and myths. *Biochemical and Biophysical Research Communications, 393*, 561–564.

Guidoni, S., & Hunter, J. J. (2012). Anthocyanin profile in berry skins and fermenting must/wine, as affected by grape ripeness level of *Vitis vinifera* cv. Shiraz/R99. *European Food Research and Technology*, *235,* 397–408.

Hafeez, B. B., Siddiqui, I. A., Asim, M., Malik, A., Afaq, F., Adhami, V. M., Saleem, M., Din, M., & Mukhtar, H. A. (2008). Dietary Anthocyanidin Delphinidin Induces Apoptosis of Human Prostate Cancer PC3 Cells In vitro and In vivo: Involvement of Nuclear Factor-KB Signaling. *Cancer Research, 68*, 20, 8564-8572.

Haslam, E. (2007). Vegetable tannins-Lessons of a phytochemical lifetime. *Phytochemistry*, *68,* 2713-2721.

He, F., Liang, N. N., Mu, L., Pan, Q. H., Wang, J., Reeves, M. J., & Duan, C. Q. (2012). Anthocyanins and Their Variation in Red Wines I. Monomeric Anthocyanins and Their Color Expression. *Molecules, 17,* 1571.

Hermosín-Gutiérrez, I, Castillo-Muñoz, N., Gómez-Alonso, S., & García-Romero, E. (2011). Flavonol profiles for grape and wine authentication. In S. E. Ebeler, G. R. Takeoka, & P. Winterhalter, *Progress in authentication of food and wine* (pp. 113-129). Washington, DC: American Chemical Society, ACS Symposium Series.

Hou, D. X. (2003). Potential mechanisms of cancer prevention. *Current Molecular Medicine, 3*, 2, 149-159.

Jackson, R. (2008). *Wines Science. Principles and applications*. (3th ed.). USA: *Elsevier.*

Jordão, A. M., Silva, R., & Laureano, O. (1998). Influência da rega na composição fenólica das uvas tintas da casta Touriga Francesa (Vitis vinifera l.). *Ciencia y Tecnología Alimentaria, 2,* 2, 60-73.

Kammerer, D., Claus, A., Carle, R., & Schieber, A. (2004). Polyphenol Screening of Pomace from Red and White Grape Varieties (*Vitis vinifera* L.) by HPLC-DAD-MS/MS. *Journal of Agriculture and Food Chemistry*, *52*, p. 4360−4367.

Karvela, E., Makris, D. P., Kalogeropoulos, N., Karathanos, V. T., & Kefalas, P. (2009). Factorial design optimisation of grape (Vitis vinifera) seed polyphenol extraction. *European Food Research Technology, 229*, 731–742.

Kenedy, J. (2002). *Understanding grape berry development*. Practical Winery and Vineyard. (pp. 14-23).

Kong, J. M., Chia,L. S., Goh, N. K., Chia, T. F., & Brouillard, R. (2003). Analysis and biological activities of anthocyanins. *Phytochemistry, 64*, 923–933.

Lafka, T., Sinanoglou, V., & Lazos, E. S. (2007). On the extraction and antioxidant activity of phenolic compounds from winery wastes. *Food Chemistry, 104*, 1206–1214.

Lago-Vanzela, E. S., Rebello, L. P. G., Ramos, A. M., Stringheta, P. C., Da-Silva, R., García-Romero, E., Gómez-Alonso, S., & Hermosín-Gutiérrez, I. (2013). Chromatic characteristics and color-related phenolic composition of Brazilian young red wines made from the hybrid grape cultivar BRS Violeta ("BRS Rúbea" × "IAC 1398-21"). *Food Research International*, *54*, 33–43.

Llobera, A., & Cañellas, J. (2007). Dietary fibre content and antioxidant activity of Manto Negro red grape (Vitis vinifera): pomace and stem. *Food Chemistry, 101*, 659–666.

López-Alarcón, C., & Denicola, A. (2013). Evaluating the antioxidant capacity of natural products: A review on chemical and cellular-based assays. *Analytica Chimica Acta. 763,* 1-10.

Makris, D. P., Boskou, G., & Andrikopoulos, N. K. (2007). Polyphenolic content and in vitro antioxidant characteristics of wine industry and other agri-food solid waste extracts. *Journal of Food Composition and Analysis, 20,*125–132.

Mamede, M. E. O., & Pastore, G. M. (2004). Compostos fenólicos do vinho: estrutura e ação antioxidante. *Boletim CEPPA, 22*, 2.

Maragkoudakis, P. A., Nardi, T., Bovo, B., D'Andrea, M., Howell, K. S., Giacomini, A., & Corich, V. (2013). Biodiversity, dynamics and ecology of bacterial community during grape marc storage for the production of grappa. *International Journal of Food Microbiology, 162*, 143-151.

Maraschin, R. P. Caracterização química de vinhos Cabernet sauvignon produzidos na serra gaúcha. Dissertação de mestrado, Universidade Federal de Santa Catarina, Brasil, 2003.

Mello, L. M. R. (*2011).* Vitivinicultura brasileira: Panorama 2010. Comunicado técnico*, ISSN 1808-6802,1-5.*

Mello, L. M. R. (*2012).* Vitivinicultura mundial: principais países e posição do Brasil. Comunicado técnico*, ISSN 1808-6802,1-8.*

Mello, L. M. R. (*2013).* Vitivinicultura brasileira: Panorama 2012. Comunicado técnico*, ISSN 1808-6802,1-5.*

Melo, E. A., & Guerra, N., B. (2002). Ação Antioxidante de Compostos Fenólicos Naturalmente Presentes em Alimentos. *Boletim da Sociedade Brasileira de Ciência e Tecnologia de Alimentos, 36*, 1-11.

Melo, P. S., Bergamaschi, K. B., Tiveron, A. P., Massarioli, A. P., Oldoni, T. L. C., Zanus, M. C., Pereira, G. E., & Alencar, S. M. (2011). Composição fenólica e atividade antioxidante de resíduos agroindustriais. *Ciência Rural, 41*, 6, 1088-1093.

Morata, A., Gómez-Cordovés, M. C., Colomo, B., & Suárez, J. A. (2005). Cell Wall anthocyanin adsorption by different *Saccharomyces* strains during the fermentation of *Vitis vinifera* L. cv Graciano grapes. *European Food Research and Technology*, *220*, 341-346.

Nixdorf, S. L., & Hermosín-Gutiérrez, I. (2010). Brazilian red wines made from the hybrid grape cultivar Isabel: Phenolic composition and antioxidant capacity. *Analytica Chimica Acta, 659*, 208–215.

Okumura, F., Soares, M. H. F. B., & Cavalheiro, E. T. G. (2002). Identificação de pigmentos naturais de espécies vegetais utilizando-se cromatografia em papel. *Química Nova, 25*, 4, 680-683.

Paradelo, R., Moldes, A. B., & Barral, M. T. (2010). Utilization of a factorial design to study the composting of hydrolyzed grape marc and vinification lees. *Journal of Agricultural and Food Chemistry*, *58,* 3085–3092.

Pérez-Serradilla, J.A., & Luque de Castro, M.D. (2008). Role of lees in wine production: A review. *Food Chemistry, 111*, 447–456.

Pertuzatti, P. B., Barcia, M. T., Jacques, A. C., Vizzotto, M., Godoy, H. T., & Zambiazi, R. C. (2012). Quantification of several bioactive compounds and antioxidant activities of six cultivars of brazilian blueberry. *The Natural Products Journal, 2,* 188-195.

Peterson, J., & Dwyer, J. (1998). Flavonoids: Dietary occurrence and biochemical activity. *Nutrition Research, 18*,12, 1995-2018.

Potter, G. H. Efeito da desfolha e do armazenamento de cachos em câmara fria antes do esmagamento em uvas e vinhos Chardonnay e Cabernet Sauvignon da região da campanha, RS. Dissertação de mestrado, Universidade Federal de Santa Maria, Brasil, 2009.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry, 53*, 4290-4302.

Rebello, L. P. G., Lago-Vanzela, E. S., Barcia, M. T., Ramos, A. M., Stringheta, P. C., Da-Silva, R., Castillo-Muñoz, N., Gómez-Alonso, S., & Hermosín-Gutiérrez, I. (2013). Phenolic composition of the berry parts of hybrid grape cultivar BRS Violeta (BRS Rubea × IAC 1398-21) using HPLC–DAD–ESI-MS/MS. *Food Research International, 54*, 354–366,

Rentzsch, M., Schwarz, M., & Winterhalter, P. (2007). Pyranoanthocyanins: an overview on structures, occurrence and pathways of formation. *Trends in Food Science & Technology, 18,* 526-534.

Ribéreau-Gayon, J., Glories, Y., Maujean, A., & Dubourdieu, D. (2006). Handbook of Enology: The chemistry of wine stabilization and treatments. (2th ed.). John Wiley & Sons, Ltda.

Rockenbach, I. I., Silva, G. L., Rodrigues, E., Kuskoski, E. M., & Fett, R. (2008) Influência do solvente no conteúdo total de polifenóis, antocianinas e atividade antioxidante de extratos de bagaço de uva (Vitis vinifera) variedades Tannat e Ancelota. *Ciência Tecnologia de Alimentos, Campinas, 28*(Supl.), 238-244.

Rockenbach, I. I., Gonzaga, L. V., Rizelio, V. M., Gonçalves, A. E. S. S., Genovese, M. I., & Fett, R. (2011). Phenolic compounds and antioxidant activity of seed and skin extracts of red grape (Vitis vinifera and Vitis labrusca) pomace from Brazilian winemaking. *Food Research International, 44*, 897-901.

Ruberto, G., Renda, A., Daquino, C., Amico, V., Spatafora, C., Tringali, C., & De-Tommasi, N. (2007). Polyphenol constituents and antioxidant activity of grape pomace extracts from five Sicilian red grape cultivars. *Food Chemistry, 100*, 203-210.

Sant'Anna, V., Brandelli, A., Marczak, L. D. F., & Tessaro, I. C. (2012). Kinetic modeling of total polyphenol extraction from grape marc and characterization of the extracts. *Separation and Purification Technology*, *100*, 82–87.

Silva, L. M. L. R. (2003). Caracterização dos subprodutos da vinificação. *Revista Millenium, 28*, 123-133.

Sluis, A. A., Dekker, M., Jager, A. De, & Jongen, W. (2001). Activity and concentration of polyphenolic antioxidants in apple: effect of cultivar, harvest year, and storage conditions. *Journal Agriculture Food Chemistry, 49*, 8, 3606-3613.

Soares, S. E. (2002). Ácidos fenólicos como antioxidantes. *Revista Nutrição, 15*, 1, 71-81.

Sobrinho, M. R. Caracterização físico-química do vinho paulista. Tese de doutorado, Universidade Estadual de Campinas, Brasil, 2013.

Spigno, G., Tramelli, L., & Faveri, D. M. (2007). Antioxidants from grape stalks and marc: Influence of extraction procedure on yield, purity and antioxidant power of the extracts. *Journal of Food Engineering, 78*, 793–801.

Taipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Byrne, D. H. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis, 19*, 669-675.

Terci, D. B. L., & Rossi, A. V. (2002). Indicadores naturais de pH: usar papel ou solução?. *Quimica Nova, 25*, 4,684-688.

Terrier, N., Poncet-Legrand, C., & Cheynier, V. (2009). Flavanols, flavonolsand dihydroflavonols. In M. V. Moreno-Arribas, & M. C. Polo (Eds.), *Wine chemistry and biochemistry* (pp. 463-496), New York: Springer.

Torres, J. L., Varela, B., Garcia, M. T., Carilla, J., Matito, C., Centelles, J. J., Cascante, M., Sort, X., & Bobe, R. (2002). Valorization of Grape (Vitis vinifera) Byproducts. Antioxidant and Biological Properties of Polyphenolic Fractions Differing in Procyanidin Composition and Flavonol Content. *Journal of Agricultural and Food Chemistry, 50*, 7548−7555.

Yilmaz, Y., & Toledo, R. (2006). Oxygen radical absorbance capacities of grape/wine industry byproducts and effect of solvent type on extraction of grape seed polyphenols. *Journal of Food Composition and Analysis, 19*, 41–48.

Zafra-Stone, S., Yasmin, T., Bagchi, M., Chatterjee, A., Vinson, J. A., & Bagchi, D. (2007). Berry anthocyanins as novel antioxidants in human health and disease prevention. *Molecular Nutrition Food Research, 51*, 675 – 683.

Zhu, L., Zhang, Y., & Lu, J. (2012). Phenolic contents and compositions in skins of red wine grape cultivars among various genetic backgrounds and originations. *International Journal of Molecular Scienc, 13*, 3492-351

ARTIGO 1

Antioxidant Capacity of Grape and Winemaking By-Products by ABTS, FRAP, ORAC and β-Carotene/Linoleic Acid Methods.

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Antioxidant Capacity of Grape and Winemaking By-Products by ABTS, FRAP, ORAC and β-Carotene/Linoleic Acid Methods.

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ABSTRACT

After the winemaking process, part of grape phytochemicals still remains in grape marc skins and seeds, and also in wine lees. Polyphenolic content and antioxidant capacity in these winemaking by-products will mainly be conditioned by grape variety and wine production conditions. Thus, this work aimed a complete evaluation of antioxidant capacity behavior using different methods (ABTS, FRAP, ORAC, and β-carotene/linoleic acid emulsion bleaching) and its correlation with total polyphenol and anthocyanin contents in freeze-dried winemaking by-products. Four different grape varieties (BRS Violeta, BRS Lorena, Cabernet sauvignon and Cabernet franc) produced in São Paulo (Brazil) in two successive years were studied. Furthermore, the polyphenolic stability of by-products obtained following two drying process (oven at 50 °C and spray-drying) in grape marcs was monitored at 0, 30 and 90 days of storage. Under testing conditions, BRS Violeta grapes showed the greatest stability and initial high levels of total phenolics (mean value, 4330 mg GAE/100g DM) and anthocyanins (mean value, 451 mg mv-3-glc/100g DM) remained almost unchanged until the end of storage period. The same behavior was observed in BRS Violeta freeze-dried skins, seeds, and lees (8557, 9520, and 4261 mg GAE/100g DM, respectively, and 829 and 257 mg mv-3-glc/100g DM in skin and lees, respectively). In all methodologies tested, BRS Violeta also showed higher values for antioxidant capacity when compared to BRS Lorena, Cabernet sauvignon and Cabernet franc. At the initial time, the dried by-products obtained in both oven and in spray-drying resulted in a reduction of 50% of total phenolic content when compared to freeze-drying process. Skin and lees anthocyanins from BRS Violeta cultivar were stable for 90 days. The above findings suggest that, despite skins, seeds and lees are winemaking by-products, they can be used as a source for extraction of antioxidant phenolic compounds.

Keywords: grape marc, lees, phenolics, anthocyanins, antioxidant capacity.

1 INTRODUCTION

Brazil, a country of great agricultural activity, is one of the biggest producers of agro-industrial residues. Then, the search of alternatives to use all organic matter generated is increasingly developed by several research centers. Grape production in Brazil is mainly used for winemaking. Thus, a sum of 60% of total grape harvested is used in wine production. After winemaking, 5% of total weight of berries will originate the first residue, the grape marcs. Moreover, at a least 4% of total wine production will precipite as lees, the second winemaking residue (Cataneo, Caliari, Gonzaga, & kuskoski, 2008, Rockenbach, Gonzaga, Rizelio, Gonçalves, Genovese, & Fett, 2011).

Grape marc (a mixture of skins and seeds) is an abundant winemaking by-products and it is responsable by problems of storage, processing and disposal at ecological and economic points of view (Shojaee-Aliabadi, Hosseini, Tiwari, Hashemi, Fadavi, & Khaksar, 2013). After fermentation, natural decantation of wine give rises to the lees (mostly comprising fine residual particles from grape and death yeasts) (Maragkoudakis, Nardi, Bovo, D'andrea, Howell, Giacomini, & Corich,et al., 2013, Paradelo, Moldes, & Barral, 2010, Cortes, Rodríguez, Salgado, & Domínguez, 2011).

Phenolic compounds from in grapes are transferred to wine depending on the characteristics of the winemaking process. However, this transference is subjected to solid/liquid partition equilibrium and, a large proportion of phenolic compounds still remains in the winemaking by-produtcs (Alonso, Guillen, Barroso, Puertas, & Garcia, 2002). Consequently, there is a great interest in exploring these winemaking by-products with regards to the various extraction processes, especially of phenolic compounds (Arvanitoyannis, Ladas, & Mavromatis, 2006). As previously reported, the major phenolic

compounds class in grape marcs are anthocyanins present in red skins (Rodrigues, Poerner, Rockenbach, Gonzaga, Mendes, & Fett, 2011), epicatechins and catechins present in greater quantities in seeds (Torres, et al., 2002, Yilmaz, & Toledo, 2004, Karvela, Makris, Kalogeropoulos, & Karathanos, 2009), together with stilbenes and phenolic acids present in seeds and skins (Cataeno et al., 2008, Karvela et al., 2009).

Phenolic compounds are recognized as potent free radical scavengers (antioxidants). The multiple mechanisms of antioxidant capacity are expressed in terms of its ability to eliminate free radicals, metal chelation, and synergism with other antioxidants (Torres et al., 2002). In general, the methods used for the determination of the total antioxidant capacity are divided into two main groups: those based on assays of single electron transfer (SET) from the reaction by means of a color change as the oxidant is reduced; and those based on assays of hydrogen atom transfer (HAT) (Huang, Ou, Ronald & Prior, 2005), measuring the activity of antioxidant by elimination of peroxyl radicals. Within the methods used for antioxidant capacity we can found: absorption capacity of the oxygen radical (ORAC), antioxidant capacity by reducing iron (FRAP), and 2,2-azinobis (3-ethyl-6-benzothiazoline sulfonic acid) (ABTS). The discoloration of β-carotene is also widely used to measure the antioxidant capacity of bioactive compounds because β-carotene is extremely susceptible to free radical oxidation mediated by linoleic acid.

The aim of this work was to study the antioxidant capacity by comparison of different methodologies and it correlations with total phenolics and anthocyanins contents in winemaking by-products produced at São Paulo (Brazil), with different grape varieties (BRS Violeta, BRS Lorena, Cabernet sauvignon and Cabernet franc).

The study was extended over two successive harvesting years in dried materials. The

control method for the drying process was freeze-drying, but other drying technologies (drying in oven at 50 °C and spray-drying) were tested for their stability over time (0, 30, and 60 days). This data represents valuable information aiming to support future studies that may allow the usage of these by-products in the food processing, pharmaceutical and chemical industry.

2 MATERIAL AND METHODS

2.1 Materials

2.1.1 Chemicals and Equipments

The chemicals: 2,20-Azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,4,6-tripyridyltriazine (TPTZ), β-carotene, acid gallic purchased from SIGMA, the 2,2'-azobis (2-amidino-propane) dihydrochloride (APPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were ALDRICH, linoleic acid was FLUKA. Buffer salts and all other chemicals were of analytical grade.

Equipments: spectrophotometer UV 1600 (Pró-Análise), automated plate reader BMG Labtech, Novo Star (Germany, S/N 700-0120), Ultrasound bath SX-20 (Arruda, Ultra-Sons LTDA., Brasil), freeze-dryer (Terroni LS-3000), oven (Nova Ética), spray-dryer (LAB PLANT SD-05, L.P. Technoloy LTDA, Leeds England), centrifuge (Harrier 18/80-SANYO-MSE), and microplate reader BMG Labtech.

2.2 Samples

Entire grapes, skins and seeds from fermented, and lees of hybrid varieties (BRS Violeta and BRS Lorena) and *Vitis vinifera* (Cabernet sauvignon and Cabernet franc) were

used in this work, belonging to 2011 and 2012 vintages for each variety (except for fermented skin of Cabernet franc and lees from BRS Lorena, both of 2011 vintage, which were not analyzed). Samples were kindly supplied by a winery in the region of São Roque, São Paulo/Brazil, at the coordinates 23º 31'44" South and 47º 08'06" West, and 771 moters above sea level as referred in datum WGS84, World Geodetic System 1984 characterized by a subtropical climate (maximum, 23.1 °C; minimum, 15.5 °C). After harvested, the samples were stored at -20 °C. For the purpose of characterization of the samples, the moisture content was determined by Adolfo Lutz (1985).

2.2.1 Sample Preparation

The samples of grapes, lees, skins, and seeds were lyophilized in order to avoid degradation. To study the effect of drying, the skins and seeds were dried in an oven (50 °C) with forced air circulation until constant weight, whereas the lees were spray-dried (180 °C, flow rate of 9 mL/min, outlet diameter 1.0 mm, and exhaust temperature 100 °C). Then, samples were crushed, homogenized and stored in vacuum sealed laminated bags.

The samples subjected to spray-drying and oven-drying (50 °C) were stored at 25 °C in BOD (biochemical oxygen demand) conditions for developing the stability study (time 0, 30 and 90 days of storage).

2.2.2 Extraction of Phenolic Compounds and Validation

The freeze-dried samples (validation range: 50 to 200 mg) were immersed in 25 mL of a solvent mixture of methanol, water, and formic acid (50:48.5:1.5 v/v/ v), and the subsequent extraction was assisted by homogenization for 1 min and then centrifuged at 2500*g* at 5 °C for 15 min. A second extraction of the resulting pellets was completed using

the same volume of the solvent mixture (25 mL), and the combined supernatants for each sample were immediately analyzed (Castillo-Muñoz, Gomez-Alonso, Garcia-Romero, Gomes, Velders, & Hermozin-Gutierrez, 2009).

The extraction was validated as reported by to the Brazilian Legislation requirements (INMETRO, 2003, Ribani, Bottoli, Collins, & Jardim, 2004). The following validation characteristics were addressed: linearity, precision and accuracy. The validation of the extraction was performed for five complex samples. The repeatability of the extraction was performed with 10 repetitions of the extraction in 1 day. Intermediate precision was determined repeating this procedure on 3 consecutive days (3 replicates each). System linearity was verified with calibration curves made up of seven points (0.01-0.07mg/ml). A lack of fit test for calibration curve was performed as recommended by Danzer and Currie (1998). In order to study the efficiency of the extraction, recovery tests were performed by spiking samples with gallic acid standard at two levels: 0.04 and 0.07 mg/mL.

2.3 Total Phenolics and Antocyanins

The total phenolic content (TPH) in the samples was determined using the Folin-Ciocalteu colorimetric method (Singleton, Orthofer, & Lamuela-Raventos, 1999, Scherer & Godoy, 2013). The absorbance was measured at 740 nm after 120 min at room temperature. The absorbance values were then compared with those of standards with known gallic acid concentrations. All values were expressed as the mean (milligrams of gallic acid equivalents per 100 g of dry sample) ± SD (deviation standard) for three replications. The standard curve was constructed using gallic acid (0.01-0.07 mg /mL).

The total monomeric anthocyanin content (ACY) of the extracts was measured

using the pH differential method (Giusti, & Wrolstad, 2001). The extracts were mixed thoroughly with 0.025 M potassium chloride buffer pH 1 in a 1:2 extract to buffer ratio. The extracts were then mixed similarly with a sodium acetate buffer pH 4.5. The absorbance at 520 and 700 nm was measured against a buffer blank at pH 1.0 and 4.5.

Absorbance readings were converted to total milligrams of malvidin 3-glucoside (M3G). The anthocyanin content was calculated on the basis of molecular weight for M3G (494) and its molar absorptivity (36400). The anthocyanin content was expressed as milligrams of M3G equivalents per 100 g of dry sample, for the triplicate extracts.

2.4 Antioxidant Capacity

2.4.1 Ferric-reducing Antioxidant Power (FRAP) Assay

The FRAP assay was carried out as previously described (Benzie, & Strain, 1996). Briefly, the FRAP reagent is composed of sodium acetate buffer (300 mM, pH 3.6), 10 mM TPTZ solution (40 mM HCl as solvent) and 20 mM iron (III) chloride solution in a volume ratio of 10:1:1 (v/v/v), respectively. The FRAP reagent was daily prepared and warmed up to 37 °C in a water bath immediately before use water (240 μL) and diluted sample (80 μL) were mixed to 2400 μL of FRAP reagent. After 15 min, the absorbance was measured at 593 nm. For quantification purposes, a standard curve of Trolox was used (160-720 μM) solution.

2.4.2 Capture of Free Radical ABTS

The ABTS assay was carried out according to the method previously established (Re, Pellegrini, Proteggente, Pannala, Yang, & Rice-Evans, 1999). Briefly, the $ABTS^{\cdot+}$ stock solution was prepared from 7 mM ABTS and 2.45 mM potassium persulphate in a

volume ratio of 1:1, and then incubated in the dark for 16 h at room temperature. The $ABTS^{\cdot+}$ working solution was prepared by diluting the stock solution with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. All samples were diluted approximately to provide 20–80% inhibition of the blank absorbance. The 30 µL of the diluted sample were mixed with 3.0 mL $ABTS^{\cdot+}$ working solution. The absorbance of the mixture was measured at 734 nm after 25 min of incubation at 30 °C temperature, and the percent of inhibition of absorbance at 734 nm was calculated. The calibration curve was constructed using Trolox (2000- 20 µM) solution.

2.4.3 Oxygen Radical Absorbance Capacity (ORAC)

The ORAC procedure used an automated plate reader with 96-well plates (Huang, Ou, Hampsch-Woodill, Flanagan, & Prior, 2002). Analyses were conducted in system composed of one indicator, fluorescein; an peroxyl radical generator, 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH); and Trolox as a control standard. All reagents were prepared in 75 mM potassium phosphate buffer (pH 7.4) just before analysis. After addition of 20 µl of diluted samples, it was added 120 µl of fluorescein (0.378 µg/mL) and 60 µL of AAPH (41.4 µg/mL) in each well, 80 minutes for reaction completion at 37 °C. Fluorescence conditions were as follows: excitation at 485nm and emission at 520 nm. The standard curve was constructed using Trolox (80-1500 µM) solution. The area under de curve (AUC), relative fluorescence *versus* incubation time, was calculated as showed in **Equation 1**. The AUC differences between the extract and blank were taken and used for calculations.

$$AUC = 1 + \frac{f2}{f1} + \frac{f3}{f1} + \frac{f4}{f1} + \frac{fn}{f1}$$, were *f* is the fluorescence reading. Equation 1

2.4.4 Antioxidant Assay using the β-carotene Bleaching Method

The prevention of ß-carotene bleaching was determined as described by Miller (1970). In brief, 300 µL (1 mg β-carotene in 1 mL chloroform) was mixed with 22 µL of linoleic acid and 200 µL of Tween-40. The chloroform was evaporated under nitrogen, then 50 mL distilled water (supersaturated oxygenic) was added and the resulting mixture was vigorously stirred. The emulsion obtained was freshly prepared before each experiment. An aliquot (250µL) of the β-carotene-linoleic acid emulsion was transferred to microplate containing 10 µL of each sample. The microplate was immediately incubated at 45 °C for 2 h. The absorbance of each sample was measured at 470 nm. A control consisted of 10µl of solution extraction instead of the sample solution. The percentage of inhibition was obtained from extracts with a concentration of 0.5 g/L.

2.5 Statistical

Data analysis was carried out with ANOVA and Tukey test focusing on significant differences in means. Statistic 7.0 software program was employed with significance level between mean differences at 5% ($p<0.05$). Correlations among data obtained were calculated using Pearson's correlation coefficient (r). All analyses were made in triplicates and the results were given as means.

It was also a tool used principal component analysis (PCA), Pirouette program 3.11, aiming to highlight similarities or differences between samples in a given data set, the data were previously auto-scaled before being subjected to principal components analysis.

3 RESULTS AND DISCUSSION

The initial moisture (%) contents of the samples were (data corresponding to BRS Violeta, BRS Lorena, Cabernet sauvignon, and Cabernet franc samples and in the years 2011 and 2012 for each grape variety, respectively): 79.8, 78.5 for grape BRS Violeta, 77.5, 73.7 for grape BRS Lorena, 78.9, 76.3 for grape Cabernet sauvignon and 74.5, 72.6 for grape Cabernet franc; 83.9, 81.2 for skins BRS Violeta, 82.1, 72.6 for skins BRS Lorena, 86.8, 55.6 for skins Cabernet sauvignon, and NA (Not analyzed), 60.6 for skins Cabernet franc; 46.7, 43.9 for seeds BRS Violeta, 43.4, 46.2 for seeds BRS Lorena, 46.1, 42.7 for seeds Cabernet sauvignon, NA and 37.9 for seeds Cabernet franc; 80.4, 69.1 for lees BRS Violeta NA, 84.9 for lees BRS Lorena 88.9, 91.1 for lees Cabernet sauvignon 94.7, and 96.5 for lees Cabernet franc.

3.1 Extraction of Total Phenolic Compounds and Validation

The validation results are summarized in **Table 1.1**. The results demonstrated that the standard curve was linear, with high values for the correlation coefficient (R = 0.99835). Intermediate precision was evaluated over 3 days using the same sample extraction under the same conditions. The RSD value of <10% for sample indicates that the intermediate precision and repeatability is acceptable. The accuracy of extraction was evaluated by calculating the percentage of recovery. The results ranged from 79.34% to 114.05%, these values are according with Ribani et al., 2004.

Table 1.1. Validation of the extraction method for the analysis of phenolics in samples of winemaking by-products.

Parameter	Skin BRS Violeta	Seed BRS Violeta	Lees BRS Violeta	Skin BRS Lorena	Seed BRS Lorena
Coef. angular (sensitivity)	12,216	12,216	12,216	12,817	12,817
Coef. Linear	-0,0274	-0,0274	-0,0274	-0,0129	-0,0129
Coef. Correlation (linearity)	0,9984	0,9984	0,9984	0,9983	0,9983
p-value (lack of fit test)[a]	0,500	0,500	0,500	0,447	0,447
Repeatability (n = 10)[b]	7,11	0,58	4,59	8,42	6,33
Intermediate precision (n=3)[b]	7,10	0,55	4,63	8,48	6,29

a The probability value of the lack of fit test should be greater than 0.05.
b The repeatability and intermediate precision parameters were evaluated by calculating the relative standard deviation, RSD (%).

3.2 Total Phenolic Compounds and Anthocyanins

Results of total phenolic compound and anthocyanin contents are shown in **Table 1.2**. Considering all sampling dates, total phenolic compounds in grape samples ranged from 477 to 4509 mg GAE/100g DM. Significant differences ($p<0.05$) were found for the BRS Lorena and Cabernet sauvignon grapes between vintages with higher amounts of phenolic compounds at 2011 than in 2012.

Table 1.2. Results of total phenolics (TPH) and total anthocyanins (ACY) in the sample of grapes, skin, seed and lees BRS Violeta, BRS Lorena, Cabernet franc and Cabernet sauvignon in the years 2011 and 2012.

Samples/Years		TPH mg GAE/100g DM		ACY mg mv-3-glc/100g DM		ACY / TPH	
		2011	2012	2011	2012	2011	2012
BRS Violeta	Grape	4509±114cA	4152±320cA	444±14bA	459±17bA	0.098	0.111
	SKin	7832±164aB	9282±207bA	677±33aB	981±26aA	0.086	0.106
	Seed	8062±114aB	10979±922aA	---	---	---	---
	Lees	4911±157bA	3612±73cB	289±9cA	225±4cB	0.059	0.062
BRS Lorena	Grape	585±15bA	477±33cB	---	---	---	---
	SKin	491±19cB	1131±31bA	---	---	---	---
	Seed	974±55aB	2056±78aA	---	---	---	---
	Lees	---	1149±102b	---	---	---	---
Cabernet franc	Grape	1672±125bA	1607±67cA	55±4bA	52±5cA	0.033	0.032
	SKin	---	2907±18b	---	85±1a	---	0.029
	Seed	---	5026±169a	---	---	---	---
	Lees	3086±71aA	2697±24bB	90±3aA	64±1bB	0.029	0.031
Cabernet sauvignon	Grape	2462±219cA	1335±94bB	53±2bA	37±2bB	0.022	0.048
	SKin	3203±71aA	1124±60bB	78±8aA	19±1cB	0.024	0.017
	Seed	6312±152aA	3245±274aB	---	---	---	---
	Lees	2909±47bB	3224±60aA	62±1aA	52±1aB	0.021	0.016

Results are mean ± standard deviation. For each grape cultivar, different lower case letters in a same column shows significant differences ($p<0.05$) among samples. Different capital letters in a same line shows significant differences between years ($p<0.05$), second test of Tukey.
mv-3-glc = malvidin-3-glucoside; GAE = equivalent acid gallic; DM = Dry Matter

Very likely, higher levels of phenolic compounds were found in grape berries samples (BRS Lorena and Cabernet sauvignon) of year 2011 due to the difference in climate conditions between years. Thus, considering the climate information about the cropping area that is available in CEPAGRI (2013), lower average temperatures and precipitation volumes were observed in the year 2012 (15.3 °C and rainfall of 133.4 mm) than in the year 2011 (temperature 20.8 to 29.8 °C and rainfall of 451.6 mm).

Therefore, the significant increase in total phenolic content between years could be a result of the plant defense mechanism at high temperatures, since these compounds were reported as able to act against UV radiation (Treutter, 2006, Barcia, Jacques, Pertuzatti, & Zambiazi, 2010).

The grape cultivar showing the highest content of total phenolics compounds in the present study was BRS Violeta, in both vintages, which can be related to the fact that this grape variety showed an intense red-purple color attributed to the high content of anthocyanins, usually associated to the high content of phenolics compounds when compared with white grapes, as described Rebello et al. (2013).

In contrast, the white grape hybrid cultivar BRS Lorena had the lowest total phenolic content in both sampling dates (585 and 477 mg GAE/100 g DM for the years 2011 and 2012, respectively). This is in agreement with a previous work in which lower levels of phenolic were found for all studied white grapes varieties (Borbalán, Zorro, Guillén, & Barroso, 2003).

However, according to Yang, Martinson and Liu (2008) the content of phenolic compounds in different grapes is mainly related to differences among varieties and not to skin color. The Cabernet franc presented a phenolic content of 427 and 440 mg GAE/100 g FW for vintages of 2011 and 2012, respectively. This result is consistent with the study published by Yang et al. (2008) which reported an average value of 425 mg FW GAE/100 g for 14 samples of Cabernet franc.

Singleton (1982) found that the distribution of phenolics in grape juice, pulp, skin, and seeds is approximately 5%, 1%, 30% and 64%. In all analyzed varieties, seed showed higher content of phenolic compounds than skins and lees. This result agrees with previous literature data (Babbar, Oberoi, Uppal, & Patil, 2011, Casazza, Aliakbarian, Faveri, Fiori, & Perego, 2012, Makris, Boskoub, & Andrikopoulosb, 2007, Rockenbach et al., 2011). According to Makris et al. (2007), seeds have the highest contribution to the phenolic content in the grape marcs, mainly in white grapes, in which the skin shows a lower

phenolic content than in purple cultivars. This finding was also observed for the BRS Lorena white grapes since higher amounts of phenolic compounds were found in seeds (**Table 1.2**) than in skin samples (491 and 1131 mg/100 g DM, for the years 2011 and 2012, respectively). Moreover, BRS Lorena seeds for the year 2012 showed a phenolic content similar to that reported by Rockenbach et al. (2011) for Isabel grape (2128 mg GAE/100 g DM), that is also a non vinifera grape cultivar.

The same work also showed lower concentration of phenolic compounds in the skin (1065 mg GAE/100 g DM) than in seed (8249 mg GAE/100 g DM) for Cabernet sauvignon. However, we observed that there was a considerable variation between vintages for the same winemaking by-product. Additionally, there is a scarce literature data about phenolic compounds in winemaking by-products. For BRS Violeta and BRS Lorena varieties, for example, no literature data were found, thus making difficult the comparison of the results obtained.

For all grape varieties, lees still showed high levels of total phenolic compounds when compared to the whole grape berry. Thus, the highest levels was observed again in the case of BRS Violeta, followed by Cabernet sauvignon and Cabernet franc, with similar contents, and finally BRS Lorena showed the lowest amount of phenolics. However, all these values were higher in lees than those found in the whole grape berry, on a basis of dry matter.

In red grape cultivars, the anthocyanins represented one group among other classes of phenolic compounds with remarkable importance. BRS Violeta showed the highest values for ACY/TPH ratio (**Table 1.2**). However, according to Moyer, Hummer, Finn, Frei and Wrolstad (2002) these values are considered low, not due to the content of

anthocyanins in grape and residues is low, but its content of total phenolic compounds is very high , especially for BRS Violeta grapes. Grape varieties showed a content of anthocyanins between 37.0 and 459 mg mv-3-glc/100 g DM (**Table 1.2**). The content of anthocyanins in white grape cultivar BRS Lorena was not measured. Among the grape cultivars having anthocyanins, BRS Violeta accounted for significantly higher levels ($p<0.05$) when compared with Cabernet franc and Cabernet sauvignon, in agreement to the observed by Abe, Da-Mota, Lajolo and Genovese (2007).

Our results showed that Cabernet franc had total anthocyanin content between 13.9 (year 2011) and 14.1 mg mv-3-glc/100 g fresh weight (year 2012), with no differences among the years ($p = 0.44$). These values were presented in fresh weight for comparison with Hogan, Zhang, Li, Zoecklein and Zhou (2009), which reported a concentration of 17 mg cyanidin-3-glc/100 g of fresh weight for the same grape, and of 13-18 mg cyanidin-3-glc/100g of fresh weight for Niagara Rosada (Abe et al., 2007). For the other grapes analyzed in this study no data were found in the literature. BRS Violeta, a grape hybrid cultivar, was the variety with higher amounts of anthocyanins, its content in fresh weight basis from 89.4 mg malvidin-3-glc/100 g FW (year 2011) to 98.6 mg malvidin-3-glc/100 g FW (year 2012). The latter results are in agreement with other studies where fourteen different *V. vinifera* grapes and hybrids were analyzed and the content of anthocyanins were higher in hybrid grapes than in *V. vinifera* grapes (Yang et al., 2008).

If compared to other anthocyanin-rich sources, as 72-128 mg cyanidin-3-glc/100 g FW and 140 mg cyanidin-3-glc/100 g FW for blueberries and blackberries, respectively (Jacques, Pertuzatti, Barcia, Zambiazi, & Chim, 2010), anthocyanin content in BRS Violeta is similar to content reported for blueberries. But lower than values reported for

blackberries.

Regarding winemaking by-products, skins presented higher content of anthocyanins than lees. An exception was observed for Cabernet sauvignon, in which there was no significant difference between skin and lees for samples of the year 2011 (**Table 1.2**), and skin samples of the year 2012 showed lower anthocyanin content than in lees. Literature data showed the amount of 96-99 mg cyanidin-3-glc/100 g FW for grape juice marc of Isabel cultivar (Sant'Anna, Brandelli, Marczak, & Tessaro, 2012), while comparing with our study, the skin of BRS Violeta cultivars revealed a higher content of anthocyanins (109-184 mg malvidin-3-glc/100 g FW). However, it is important to note that there are differences between winemaking by-products and the juice processing by-products, which was the material used by Sant'Anna et al. (2012), thus, comparison must be carefully considered.

Another important finding was reported by Rebello et al. (2013) showing that in the fresh skin of BRS Violeta, anthocyanins accounted for 393 mg mv-3, 5-diglc/100 g FW, a value higher than that found in our study. However, we believe that anthocyanins were transferred from the skin to the wine. Vatai, Skerget, Knez, Kareth, Wehowski and Weidner (2008) analyzed different solvents and different temperatures for the extraction of bioactive compounds in grape residue of Refosk cultivar. They found an anthocyanin content between 50 and 70 mg cyanidin-3-glc/100 g DM in the extract obtained with 50% acetone, which resembles to the content of anthocyanins in Cabernet sauvignon grape residues.

3.3 Antioxidant Capacity

For all samples, the ORAC values of antioxidant capacity were higher than their corresponding FRAP, ABTS, and β-carotene/linoleic acid bleaching test (**Table 1.3**). These

results very likely suggest that grape and winemaking by-product phenolic compounds have an intense activity as scavenger of peroxyl radicals, and in a less extension by mechanisms of ABTS catium radical neutralization, electron donation to ferric ions, or prevention of carotenoid bleaching by lipid peroxyl radicals.

Table 1.3. Results of antioxidant capacity by FRAP, ABTS, ORAC, and β-carotene/linoleic acid in the samples of grape, skin, seed, and lees BRS Violeta, BRS Lorena, Cabernet sauvignon and Cabernet franc in the years 2011 and 2012.

Samples/Years		FRAP μM TE/g DM		ABTS μM TE/g DM		ORAC μM TE/g DM		β carotene % inhibition	
		2011	2012	2011	2012	2011	2012	2011	2012
BRS Violeta	Grape	374±10bA	401±34bA	344±18bA	292±10cB	1408±71cA	1336±36bA	14±1dB	21±2dA
	Skin	639±16aB	880±32aA	554±65aA	618±20bA	3132±75aA	2901±113aB	29±6cB	51±8cA
	Seed	593±54aB	958±102aA	636±49aB	833±76aA	2487±75bB	3370±330aA	91±6aA	111±15bA
	Lees	446±4bA	374±11bB	355±4bA	73±1bB	1568±114cA	781±80cB	58±3bB	133±1Aa
BRS Lorena	Grape	62±4bA	34±3dB	60±3A	27±1cB	186±14bA	117±12dB	7.2±0.4bA	6.3±1cA
	Skin	54±3bB	127±3bA	47±6cA	21±1cB	203±16abB	257±18cA	4.9±0.3cA	1.1±0.2dB
	Seed	80±4aB	174±3aA	93±6aA	44±2bB	233±10aB	473±26abA	19±1aA	18±1aA
	Lees	---	63±6c	---	65±6a	---	372±17	---	8.7±0.4b
Cabernet franc	Grape	116±2bB	131±5dA	64±7bB	121±4cA	339±58bA	400±37cA	18±1bA	19±3bA
	Skin	---	257±2b	---	60±1d	---	661±14b	---	24±1b
	Seed	---	509±30a	---	449±16a	---	477±49c	---	21±3b
	Lees	265±4aA	215±5cB	140±3aB	182±9bA	832±37aA	815±21aA	57±9aB	73±2aA
Cabernet sauvignon	Grape	176±18cA	115±9bB	155±13bA	96±4cB	460±68cA	331±18cB	17±1cA	19±2cA
	Skin	2534bA	95±11bB	163±7bA	37±3dB	976±94aA	482±7bB	23±2cA	18±0.2cB
	Seed	551±11A	241±41aB	457±20aA	188±11bB	997±59aA	446±6bB	90±8bA	104±1aA
	Lees	202±6cB	246±10aA	137±3bB	217±13aA	716±8bA	794±71aA	65±4aA	71±9A

For each grape cultivar, different lower case letters in a same column shows significant differences ($p<0.05$) among samples. Different capital letters in a same line shows significant differences between years ($p<0.05$), the second test of Tukey. TE = equivalente Trolox; DM = Dry Matter

BRS Violeta grape variety has the greatest antioxidant capacity which is in agreement with the high content of phenolic compounds observed in these samples (**Table 1.2**). This hypothesis was reinforced by the high positive correlation coefficients shown in **Table 1.4** that were significant values between total polyphenol content ($p<0.05$) and FRAP, ABTS, and ORAC values (0.99, 0.94, and 0.97, respectively). Similar positive correlations were found between total anthocyanin content and antioxidant capacity measures (0.96, 0.92, and 0.93 for FRAP, ABTS, and ORAC values, respectively), an expectable result given the also good correlation between TPH and ACY (0.95).

Table 1.4. Pearson's correlation coefficients of antioxidant capacity, total phenolics, and total anthocyanins content.

Trait*	TPH	ACY	FRAP	ABTS	ORAC
ACY	0.95				
FRAP	0.99	0.96			
ABTS	0.94	0.92	0.91		
ORAC	0.97	0.93	0.94	0.95	
CAROTENE	0.15	0.01	0.20	-0.03	0.04

*TPH= total phenolics; ACY= total anthoyanins; FRAP= antioxidant capacity based on FRAP assay; ABTS= antioxidant capacity based on ABTS assay; ORAC= antioxidant capacity based on ORAC assay; CAROTENE= antioxidant capacity based on β-carotene/linoleic acid.

Thaipong, Boonprakoba, Crosbyb, Cisneros-Zevallosc and Byrne (2006) reported high correlation of FRAP, ABTS, and ORAC values with TPH content in guava fruits. It was argued that phenolic compounds are mainly secondary metabolites in these fruits. However, there was no significant correlation between bleaching of β-carotene/linoleic acid emulsions and any of the phenolic compound concentrations nor with any other antioxidant methods tested (**Table 1.4**). Indeed, all grape varieties showed lower antioxidant capacity to inhibit bleaching in β-carotene/linoleic acid emulsion. Values ranged from 6.34% for

BRS Lorena grapes of the year 2012 and 20.79% for BRS Violeta grapes of the same year (**Table 1.3**).

Results were in agreement with previous reported values. Recently, Rodrigues et al. (2011) published values of 19.5% for a blueberries cultivar variety with the highest antioxidant capacity by other methods. BRS Lorena grapes showed similar results than previous reports for acerola and papaya Formosa variety (Melo, Maciel, Lima, & Nascimento, 2008). Similar values were found for BRS Violeta, Cabernet sauvignon, and Cabernet franc in this work. However, all grapes berries showed inhibition percentages lower than 50% and can be considered weak antioxidants by this methodology (Melo et al., 2008).

The antioxidant capacity of winemaking by-products (**Table 1.3**) reached the highest values for seeds ($p<0.05$) for all studied varieties. This can be explained by the high level of phenolic compounds found in this grape part (**Table 1.2**). However, this behavior has not been always reported for fruits in general, since there are some previous works that showed high levels of phenolic compounds in fruit seed but the highest antioxidant capacity was found in other fruit part, using ABTS method (Babbar et al., 2011). It was hypothesized that it could be due to the presence of other non-phenolic antioxidants like ascorbate, carotenoids, or terpenes.

3.4 Principal Component Analysis (PCA)

Principal component analysis (PCA) provides a multivariate study of experimental data, which facilitates the visualization of the correlation between samples and variables.

However, to facilitate this view was necessary pre-processing, specifically self-

scaling. For this, the data focused on the middle and each one was divided by the standard deviation, so that all variables (phenolics, anthocyanins, ORAC, ABTS, FRAP, and β-carotene/linoleic acid assays) were given the same importance, that also contributes to the dataset variance.

PC1 explained 74.1% of the total variance, PC2 16.7%, PC3 6.9%, while PC4 explained only 1.6% of the total variance. However, PC4 vs. PC1 were important for the separation of the samples (**Figures 1.1a** and **1.1b**). First of all, it can be noted that all samples of BRS Violeta (grape, skins, seeds, and lees) had highly positive scores on PC1 (mainly related to ORAC, FRAP, ABTS, and total phenolic content) and separation of these samples was possible along the positive axe of PC1 (**Figure 1.1a**). The samples of the also hybrid variety BRS Lorena, were separated by PC1 and appeared grouped in the most negative part along the PC1 axe, that corresponded to the lowest values of phenolic compounds, ABTS, FRAP, and ORAC. Cabernet sauvignon and Cabernet franc were not well separated as they showed similar phenolic composition and antioxidant behavior. The PC2 was mainly related to the content of total anthocyanins (**Figure 1.1d**) and made possible the separation of samples of BRS Violeta grape and lees, and also samples of BRS Violeta skin and seeds.

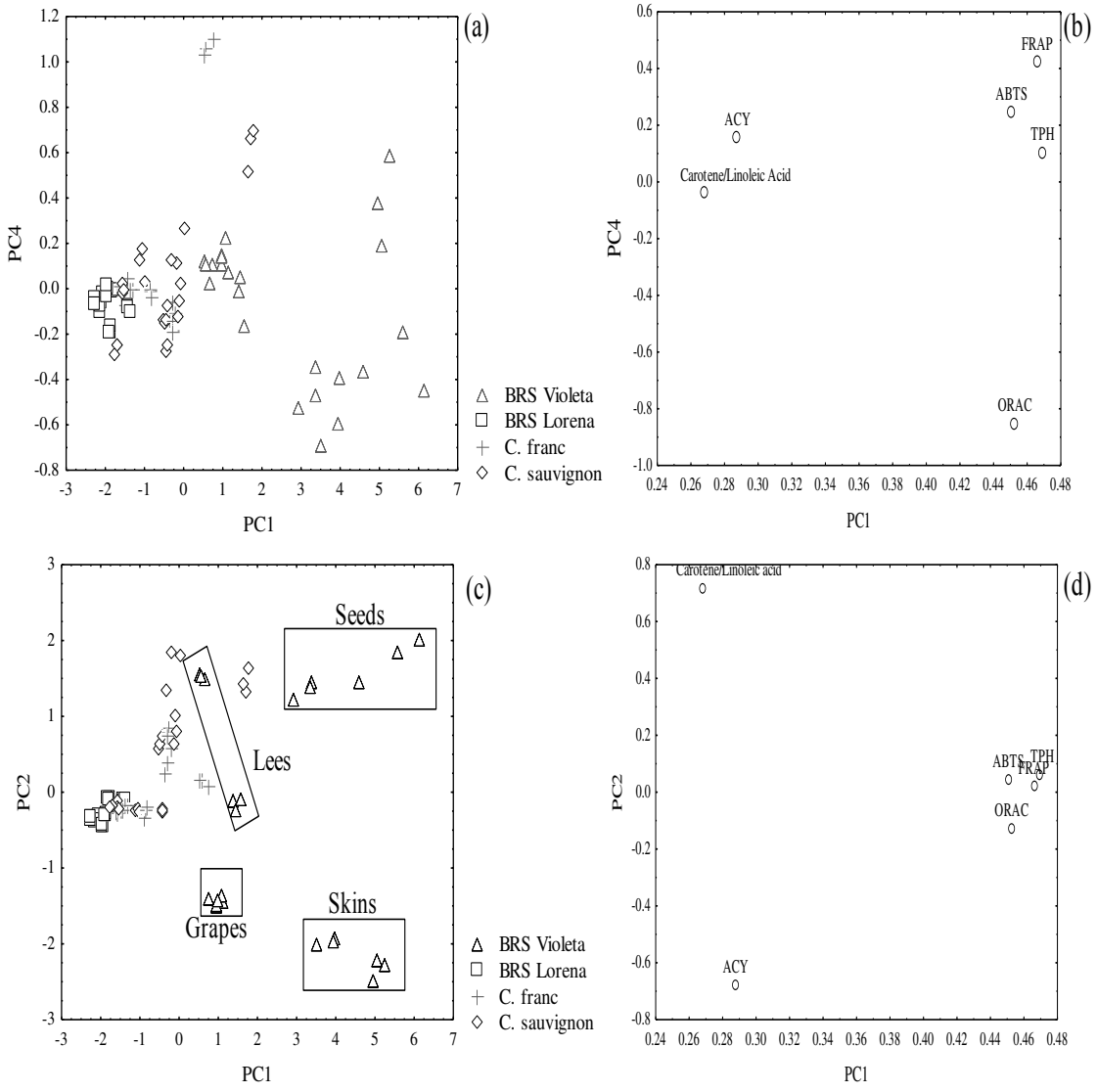

Figure 1.1. Principal component analysis (PCA) on grape, skin, seed, and lees of four varieties BRS Violeta, BRS Lorena, Cabernet sauvignon and Cabernet franc belonging harvests of 2011 and 2012 (a) Graph of scores for PC1 (factor 1) and PC4 (factor 4), differences between classes (grape varieties), (b) Graph of loadings for PC1 (factor 1) and PC4 (factor 4), (c) Graph of scores for PC1 (factor 1) and PC2 (factor 2), (d) Graphics loadings for PC1 (factor 1) and PC2 (factor 2).

3.5 Stability of Winemaking By-Products

The content of phenolics and anthocyanins, as well as the four assays of antioxidant capacity were monitored over the storage of the samples dried after processes oven-drying (skins and seeds samples) and spray-drying (only lees samples) (**Figure 1.2**). With the

exception of BRS Violeta by-products the other significant difference ($p<0.05$) was the content of bioactive compounds and the antioxidant capacity during the interval of 90 days of storage.

The phenolic compounds are highly unstable and can be lost during processing, particularly when thermal treatment is involved (Srivastava, Akoh, Yi, Fischer, & Krewer, 2007), as was observed in this study when compared data from **Figure 1.2a** with those in **Table 1.2**. A reduction of up to 50% in the total phenolic content of different by-products of the four grape varieties compared to initial time was measured. This reduction could be attributed to oxidative conditions during storage, as reported by other researchers (Schmidt, Eerdman, & Lila 2005, Srivastava et al., 2007).

Anthocyanins of BRS Violeta remained stable during the ninety-day storage, both in the skin and the lees of red grape cultivars. In contrast, the anthocyanin content in the lees of Cabernet franc already decreased by 39% after 30 days and remained almost unchanged after 90 days of storage. Similar result was found for lees of Cabernet sauvignon (reduction of 40% after 30 days) but significant reduction ($p<0.05$), was also observed after 90 days of storage up to reach 21% of the initial value. Finally, the anthocyanin content of skins of Cabernet sauvignon only decreased after 90 days (56% of the initial value).

Regarding the stability of the antioxidant, the observed behavior differed from that observed for total contents of phenolic compounds and anthocyanins but also differed according to the type of determination (**Figures 1.2c**, **1.2d**, **1.2e**, and **1.2f**). FRAP values did not change so much during storage for all samples (**Figure 1.2c**). In the case of ORAC determinations, little variations (increasing or decreasing trends) and even maintenance of antioxidant capacity were observed (**Figure 1.2e**).

The BRS Violeta by-products increased their ABTS (**Figure 1.2d**) and β-carotene/linoleic acid (**Figure 1.2d**) values after 30 days of storage and, after 90 days, the ABTS values decreased for skin and seed samples (but maintained for lees) and the β-carotene/linoleic acid values increased for seeds and lees (but maintained for skins). The behavior for Cabernet sauvignon by-products was different: after 30 days, all ABTS values and β-carotene/linoleic acid values for skins and lees increased, but β-carotene/linoleic acid value for seed maintained; after 90 days, ABTS of skins and β-carotene/linoleic acid values for seeds and lees decreased, ABTS values for seeds and lees maintained, and β-carotene/linoleic acid value for skins increased. The behavior of Cabernet franc lees was similar to that of lees form Cabernet sauvignon. The singular behavior shown by BRS Violeta winemaking by-products could be very likely linked with their high contents in anthocyanins that dominated the pool of total phenolic compounds, as it has been reported for the detailed phenolic composition of the grapes of this hybrid cultivar (Rebello et al., 2013).

Some studies claim that prolonged storage at high temperatures can affect glycosylation and hydroxylation of phenolic compounds (Srivastava, et al., 2007), besides an increase in glycosidic substitution, acylation, and methoxylation tends to improve the stability of anthocyanins (Rice-Evans, Miller, & Pagana, 1996), which may have influenced so that there was an increase in antioxidant capacity in most of the samples analyzed in this study.

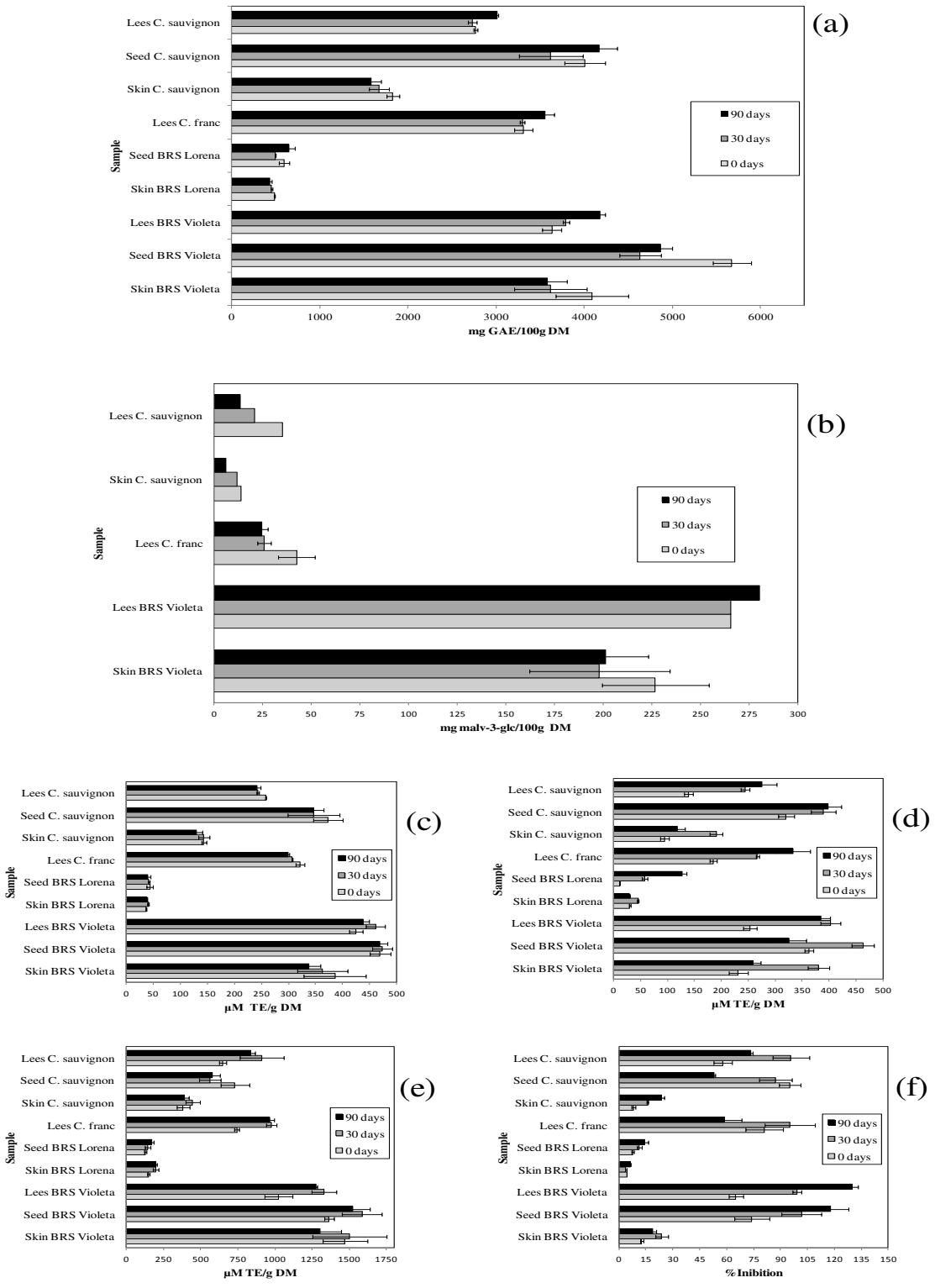

Figure 1.2. Phenolic compounds (a), anthocyanins (b), antioxidant capacity by FRAP (c), ABTS (d), and ORAC (e) β-carotene/linoleic acid (f) in skin, seed (drying oven-dried at 50 °C) and lees (spray-drying) at 0, 30 and 90 days of storage.

4 CONCLUSION

The grape cultivar BRS Violeta and their corresponding winemaking residues had the highest content of total phenolic compounds and anthocyanins, thus contributing to an important enhancement of their antioxidant capacity measured by four methods.

The winemaking by-products of all the studied grape varieties can be suggested as interesting sources for the extraction of bioactive compounds, with the seed, regardless of the variety, the largest source of phenolic compounds.

With regard to stability of phenolic compounds present in winemaking by-products, the behavior shown by BRS Violeta was the most interesting. The total phenolic and anthocyanin contents were almost maintained over 90 days of storage, in parallel with maintenance and even increase of antioxidant capacity.

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6 REFERENCES

Abe, L.T., Da-Mota, R.V. Lajolo, F.M., & Genovese, M.I. (2007). Compostos fenólicos e capacidade antioxidante de cultivares de uvas *Vitis labrusca* L. e *Vitis vinifera* L.. *Ciência e Tecnologia de Alimentos, 27*, 2, 394-400.

Alonso, A.M., Guillen, D.A., Barroso, C.G., Puertas, B., & Garcia, A. (2002). Determination of Antioxidant Activity of Wine Byproducts and Its Correlation with Polyphenolic Content. *Journal of Agricultural and Food Chemistry, 50*, 5832−5836.

Arvanitoyannis, I.S., Ladas, D., & Mavromatis, A. (2006). Potential uses and applications of treated wine waste: a review. *International Journal of Food and Technology, 41*, 475-487.

Babbar, N., Oberoi, H.S., Uppal, D.S., & Patil, R.T. (2011). Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. *Food Research International, 44,* 391–396.

Barcia, M. T., Jacques, A. C., Pertuzatti, P. B., & Zambiazi, R. C. (2010). Determinação de ácido ascórbico e tocoferóis em frutas por CLAE. *Semina: Ciências Agrárias, 31*, 2, 381-390.

Benzie, I.F.F., & Strain, J.J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a Measure of ‘‘Antioxidant Power’’: The FRAP Assay. *Analytical Biochemistry, 239*, 70 – 76.

Borbalán, A. M. A., Zorro, L., Guillén, D. A., & Barroso, C. G. (2003). Study of the polyphenol content of red and white grape varieties by liquid chromatography–mass spectrometry and its relationship to antioxidant power. *Journal of Chromatography A, 1012*, 1, 31-38.

Casazza, A. A., Aliakbarian, B., Faveri, D., Fiori, L., & Perego, P. (2012). Antioxidants from winemaking wastes: a study on extraction parameters using response surface methodology. *Journal of Food Biochemistry, 36*, 28–37.

Castillo-Muñoz, N., Gomez-Alonso, S., Garcia-Romero, E., Gomes, M.A., Velders, A.H., & Hermosín-Gutiérrez, I. (2009). Flavonol 3-*O*-Glycosides Series of *Vitis vinifera* Cv.Petit Verdot Red Wine Grapes. *Journal Agricultural and Food Chemistry, 57*, 209-219.

Cataneo, C.B., Caliari, V., Gonzaga, L.V., Kuskoski, E.M., & Fett, R. (2008). Atividade antioxidante e conteúdo fenólico do resíduo agroindustrial da produção de vinho. *Semina: Ciências agrárias, 29*, 1, 93-102.

CEPAGRI (*Centro de Pesquisas Meteorológicas e Climáticas Aplicadas à Agricultura*). Available in: <http://www.agritempo.gov.br/agritempo/jsp/PesquisaClima/index.jsp?siglaUF=SP>. Accessed on: 04 September 2013.

Cortés, S., Rodríguez, R., Salgado, J.M., & Domínguez, J.M. (2011). Comparative study between Italian and Spanish grape marc spirits in terms of major volatile compounds. *Food Control, 22*, 673-680.

Danzer, K., & Currie, L. (1998). Guidelines for calibration in analytical chemistry. *Pure Applied Chemistry, 70*, 4, 993-1014.

Giusti, M.M., & Wrolstad, R.E. (2001). Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy. *Current Protocols in Food Analytical Chemistry*, F1.2.1-F1.2.13.

Hogan, S., Zhang, L., Li, J., Zoecklein, B., & Zhou, K. (2009). Antioxidant properties and bioactive components of Norton (*Vitis aestivalis*) and Cabernet Franc (*Vitis vinifera*) wine grapes. *LWT - Food Science and Technology, 42*, 1269–1274.

Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J.A., & Prior, R.L. (2002). High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *Journal of Agricultural and Food Chemistry, 50*, 4437-4444.

Huang, D., Ou, B., Ronald, D., & Prior, L. (2005). The Chemistry behind Antioxidant Capacity Assays. *Journal of Agricultural and Food Chemistry, 53*, 1841-1856.

INMETRO. *Instituto Nacional de Metrologia, Normalização e Qualidade Industrial.* (2003). Orientações sobre Validação de Métodos de Ensaios Químicos, DOQ-CGCRE-008.

INSTITUTO ADOLFO LUTZ (1985). *Normas analíticas do Instituto Adolfo Lutz*: métodos químicos e físicos para análise de alimentos. 2 ed. São Paulo, 371p.

Jacques, A. C., Pertuzatti, P. B., Barcia, M. T., Zambiazi, R. C., & Chim, J. F. (2010). Estabilidade de compostos bioativos em polpa congelada de amora-preta (*Rubus fruticosus*) cv. Tupy. *Química Nova, 33*, 8, 1720-1725.

Karvela, E., Makris, D.P., Kalogeropoulos, N., Karathanos, V.T., & Kefalas, P. (2009). Factorial design optimisation of grape (*Vitis vinifera*) seed polyphenol extraction. *European Food Research Technology, 229*, 731–742.

Lafka, T-I., Sinanoglou, V., & Lazos, E.S. (2007). On the extraction and antioxidant activity of phenolic compounds from winery wastes. *Food Chemistry, 104*, 1206–1214.

Makris, D.P., Boskoub, G., & Andrikopoulosb, N.K. (2007). Polyphenolic content and in vitro antioxidant characteristics of wine industry and other agri-food solid waste extracts. *Journal of Food Composition and Analysis, 20*, 125–132.

Maragkoudakis, P.A., Nardi, T., Bovo, B., D'andrea, M., Howell, K.S., Giacomini, A., & Corich, V. (2013). Biodiversity, dynamics and ecology of bacterial community during grape marc storage for the production of grappa. *International Journal of Food Microbiology, 162*, 2, 143-151.

Melo, E.A., Maciel, M.I.S., Lima, V.L.A.G., & Nascimento, R.J. (2008). Capacidade antioxidante de frutas. *Brazilian Journal of Pharmaceutical Sciences, 44*, 2, 193-201.

Miller, H.E.A. (1970). A simplified method for the evaluation of antioxidants. *Journal of the American Oil Chemists' Society, 48*, 2, 91. In: Marco, G.J. (1968). A rapid method for evaluation of antioxidants. *Journal of the American Oil Chemists' Society, 45*, 594-598.

Moyer, R.A., Hummer, K.E., Finn, C.E., Frei, B., & Wrolstad, R.E. (2002). Anthocyanins, Phenolics, and Antioxidant Capacity in Diverse Small Fruits: *Vaccinium*, *Rubus*, and *Ribes. Journal of Agricultural and Food Chemistry, 50,* 519-525.

Paradelo, R., Moldes, A.B., & Barral, M.T. (2010). Utilization of a Factorial Design To Study the Composting of Hydrolyzed Grape Marc and Vinification Lees. *Journal Agricultural and Food Chemistry, 58*, 3085-3092.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved abts radical cation decolorization assay. *Free Radical Biology & Medicine, 26*, 9, 1231-1237.

Rebello, L.M.G., Lago-Vanzela, E.S., Barcia, M. T., Ramos, A.M., Stringheta, P. C., Da-Silva, R., Castillo-Muñoz, N., Gómez-Alonso, S., & Hermosín-Gutiérrez I. (2013). Phenolic composition of the berry parts of hybrid grape cultivar BRS Violeta (BRS Rubea × IAC 1398-21) using HPLC–DAD–ESI-MS/MS. *Food Research International, 54*, 354–366.

Ribani, M., Bottoli, C., Collins, C., & Jardim, I. (2004). Validação em métodos cromatográficos e eletroforéticos. *Quimica Nova, 27*, 5, 771-780.

Rice-Evans, C.A., Miller, N.J., & Pagana, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology & Medicine, 20*, 933-956.

Rockenbach, I.I., Gonzaga, L.V., Rizelio, V.M., Gonçalves, A.E.S.S., Genovese, M.I., & Fett, R. (2011). Phenolic compounds and antioxidant activity of seed and skin extracts of red grape (*Vitis vinifera and Vitis labrusca*) pomace from Brazilian winemaking. *Food Research International, 44*, 897–901.

Rodrigues, E., Poerner, N., Rockenbach, I.I., Gonzaga, L.V., Mendes, C.R., & Fett, R. (2011). Phenolic compounds and antioxidant activity of blueberry cultivars grown in Brazil. *Ciência e Tecnologia de Alimentos, 31*, 4, 911-917.

Sant'Anna, V., Brandelli, A., Marczak, L.D.F., & Tessaro, I.C. (2012). Kinetic modeling of total polyphenol extraction from grape marc and characterization of the extracts. *Separation and Purification Technology, 100*, 82–87.

Scherer, R.; Godoy, H. T. (2013). Effects of extraction methods on the phenolics compounds and antioxidant activity of Xanthium srumarium L. Braz. J. Med. Plants, in press.

Schmidt, B.M., Eerdman, J.W., & Lila, M.A. (2005). Effects of Food Processing on Blueberry. Antiproliferation and Antioxidant Activity. *Journal of Food Science, 70*, 6, 389-394.

Shojaee-Aliabadi, S., Hosseini, S. M., Tiwari, B., Hashemi, M., Fadavi, G., & Khaksar, R. (2013). Polyphenols content and antioxidant activity of Ghure (unripe grape) marc extract: influence of extraction time, temperature and solvent type. *International Journal of Food Science and Technology, 48*, 412–418.

Singleton, V.L. (1982). Grape and wine phenolics: Background and prospects. In: *Grape and Wine Centennial Symposium Proceedings*, University of California, Davis, (A. D. Webb, ed.), UC Davis, Davis, CA, 215–227.

Singleton, V.L., Orthofer, R., & Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology, 299*, 152-178.

Srivastava, A., Akoh, C.C., Yi, W., Fischer, J., & Krewer, G. (2007). Effect of Storage Conditions on the Biological Activity of Phenolic Compounds of Blueberry Extract Packed in Glass Bottles. *Jounal of Agricultural and Food Chemistry, 55*, 2705-2713.

Treutter. D. (2006). Significance of Flavonoid in Plant Resistance: a Review. *Environmental Chemistry Letters, 4*, 147-157.

Thaipong, K., Boonprakoba, U., Crosbyb, K., Cisneros-Zevallosc, L., & Byrne, D.H. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis, 19*, 669–675.

Torres, J.L., Varela, B., Garcia, M.T., Carilla, J., Matito, C., Centelles, J.J., Cascante, M., Sort, X., & Bobe, R.J. (2002). Valorization of Grape (*Vitis vinifera*) Byproducts. Antioxidant and Biological Properties of Polyphenolic Fractions Differing in Procyanidin Composition and Flavonol Content. *Journal of Agricultural and Food Chemistry*, *50*, 7548−7555.

Vatai, T., Skerget, M., Knez, Z., Kareth, S., Wehowski, M., & Weidner, E. (2008). Extraction and formulation of anthocyanin-concentrates from grape residues. *Journal of Supercritical Fluids, 45*, 32–36.

Yang, J., Martinson, T.E., & Liu, R.H. (2008). Thermal degradation kinetics of aqueous anthocyanins and visual color of purple corn (*Zea mays L.*) cob. *Food Chemistry, 116*, 332–339.

Yilmaz, Y., & Toledo, R.T. (2004). Major Flavonoids in Grape Seeds and Skins: AntioxidantCapacity of Catechin, Epicatechin, and Gallic Acid. *Journal Agricultural and Food Chemistry, 52*, 255−260.

ARTIGO 2

Phenolic Composition of Grape and Winemaking By-Products of Brazilian Hybrid Cultivars BRS Violeta and BRS Lorena.

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Phenolic Composition of Grape and Winemaking By-Products of Brazilian Hybrid Cultivars BRS Violeta and BRS Lorena.

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ABSTRACT

The grapes and winemaking by-products (skins from grape marc, and lees) from Brazilian hybrid cultivars BRS Violeta (red) and BRS Lorena (white) was studied for characterize the phenolic compounds. Two vintages, four classes of phenolic compounds, and recovery yields using three dehydration techniques were considered: oven-drying at 50 ºC (D50); spray-drying (SD); and freeze-drying (FD). Recovery yields were higher using FD, although less expensive SD was a good alternative for BRS Violeta lees. D50 caused great recovery reduction in BRS Violeta but yielded similar results for BRS Lorena. BRS Violeta winemaking by-products were excellent sources of anthocyanins (mainly non-acylated and *p*-coumaroylated diglucosides), flavonols (mainly myricetin-based) and hydroxycinnamic derivatives (mainly caffeic-based). BRS Lorena winemaking by-products contained lesser amounts of phenolic compounds, around a tenth of the values found in BRS Violeta grapes for flavonols (mainly quercetin-based) and hydroxycinnamic derivatives (mainly caffeic-based). BRS Lorena cultivar contained small amounts of *trans*-resveratrol and its 3-glucoside, which were missing in BRS Violeta cultivar.

Keywords: anthocyanins, pyranoanthocyanins, phenolic acids, flavonols, grape marc, lees, non-vinifera grape

1 INTRODUCTION

Polyphenolic compounds from grapes can be transfered to the wine during the winemaking maceration phase until reach to an equilibrium condition. It will depend on the process characteristics (e.g., maceration time, temperature, intensity and duration of pressing, using of macerating enzymes, yeast strain, and used SO_2 doses). As the major part of grape polyphenols comes from solid grape parts, high proportion of polyphenols still can remains in the solid winemaking by-products or grape marc (Gallego, García-Carpintero, Sánchez-Palomo, Viñas, & Hermosín-Gutiérrez, 2012).

Grape marc (a mixture made mainly of grape skin and seeds) is one of the most abundant by-products of the winemaking process, with a significant negative ecological and economical impact. However, it is considered as a low-cost source of natural antioxidants (Shojaee-Aliabadi, Hosseini, Tiwari, Hashemi, Fadavi, & Khaksar, 2013). Grape marc is produced after pressing the previously crushed grapes, in white wine production technology, or after the maceration phase concurrent with fermentation step, in red wine production technology. After must fermentation, a decanting process takes place in which the supernatant wine is separated from lees which is mainly constituted of grape particle residues and death yeasts (Maragkoudakis et al., 2013, Paradelo, Moldes, & Barral, 2010). Moreover, this solid residue can contain tartaric acid, inorganic matter and grape phenolic compounds (Pérez-Serradilla, & Luque de Castro, 2008).

The literature dealing with this topic has revealed an adsorption capacity of yeast cell wall with regards to anthocyanins and other phenolic compounds, like flavonols or hydroxycinnamic acids (Morata, Gómez-Cordovés, Colomo, & Suárez, 2005, Pérez-Serradilla, & Luque de Castro, 2008 and 2011). It is well known that grape residue from

winemaking (seeds, skins) contain a high amount of secondary metabolites including phenolic acids, flavan-3-ols and anthocyanins (Makris, Boskou, & Andrikopoulos, 2007). However, many studies have been focused on winemaking by-products from *Vitis vinifera* varieties and fewer information is available about the polyphenol content of non-vinifera grape varieties (Sant'Anna, Brandelli, Marczak, & Tessaro, 2012).

The non-vinifera hybrid grape varieties are produced with the purpose of increase productivity and resistance to plant diseases that generally affect *Vitis vinifera* grape varieties (Abe, Mota, Lajolo, & Genovese, 2007). The Brazilian Agricultural Research Corporation for Grape and Wine (Embrapa Uva e Vinho) have developed hybrid grape varieties adapted to the specific conditions required by emerging new crop regions for grape in Brazil. The red grape cultivar BRS Violeta is a complex hybrid obtained from the cross between BRS Rúbea × IAC 1398-21. It maintains the general characteristics of *Vitis labrusca* grapes regarding to the vine morphology and grape taste. The BRS Violeta grape was created as an alternative to increase quality and competitiveness of table wine and grape juice produced in Brazil, due to the high level of sugar and intense color. It is especially suitable for cultivation in subtropical climate zones as in São Paulo state regions (Camargo, Maia, & Nachtigal, 2005). The white hybrid grape cultivar BRS Lorena (Malvasia Bianca × Seyval) was developed in 2001 as an alternative for elaboration of white wines. It also has a high productivity, rich content of sugars and, it is relatively rich in acids must being a kind of suitable for production of a well-balanced taste sparkling and still wines (Camargo, & Guerra, 2001).

Thus, studies with this type of by-products can be a promising option for verifying the presence of phenolic compounds with importance to human health. The chemical

characterization of phenolic compounds from winemaking by-products (grape marc and lees) are basic information about the viability of using this food by-products in foodstuff, chemical, and pharmaceutical industries. For this reason, the aim of this work was to study the phenolic composition of red and white winemaking by-products (skins from grape marc, and lees) from Brazilian hybrid grape varieties (BRS Violeta and BRS Lorena). The study of different phenolic classes (anthocyanins and some pyranoanthocyanins, flavonols, hydroxycinnamic acid derivatives, and stilbenes) was performed over two vintages. Furthermore, the effect of three dehydration techniques applied to the winemaking by-products has been considered: oven-drying at 50 °C, spray-drying, and freeze-drying, to improve the maintenance and stability of phenolic compounds.

2 MATERIAL AND METHODS

2.1 Chemicals

All solvents were of HPLC quality and all chemicals of analytical grade (> 99 %). Water was of ultrapure quality (Milli-Q). Anthocyanins were quantified as equivalents of malvidin 3,5-diglucosides (g/kg sample) (Phytolab, Vestenbergsgreuth, Germany). Flavonols, hydroxycinnamic acid and stilbenes (as mg/kg sample) were quantified using the calibration curve of the respective representative standards: quercetin 3-glucoside (Extrasynthese, Genay, France), *trans*-caftaric acid (Phytolab, Vestenbergsgreuth, Germany) and *trans*-resveratrol (Sigma, Madrid, Spain). Other standards used for identification were: malvidin 3-glucoside, peonidin 3,5-diglucosides, caffeic acid, *p*-coumaric acid, *trans*-piceid (Phytolab, Vestenbergsgreuth, Germany); cyanidin 3-glucoside, cyanidin 3,5-diglucosides, kaempferol, quercetin, isorhamnetin, myricetin, syringetin, and

the 3-glucosides of kaempferol, quercetin, isorhamnetin, and syringetin (Extrasynthese, Genay, France); myricetin 3-glucoside, quercetin 3-glucuronide, and laricitrin 3-glucoside were previously isolated from Petit Verdot grape skins (Castillo-Muñoz, Fernández-Gonzales, Gómez-Alonso, García-Romero, & Hermosín-Gutiérrez, 2009).

2.2 Samples

It was studied grape berries samples and its winemaking by-products (skins and lees) of two Brazilian hybrid varieties, BRS Violeta (BRS Rúbea × IAC 1398-21) and BRS Lorena (Malvasia Bianca × Seyval). The samples were collected in triplicate during the harvest of 2011 and 2012. The winery is located in São Roque region (São Paulo, Brazil). Grape berry samples were frozen at −20 °C for 24 h, cut in two halves, re-frozen at −20 °C for 24 h, and further freeze-dried for 48 h. Samples of fermented grape marc from BRS Violeta, fresh grape marc from BRS Lorena, and lees from both grape varieties were frozen at −20 °C for 24 h and then freeze-dried for 48 h. After that, skins were manually separated from dried grape marc samples. Skins dried samples obtained from grape marcs and lees were also dried by oven at 50 °C with forced air flow (until constant weigh). Finally, a third drying treatment was applied to lees: spray-drying. After drying, samples of entire grapes and skins were crushed and homogenized. All dried samples were stored at −18 °C until analyses.

2.3 Extraction of Phenolic Compounds

Dried samples in amounts of 2.5, 1.0, and 0.25 g (grapes, skins and lees of BRS Lorena, respectively) and 2.0, 0.25, and 0.25 g (grapes, skins and lees of BRS Violeta, respectively) were extracted with 50 mL for grape and lees, and 75 mL for skins of a mixture 50:48.5:1.5 (v/v/v) of Methanol/Water/Formic acid. Extraction was performed in

an ultrasonic bath for 2 min. The mixture was centrifuged at 5000*g* at 5 °C for 5 min. A second and third extractions in sample pellets yielded nearly 99% of total phenolic content, as confirmed by HPLC of successive extractions (up to five). All supernatants were mixed up and stored at −18 °C. For analysis of non-anthocyanin phenolics, an anthocyanin-free fraction was obtained from entire extract using solid phase extraction as previously described by Lago-Vanzela, Da-Silva, Gomes, García-Romero, and Hermosín-Gutiérrez (2011a).

2.4 Identification and Quantification of Phenolic Compounds by HPLC-DAD-ESI-MS/MS

2.4.1 Analysis of Anthocyanins and Derived Compounds

HPLC separation, identification, and quantification of anthocyanins and pyranoanthocyanins were performed in an Agilent 1100 Series system (Agilent, Germany), equipped with DAD (G1315B) and a LC/MSD Trap VL (G2445C VL) electrospray ionization mass spectrometry (ESI-MS^n) system, and coupled to an Agilent ChemStation (version B.01.03) data-processing station. The mass spectra data were processed with the Agilent LC/MS Trap software (version 5.3). Sample extracts (grape, skin: 1mL, lees: 2mL) were dried in a rotary evaporator (35 °C) and re-dissolved in 1 mL of 0.1 M HCl before injection (10 μL) on a reversed-phase column Zorbax Eclipse XDB-C18 (2.1 × 150 mm; 3.5 μm particle; Agilent, Germany), thermostated at 40 °C. The solvent system was based on mixtures of water, acetonitrile, and formic acid (88.5:3:8.5, v/v/v, solvent A; 41.5:50:8.5, v/v/v, solvent B), and the flow rate was 0.19 mL/min. A linear gradient for solvent B was performed as follows: 0 min, 6%; 10 min, 30%; 30 min, 50%; 34 min, 100%; 36 min, 10%; 42 min, 6%. For identification, ESI-MS^n was used setting the following

parameters: positive ionization mode; dry gas, flown N_2, 11 mL/min; drying temperature, 350 °C; nebulizer, 65 psi; capillary, -2500 V; capillary exit offset, 70 V; skimmer 1, 20 V; skimmer 2, 6 V; compound stability, 100%; scan range, 50-1200 m/z. For quantification, DAD-chromatograms were extracted at 520 nm and their total concentrations were expressed as equivalents of malvidin-3,5-diglucosides.

2.4.2 Analysis of Non-Anthocyanin Phenolic Compounds

HPLC separation, identification, and quantification of non-anthocyanin phenolic compounds were performed on the same chromatographic system as that formerly described for anthocyanins. The anthocyanin-free fractions (3 mL) were concentrated in a rotaevaporator at 35 ° C and then re-dissolved in 20% methanol in water, and 20 μL injected on a reversed-phase column Zorbax Eclipse XDB-C18 (2.1 × 150 mm; 3.5 μm particle; Agilent, Germany), thermostated at 40 °C. The solvents were as follows: solvent A (acetonitrile/water/formic acid, 3:88.5:8.5, v/v/v), solvent B (acetonitrile/water/formic acid, 50:41.5:8.5, v/v/v), and solvent C (methanol/water/formic acid, 90:1.5:8.5, v/v/v). The flow rate was 0.19 mL/min. The linear solvents gradient was as follows: 0 min, 98% A, and 2% B; 8 min, 96% A, and 4% B; 37min, 70% A, 17% B, and 13% C; 51 min, 50% A, 30% B, and 20% C; 51.5 min, 30% A, 40% B, and 30% C; 56 min, 50% B, and 50% C; 57 min, 50% B, and 50% C; 64 min, 98%A, and 2% B. For quantification, DAD chromatograms were extracted at 360 nm (flavonols), and 320 nm (hydroxycinnamic acid derivatives and resveratrol forms), and their concentrations were expressed as equivalents of quercetin 3-glucoside (flavonols), *trans*-caftaric acid (hydroxycinnamic acid), and *trans*-resveratrol (resveratrol forms).

2.4.3 Statistics

The post-hoc Tukey test (STATISTIC 7.0, $p<0.05$) was used for mean value comparison. In addition, Principal Component Analysis (Pirouette 3.11.) was applied to the data matrix in order to highlight similarities and/or differences within samples in a determined set of data. Data was previously pre-escalated before submitting them to statistics analysis.

3 RESULTS AND DISCUSSION

3.1 Anthocyanins, Pyranoanthocyanins, Flavonols, Hydroxycinnamic Acid Derivatives (HCAD) and Stilbenes in BRS Violeta Grape and its Winemaking By-Products

Anthocyanins only occurred in the red grape cultivar BRS Violeta; however, because it is a teinturier variety, it was present in both fruit part, skin and pulp. The results for freeze-dried samples of BRS Violeta grape, skin and lees corresponding to years 2011 and 2012 are presented in **Table 2.1**. With the help of extracted ion chromatograms (EIC) at the *m/z* ratios corresponding to the different anthocyanidins (aglycons), we were able to detect a total of 27 anthocyanins and 3 pyranoanthocyanins (**Figure 2.1**). The identification of the anthocyanins was completed on the basis of coincident spectral data, with authentic standards and with data reported previously in the literature (Castillo-Muñoz et al., 2009, Lago-Vanzela et al., 2011a, Nixford, & Hermosín-Gutiérrez, 2010).

Table 2.1. Phenolic compounds identified and quantified in freeze-dried samples of BRS Violeta grapes, their fermented skins and lees, in years 2011 and 2012.

peak	phenolic compound	R_t (min)	molecular ion; product ions (*m/z*)*	grape 2011 (molar %)	grape 2012 (molar %)	skin 2011 (molar %)	skin 2012 (molar %)	lees 2011 (molar %)	lees 2012 (molar %)
	Anthocyanins and Pyranoanthocyanins								
	total (g /kg sample)**			31.01±2.53 $^{a B}$	23.90±0.45 $^{b \beta}$	57.74±7.57 $^{b A}$	76.48±2.67 $^{a \alpha}$	20.39±0.55 ^{B}	17.78±2.04 $^{\gamma}$
1	dp-3,5diglc	4.03	627; 465, 303	18.86±0.68 ^{A}	18.07±0.07 $^{\alpha}$	15.88±1.16 ^{B}	14.89±0.23 $^{\gamma}$	15.10±1.03 ^{B}	16.07±0.36 $^{\beta}$
2	cy-3,5diglc	5.87	611; 449, 287	6.68±0.19 $^{a B}$	5.68±0.11 $^{b \beta}$	7.16±0.04 $^{a A}$	6.11±0.18 $^{b \alpha}$	5.10±0.09 $^{b C}$	6.14±0.10 $^{a \alpha}$
3	pt-3,5diglc	8.15	641; 479, 317	11.65±0.76 ^{A}	11.64±0.78 $^{\alpha}$	12.91±0.80 ^{A}	11.87±0.30 $^{\alpha}$	8.60±0.41 ^{B}	8.57±0.37 $^{\beta}$
4	pn-3,5diglc	10.68	625; 463, 301	6.68±0.19 $^{a B}$	6.08±0.05 $^{b \beta}$	8.29±0.69 $^{a A}$	6.69±0.03 $^{b \alpha}$	4.67±0.38 ^{C}	5.09±0.17 $^{\gamma}$
5	mv-3,5diglc	11.26	655; 493, 331	12.67±0.43 ^{B}	12.25±0.31 $^{\beta}$	15.99±0.28 $^{a A}$	14.36±0.16 $^{b \alpha}$	9.76±0.13 $^{a C}$	9.08±0.18 $^{b \gamma}$
6	dp-3acglc-5glc	11.60	669; 507, 465, 303	0.62±0.02 ^{A}	0.60±0.01 $^{\alpha}$	0.33±0.01 $^{a C}$	0.29±0.00 $^{b \gamma}$	0.44±0.00 $^{a B}$	0.41±0.01 $^{b \beta}$
7	cy-3acglc-5glc	12.90	653; 491, 449, 287	0.06±0.01 ^{B}	0.05±0.01 $^{\alpha}$	0.08±0.01 $^{a A}$	0.05±0.01 $^{b \alpha}$	0.06±0.00 $^{a B}$	0.03±0.00 $^{b \alpha}$
8	pt-3acglc-5glc	13.84	683; 521,479, 317	0.21±0.02 ^{A}	0.19±0.03 $^{\alpha}$	0.16±0.01 $^{a B}$	0.09±0.02 $^{b \beta}$	0.11±0.00 $^{a C}$	0.09±0.00 $^{b \beta}$
9	pn-3-acglc-5-glc	15.30	667; 505, 463, 301	0.02±0.00 $^{a C}$	0.01±0.00 $^{b \beta}$	0.06±0.01 $^{a B}$	0.03±0.01 $^{b \alpha}$	0.11±0.01 $^{a A}$	0.04±0.00 $^{b \alpha}$
10	mv-3acglc-5glc	15.90	697; 535, 493, 331	0.06±0.01 $^{a B}$	0.04±0.00 $^{b \beta}$	0.05±0.00 ^{B}	0.04±0.01 $^{\beta}$	0.10±0.01 ^{A}	0.11±0.02 $^{\alpha}$
11	dp-3glc	6.60	465; 303	6.61±0.24 ^{A}	6.39±0.15 $^{\alpha}$	4.01±0.26 $^{b B}$	6.41±0.12 $^{a \alpha}$	2.88±0.13 $^{a C}$	1.57±0.03 $^{b \beta}$

Continuation…

12	cy-3glc	9.70	449; 287	1.19±0.06 ^{A}	1.14±0.11 $^{\alpha}$	0.42±0.02 $^{b\,B}$	0.76±0.05 $^{a\,\beta}$	0.24±0.01 $^{a\,C}$	0.05±0.00 $^{b\,\gamma}$
A	pt-3glc	11.66	479; 317	0.90±0.03 ^{A}	0.87±0.02 $^{\alpha}$	0.33±0.01 $^{a\,C}$	0.30±0.03 $^{b\,\gamma}$	0.77±0.01 $^{a\,B}$	0.71±0.01 $^{b\,\beta}$
13	pn-3glc	13.60	463; 301	0.15±0.00 ^{A}	0.18±0.02 $^{\alpha}$	0.08±0.00 $^{b\,C}$	0.15±0.01 $^{a\,\alpha}$	0.11±0.00 $^{a\,B}$	0.06±0.01 $^{b\,\beta}$
14	mv-3glc	14.34	493; 331	0.17±0.01 ^{C}	0.16±0.01 $^{\beta}$	0.23±0.02 $^{a\,B}$	0.16±0.04 $^{b\,\beta}$	0.66±0.02 $^{a\,A}$	0.44±0.01 $^{b\,\alpha}$
15	dp-3cfglc-5glc	15.03	789; 627, 465, 303	0.20±0.02 ^{B}	0.23±0.02 $^{\beta}$	0.83±0.05 $^{b\,A}$	0.97±0.04 $^{a\,\alpha}$	0.27±0.01 $^{a\,B}$	0.17±0.01 $^{b\,\beta}$
16	cy-3cfglc-5glc	16.89	773; 611, 449, 287	ND	ND	0.54±0.01	0.34±0.01	ND	ND
17	dp-3cmglc-5glc	16.65	773; 611, 465, 303	15.80±0.92 $^{b\,B}$	17.93±0.43 $^{a\,\alpha}$	13.56±0.35 $^{b\,B}$	15.92±0.07 $^{a\,\beta}$	19.99±2.06 ^{A}	18.39±0.45 $^{\alpha}$
18	cy-3cmglc-5glc	18.07	757; 595, 449, 287	2.97±0.30 ^{B}	2.74±0.34 $^{\beta}$	3.31±0.09 $^{a\,B}$	2.97±0.07 $^{b\,\beta}$	3.79±0.08 ^{A}	3.88±0.13 $^{\alpha}$
19	pt-3cmglc-5glc	18.78	787; 625, 479, 317	3.65±0.28 ^{A}	4.11±0.17 $^{\beta}$	4.54±0.69 ^{A}	4.59±0.04 $^{\alpha}$	3.71±0.36 ^{A}	3.42±0.22 $^{\gamma}$
20	pn-3cmglc-5glc	20.72	771; 609, 463, 301	0.95±0.08 ^{A}	0.96±0.05 $^{\alpha\,\beta}$	0.95±0.06 $^{a\,A}$	0.84±0.03 $^{b\,\beta}$	1.06±0.02 ^{A}	1.15±0.17 $^{\alpha}$
21	mv-3cmglc-5glc	21.30	801; 639, 493, 331	2.14±0.17 $^{b\,B}$	2.46±0.03 $^{a\,\beta}$	2.19±0.11 $^{b\,B}$	2.53±0.05 $^{a\,\beta}$	3.35±0.23 ^{A}	3.58±0.07 $^{\alpha}$
22	dp-3cmglc	19.13	611; 303	5.45±0.73 ^{B}	5.70±0.06 $^{\gamma}$	5.92±0.14 $^{b\,B}$	6.95±0.17 $^{a\,\beta}$	13.95±0.87 ^{A}	14.98±0.06 $^{\alpha}$
B	cy-3cmglc	21.30	595; 287	1.43±0.11 $^{b\,B}$	1.64±0.02 $^{a\,\beta}$	1.33±0.06 $^{b\,B}$	1.62±0.03 $^{a\,\beta}$	2.24±0.15 ^{A}	2.39±0.05 $^{\alpha}$
23	pt-3cmglc	22.41	625; 317	0.65±0.12 ^{B}	0.64±0.09 $^{\gamma}$	0.58±0.09 $^{b\,B}$	0.77±0.03 $^{a\,\beta}$	1.46±0.06 ^{A}	1.42±0.01 $^{\alpha}$
24	pn-3cmglc	26.11	609; 301	0.07±0.01 ^{B}	0.09±0.00 $^{\beta}$	0.12±0.01 $^{a\,B}$	0.10±0.01 $^{b\,\beta}$	0.84±0.06 $^{b\,A}$	1.28±0.07 $^{a\,\alpha}$
25	mv-3cmglc	27.20	639; 331	0.15±0.02 ^{B}	0.16±0.01 $^{\beta}$	0.15±0.01 $^{b\,B}$	0.19±0.01 $^{a\,\beta}$	0.36±0.02 $^{a\,A}$	0.28±0.03 $^{b\,\alpha}$
26	10-HP-pydp-3glc	20.30	581; 419	ND	ND	ND	ND	0.16±0.00 ^{b}	0.34±0.02 ^{a}

Continuation…

27	10-HP-pycy-3cmglc	30.10	711; 403	ND	ND	ND	ND	0.06±0.00 ^{b}	0.14±0.02 ^{a}
28	10-HP-pypt-3cmglc	32.00	741; 433	ND	ND	ND	ND	0.06±0.00 ^{b}	0.11±0.01 ^{a}
	Flavonols								
	total (mg/kg sample)***			936.25±120.56 ^{C}	885.77±98.82 $^{\beta}$	1387.54±164.08 $^{b\,B}$	2018.85±124.66 $^{a\,\alpha}$	2824.62±122.81 $^{a\,A}$	1961.95±231.07 $^{b\,\alpha}$
29	M-3-glcU	17.16	493; 317	2.28±0.23 ^{B}	2.26±0.16 $^{\beta}$	2.97±0.04 $^{b\,A}$	3.37±0.04 $^{a\,\alpha}$	2.30±0.28 ^{B}	2.29±0.23 $^{\beta}$
30	M-3-glc	18.84	479; 317	74.68±1.12 $^{b\,A}$	77.42±0.68 $^{a\,\alpha}$	63.02±1.18 $^{b\,B}$	70.69±1.60 $^{a\,\beta}$	17.24±0.29 $^{b\,C}$	44.56±2.53 $^{a\,\gamma}$
31	Q-3-glcU	25.45	477; 301	1.95±0.05 ^{B}	1.88±0.12 $^{\gamma}$	5.10±0.66 ^{A}	4.92±0.33 $^{\alpha}$	2.00±0.10 $^{b\,B}$	2.75±0.11 $^{a\,\beta}$
32	Q-3-glc	26.84	463;301	7.47±0.75 ^{B}	6.82±0.41 $^{\beta}$	10.11±0.90 $^{a\,A}$	7.86±0.23 $^{b\,\alpha}$	0.54±0.06 $^{b\,C}$	3.41±0.23 $^{a\,\gamma}$
33	free M	29.57	317	9.45±1.33 $^{a\,B}$	6.74±0.64 $^{b\,\beta}$	9.99±1.06 $^{a\,B}$	4.45±0.76 $^{b\,\beta}$	65.18±0.36 $^{a\,A}$	37.02±1.97 $^{b\,\alpha}$
34	L-3-glc	33.40	493;331	2.78±0.12 $^{b\,A}$	3.42±0.04 $^{a\,\beta}$	2.06±0.19 $^{b\,B}$	4.49±0.47 $^{a\,\alpha}$	ND	ND
35	I-3-glc	36.48	477;315	0.98±0.06 ^{B}	0.99±0.05 $^{\beta}$	2.74±0.16 ^{A}	2.32±0.15 $^{\alpha}$	0.87±0.06 ^{B}	0.93±0.12 $^{\beta}$
36	S-3-glc	38.40	507;345	0.42±0.07 ^{B}	0.47±0.06 $^{\beta}$	1.29±0.17 $^{a\,A}$	0.90±0.08 $^{b\,\alpha}$	0.17±0.03 $^{b\,B}$	0.50±0.06 $^{a\,\beta}$
37	free Q	40.13	301	ND	ND	2.73±0.30 $^{a\,B}$	0.99±0.11 $^{b\,\beta}$	10.19±0.31 $^{a\,A}$	7.80±0.98 $^{b\,\alpha}$
38	free L	44.57	331	ND	ND	ND	ND	0.94±0.10 ^{a}	0.25±0.02 ^{b}
39	free I	52.92	315	ND	ND	ND	ND	0.24±0.01	0.29±0.04
40	free S	54.64	345	ND	ND	ND	ND	0.33±0.06 ^{a}	0.21±0.02 ^{b}
	Hydroxycinnamic acid derivatives								
	total (mg/kg sample)****			922.77±177.50 ^{B}	747.42±37.23 $^{\beta}$	872.01±85.71 $^{a\,B}$	563.63±68.15 $^{b\,\beta}$	4379.72±140.68 ^{A}	3845.63±317.59 $^{\alpha}$

Continuation…

41	*trans*-caftaric acid	3.66	311; 179, 149	49.74±0.63 $^{b\,A}$	57.33±3.64 $^{a\,\beta}$	40.37±0.42 $^{a\,B}$	34.58±1.86 $^{b\,\gamma}$	50.91±1.96 $^{b\,A}$	64.78±0.39 $^{a\,\alpha}$
42	*trans*-1-glc-caffeic acid	4.51	341; 179, 161, 135	8.44±0.18 $^{a\,B}$	2.85±0.18 $^{b\,\beta}$	16.67±2.58 ^{A}	12.21±2.10 $^{\alpha}$	ND	ND
43	*trans*-coutaric acid	5.13	295; 163, 149	13.20±0.43 $^{a\,A\,B}$	10.24±0.38 $^{b\,\beta}$	10.45±2.22 ^{B}	6.83±1.31 $^{\gamma}$	14.97±0.40 $^{a\,A}$	13.07±0.54 $^{b\,\alpha}$
44	*trans*-caffeic acid	5.30	179	ND	ND	2.94±0.26 $^{b\,B}$	8.26±1.76 $^{a\,\alpha}$	6.98±0.18 $^{a\,A}$	4.46±0.30 $^{b\,\beta}$
45	*trans*-fertaric acid	7.36	325; 193, 149	24.46±0.68 ^{A}	24.18±3.83 $^{\alpha}$	21.90±1.75 ^{A}	29.00±4.23 $^{\alpha}$	3.44±0.15 ^{B}	3.40±0.20 $^{\beta}$
46	*cis*-fertaric acid	9.30	325; 193, 149	4.15±0.03 $^{b\,B}$	5.41±0.34 $^{a\,\beta}$	7.66±1.33 ^{A}	9.11±1.57 $^{\alpha}$	ND	ND
47	*p*-coumaric acid	11.07	163	ND	ND	ND	ND	17.97±1.93 ^{a}	8.55±0.67 ^{b}
48	ethyl *p*-coumarate	49.30	191; 163	ND	ND	ND	ND	5.74±0.33	5.75±0.33

Nomenclature abbreviations: dp, delphinidin; cy, cyanidin; pt, petunidin; pn, peonidin; mv, malvidin; glc, glucoside; diglc, diglucosides; acglc, 6”-acetyl-glucoside; cfglc, 6”-caffeoyl-glucoside; cmglc, 6”-*p*-coumaroyl-glucoside; pydp, pyranodelphinidin; pycy, pyranocyanidin; pypt, pyranopetunidin; 10-HP, 10-(4‴-hydroxy)-phenyl.; M, myricetin; Q, quercetin; L, laricitrin; I, isorhamnetin; S, syringetin; glcU, glucuronide. *Positive ionization mode for anthocyanins and pyranoanthocyanins; negative ionization mode for the other phenolic compounds. **As malvidin 3,5-diglucosides equivalents (mv-3,5-diglc). ***As quercetin-3-glucoside equivalents (Q-3-glc). ****As caftaric acid. ND, not detected. Different low case letters in a line for the samples of different vintages (2011 and 2012) of each kind of sample (grape, skin, or lees) means significant differences (ANOVA, $p< 0.05$). Different capital or Greek letters for the three samples (grape, fermented skin, and lees) of each vintage (years 2011 or 2012, respectively) means significant differences (ANOVA, $p< 0.05$). Molar percentages given as mean value ± standard deviation (n=3).

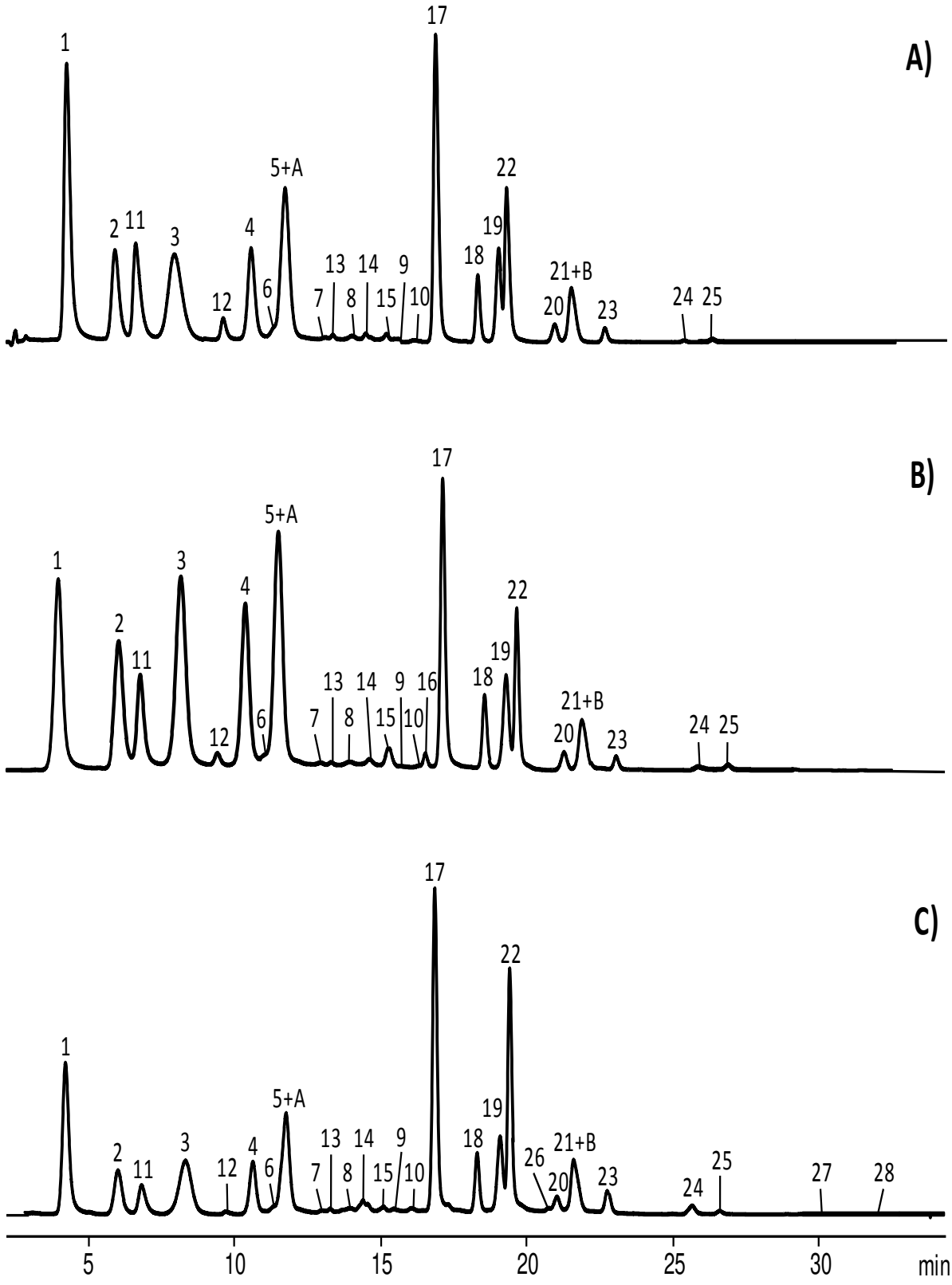

Figure 2.1. DAD-chromatograms corresponding to the anthocyanin profiles (detection at 520 nm), and identification of the peaks referred to Table 2.1, of BRS Violeta samples: A) grapes; (B) fermented skins, (C) wine lees.

The anthocyanin profile (molar percentage of individual anthocyanins) of BRS Violeta was dominated by diglucosides derivatives (ca. 80%) in two main forms: 3,5-diglucosides (3,5-diglc) and their *p*-coumaroylated derivatives, 3-(6''-coumaroyl)-glucoside-5-glucosides (3-cmglc-5-glc) of the anthocyanidins delphinidin, cyanidin, petunidin, peonidin, and malvidin (dp, cy, pt, pn, and mv, respectively). The occurrence of anthocyanidin diglucosides is characteristic of non-vinifera and hybrid (vinifera × non-vinifera) grape cultivars (Nixford et al., 2010). The main individual anthocyanins were dp-3,5-diglc (mean 16.47%) and dp-3cmglc-5glc (mean 16.93%). The acetyl and caffeoyl derivatives of 3,5-diglc (3-acglc-5-glc and 3-cfglc-5-glc, respectively) were also found in the minor concentracions (< 1 %). BRS Violeta also contained anthocyanin monoglucosides that are the only anthocyanins occurring in *Vitis vinifera* grape cultivars, and their *p*-coumaroylated derivatives (3-cmglc), but they occurred as minor compounds (< 10 % in total). The aforementioned results confirmed previous results obtained by our group about the anthocyanin composition of BRS Violeta grape (Rebello et al., 2013) and were in agreement with the anthocyanin composition showed by the *Vitis labrusca* grape cultivar Bordô, (predominance of anthocyanidin-3,5-diglc and anthocyanidin-3-cmglc-5glc) (Lago-Vanzela, et al., 2011a). The same anthocyanins were found in the grape and the two considered winemaking by-products (fermented skins and lees), with exception of the cy-3cfglc-5glc that was only detected in the fermented skins samples. However, differences in anthocyanin distribution (anthocyanin profile) were observed among the three types of samples. It is commonly accepted that the anthocyanin profile of a given variety is closely linked to its genetic heritage, although environmental factors may have some influence on this profile (Poudel, Mochioka, Beppu, & Kataoka, 2009). Therefore, differences between the anthocyanin profiles of grapes, fermented skins and lees should be likely attributable in

solid/liquid partition coefficients and solubility of the phenolics in the wine medium which modulates the anthocyanin transference from grape to wine.

Pyranoanthocyanins can only be formed from anthocyanidin-3-glucosides and they can be easily identified by their characteristic UV-vis and MS^n spectra (Nixford et al., 2010, Blanco-Vega, López-Bellido, Alía-Robledo, & Hermosín-Gutiérrez, 2011). In red wine made from *Vitis vinifera* grape cultivars pyranoanthocyanins are early formed in the first steps of fermentation, mainly derived from the reaction of anthocyanins with pyruvic acid and acetaldehyde, two yeast intermediate metabolites (Blanco-Vega et al., 2011). These compounds are known as vitisin-like pyranoanthocyanins. Therefore, it is not surprising to find such a kind of pyranoanthocyanins in winemaking by-products as been reported for Sicilian grape cultivar Nerello Mascalese grape marc (Amico, Napoli, Renda, Ruberto, Spatafora, & Tringali, 2004). However, vitisin-like pyranoanthocyanins were not detected in any of the samples of winemaking by-products, very likely due the low probability of their formation because of the low contribution of anthocyanidin-3-glucosides to the anthocyanin profile of BRS Violeta or even they were formed but mainly remained dissolved in wine. Hydroxyphenyl-pyranoanthocyanins are other type of pyranoanthocyanins and they derive from the reaction of anthocyanidin-3-glucosides with free hydroxycinnamic acids or their decarboxylation products (Blanco-Vega et al., 2011). In that case, it was possible to identify trace amounts (< 0.4 %) of three hydroxyphenyl-pyranoanthocyanins derived from *p*-coumaric acid (10-hydroxyphenyl-pyranoanthocianins, 10-HP-pyant), but only in lees: 10-HP-pydp-3glc, 10-HP-pycy-3cmglc, 10-HP-pypt-3cmglc. Maybe they were formed during wine elaboration and further adsorbed on the lees (Morata et al., 2005).

The anthocyanin profile of BRS Violeta grape showed significant differences among the two vintages were detected for some of the minor or very minor contributors. Thus, grapes from vintage of 2012 showed slightly lower proportions of cy-3,5-diglc (5.68%, vs. 6.68% in 2011), pn-3,5-diglc (6.08%, vs. 6.68% in 2011), pn-3acglc-5-glc (0.01% vs. 0.02% in 2011), and mv-3acglc-5-glc (0.04% vs. 0.06% in 2011), together with slightly higher proportions of mv-3cmglc-5-glc (2.46% vs. 2.14% in 2011) and cy-3cmglc (1.64% vs. 1.43% in 2011). In addition, grapes from 2012 accounted for lower total amount of anthocyanins (23.90 g/kg, vs. 31.01 g/kg in 2011). On one hand, the latter results support the commonly accepted suggestion of usefulness of anthocyanin profiles for varietal characterization purposes, because of the slight variations observed among vintages. On the other hand, the vintage effect (mostly climatological effects) is clearly demonstrated in the differences observed in total amount of anthocyanins accumulated in grapes.

The anthocyanin transference from grape to wine is rather limited, and values lower than 40% has been suggested (Boulton, 2001). Therefore, most of grape anthocyanins will remain in grape marc, and even the adsorption of anthocyanins on lees have been previously reported (Morata et al., 2005). The fermented skins separated from grape marc still retained important amounts of anthocyanins and the content was higher in 2012 (76.48 g/kg) vs. 2011 (57.74 g/kg) when the initial grapes accounted for less total anthocyanins. It is problaby due to the fact that anthocyanin transference during winemaking is not a simple partition process and other physic-chemical processes have been suggested to modulate such transference, for instance co-pigmentation (Boulton, 2001). Our results were in agreement with those reported by Kammerer, Claus, Carle and Shieber (2004), who found variations among years in the anthocyanin content of grape marc from Cabernet Mitos

(38% of the total amounts of 2002 were found in the skins of 2001), Spätburgunder, and Trollinger grape varieties. In contrast, lees from two vintages accounted for not significantly different content of anthocyanins (average of 19.09 g/kg), very likely because of the only involved process was adsorption on yeast cell wall and the same yeast strain and doses was used in both vintages.

Flavonols detected in BRS Violeta grape and its winemaking by-products (**Table 2.1, and Figure 2.2**) derived from five of the six flavonoid structures commonly reported for *Vitis vinifera* and non-vinifera (and also their hybrids) grape cultivars (Hermosín-Gutiérrez, Castillo-Muñoz, Gómez-Alonso, & García-Romero, 2011, Lago-Vanzela, Da-Silva, Gomes, García-Romero, & Hermosín-Gutiérrez, 2011b): the B-ring di-substituted quercetin (Q) and isorhamnetin (I); and the B-ring tri-substituted myricetin (M), laricitrin (L), and syringetin (S). None kaempferol (B-ring mono-substituted flavonoid structure) derivative was detected. BRS Violeta grape flavonols occurred mainly as 3-glycosides (3-glucosides for all aforementioned flavonoid structures and also 3-glucuronides for myricetin and quercetin) and a lower proportion of free myricetin was also observed.

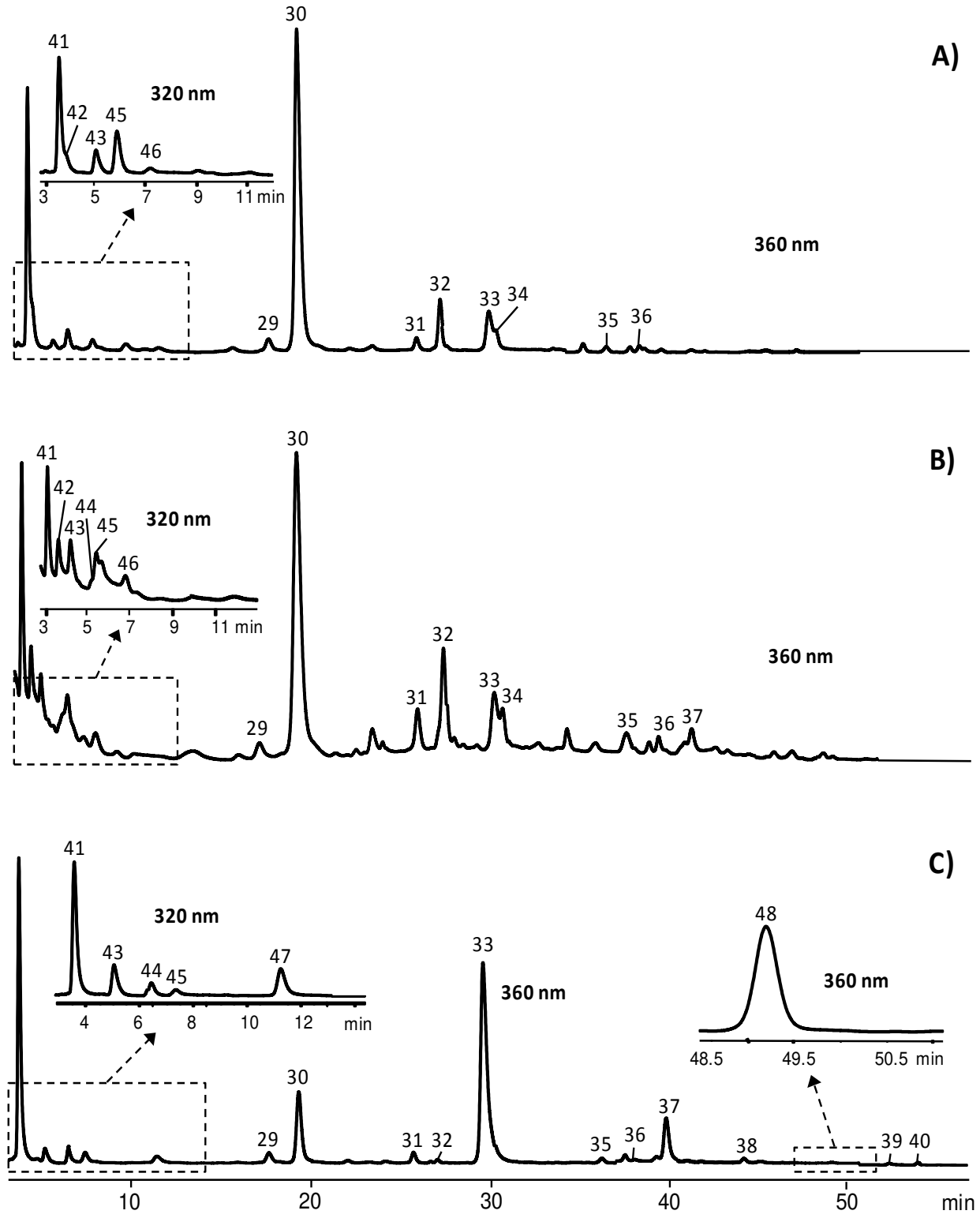

Figure 2.2. DAD-chromatograms corresponding to the profiles of flavonols (detection at 360 nm) and hydroxycinnamic acid derivatives (figure enlargements with detection at 320 nm), and identification of the peaks referred to Table 2.1, of BRS Violeta samples: A) grapes; (B) fermented skins, (C) wine lees.

The lack of kaempferol-based flavonols in BRS Violeta grape had been previously observed by our group together with the occurrence lower proportions of 3-galactoside derivatives of myricetin and quercetin (less than 2 % each). However, it was not detected in this work (Rebello et al., 2013). The same flavonols were found in BRS Violeta winemaking by-products together with higher proportions of free aglycons, especially in wine lees, which could be due to the hydrolysis of flavonol-3-glycosides during winemaking (Castillo-Muñoz et al., 2009, Hermosín-Gutiérrez et al., 2011). In the case of wine lees it was also observed the absence of laricitrin-3-glucoside. In all samples, myricetin-based flavonols were predominant: total sum of around 84% in grapes, 76-79% in fermented skins, and 84-85% in lees. Quercetin-type were the second type of more accounting flavonols (total sum of 9-18%), followed by laricitrin- (< 4.5%), isorhamnetin- (< 2.8%), and syringetin-based (< 1.5%) flavonols.

Flavonol profiles are also considered a varietal characteristic for grapes (Hermosín-Gutiérrez et al., 2011). Results showed only significant differences in the molar percentages of myricetin-3-glucoside, free myricetin, and laricitrin-3-glucoside of grape samples from vintages of 2011 and 2012 (**Table 2.1**). Usually, grape samples only contain flavonol-3-glycosides and the occurrence of free flavonol aglycons is considered an artifact formed during the extraction in acidic conditions (Hermosín-Gutiérrez et al., 2011). Moreover, this differences disappeared when total sum of myricetin-3-glucoside and free myricetin was considered (84.13 and 84.16%, respectively, for 2011 and 2012 samples). In contrast, more significant differences in flavonol profiles were found between winemaking by-product samples from the two vintages and among the three types of samples of the same vintage. In this case, the observed differences could be related, at least, with three factors: the

already mentioned hydrolysis of flavonol-3-glycosides during winemaking; the higher polarity of myricetin-based compounds (considering similar flavonol structures, as their 3-glucosides or the free aglycons, myricetin-based flavonols eluted first in reversed-phase chromatographic columns); and the very low solubility of released free aglycons with regards to their respective 3-glycosides (Boulton, 2001). The two latter reasons could explain why fermented skins diminished the proportion of myricetin-based flavonols when compared to grapes, which could be a result of their easier transference from grape to wine during maceration phase of winemaking. In addition, it also could explain why lees contained higher proportions of free flavonol aglycons, as a result of their precipitation concurrent with sedimentation of lees. Finally, differences in flavonol profiles of fermented skins and wine lees according to vintage year could be a combination of the aforementioned factors.

Regarding the total content of flavonols, significant variations between different vintage years were observed for fermented skin and wine lees, but not for grapes. The biosynthesis of grape flavonols and other flavonoids, as anthocyanins, is under genetic control but the expression of the involved genes is modulate by external, mainly agronomical (e.g., canopy management or irrigation) and climatic (e.g., temperature or sunlight exposition), factors (Poudel et al., 2009). It has been found that anthocyanin content in grapes greatly differ between vintages 2011and 2012. One important factor modulating anthocyanin biosynthesis is temperature. However, flavonol biosynthesis is strongly affected by sunlight exposure and the variation in its total content among vintages does not necessarily have to follow the same way than anthocyanins. Winemaking by-products, especially wine lees, revealed as important sources of flavonols because it

contained higher amounts than in grapes: average of 2394 mg/kg for wine lees; 1704 mg/kg for fermented skins; and 911 mg/kg for grapes, expressed as quercetin-3-glucoside.

Hydroxycinnamic acids derivatives (HCAD) and their chromatographic and spectral characteristics are presented in **Table 2.1, and Figure 2.2**. The expected hydroxycinnamoyl-tartaric acids, which are also known as caftaric (from caffeic acid), coutaric (from *p*-coumaric acid), and fertaric (from ferulic acid) acids were found in grapes, together with a glucose ester of caffeic acid, previously reported in Bordô grape (Lago-Vanzela et al., 2011a) and also found in a previous study with BRS Violeta grape (Rebello et al., 2013). The aforementioned HCAD were also detected in fermented skins and wine lees, with the exception of glucose ester of caffeic acid and *cis*-fertaric acid in wine lees. It is already known that hydroxycinnamoyl-tartaric acids can be hydrolyzes during winemaking process and released free hydroxycinnamic acids, which can also react with ethanol to form ethyl esters. Therefore, free caffeic acid was found in fermented skins and wine lees samples, together with free *p*-coumaric acid and ethyl *p*-coumarate in the case of wine lees samples. In all samples, caffeic acid derivatives were predominant and significant differences were found between samples of the same type but from different vintages and also among samples of different type from the same vintage. Quantitatively, wine lees accounted for the highest contents of HCAD, with values of one order of magnitude higher than those of the other samples: average of 4113 mg/kg, as caftaric acid, in wine lees, vs. 835 and 718 mg/kg in grape and fermented skins, respectively.

Finally, grape stilbenes usually can be analyzed in the same DAD-chromatograms at 320 nm obtained for DAHC analysis. However, in the case of samples BRS Violet in this study, these compounds showed concentrations below the limit of quantification. The same

was observed in the work of Rebello et al. (2013), but these authors analyzed estibenes using another methodology and observed that the grape has BRS Violet stilbenes isomers as piceid, and low concentration because it shows 0.058 ± 0.040 (*trans* isomer), and 0.080 ± 0.042 mg/kg fresh sample (*cis* isomer).

The application of Principal Components (PC) analysis to the matrix data comprised by the anthocyanin composition of all the freeze-dried samples (grapes, skins, and lees) allowed better visualization of main affinities and differences among samples and their relationships with anthocyanin composition. Grape and fermented skin samples were separated from lees samples along PC-1 (explaining 58.73% of total variance) axe, whereas grape samples separated from fermented skin samples along PC-2 (explaining 21.34% of total variance) axe (**Figure 2.3A**). Thus, the three kinds of samples were well separated in differentiated groups and even fermented skin samples showed some differentiation with regards to vintage year, as previously suggested their anthocyanin profiles shown in **Table 2.1**. The main features of the anthocyanin profiles that contributed to this differentiation were (**Figure 2.3B**): grape samples accounted for the highest proportions of non-acylated anthocyanidin-3-glucosides, the main non-acylated anthocyanidin-3,5-diglucosides (dp-3,5-diglc), and some minor acetylated 3,5-diglucosides; fermented skin samples contained higher proportions of the rest of non-acylated anthocyanidin-3,5-diglucosides and some of their acylated derivatives; finally, the highest proportions of acylated anthocyanidin-3,5-diglucosides, especially the *p*-coumaroylated derivatives, occurred in lees samples, in agreement with reported strong adsorption of *p*-coumaroylated anthocyanins on yeast cell wall in *Vitis vinifera* wine lees (Morata et al., 2005).

The Principal Component (PC) analysis of flavonol and hydroxycinnamic acid

derivatives data was able to clearly differentiate among samples of grape, fermented skins, and wine lees, and even between vintage year for the latter samples (**Figure 2.3C**). PC-1 explained the 68.11% of total variance, whereas PC-2 contributed with the 19.66% of total explained variance. Wine lees were separated, along the PC-1 axe, from the rest of samples mainly by their higher proportions of free myricetin and, in a lesser extent, by higher percentages of *trans*-caftaric and *trans*-coutaric acids (**Figure 2.3D**). It also showed enough different proportions of free myricetin, and correspondingly in their proportions of myricetin-3-glucoside, to be differentiate by vintage year.

Fermented skin samples were separated from grape samples due to the higher percentages of 3-glucuronide derivatives and 3-glucosides of isorhamnetin and syringetin (PC-1 axe), as well as by lower percentages of myricetin-3-glucoside and *trans*-caftaric acid (PC-2).

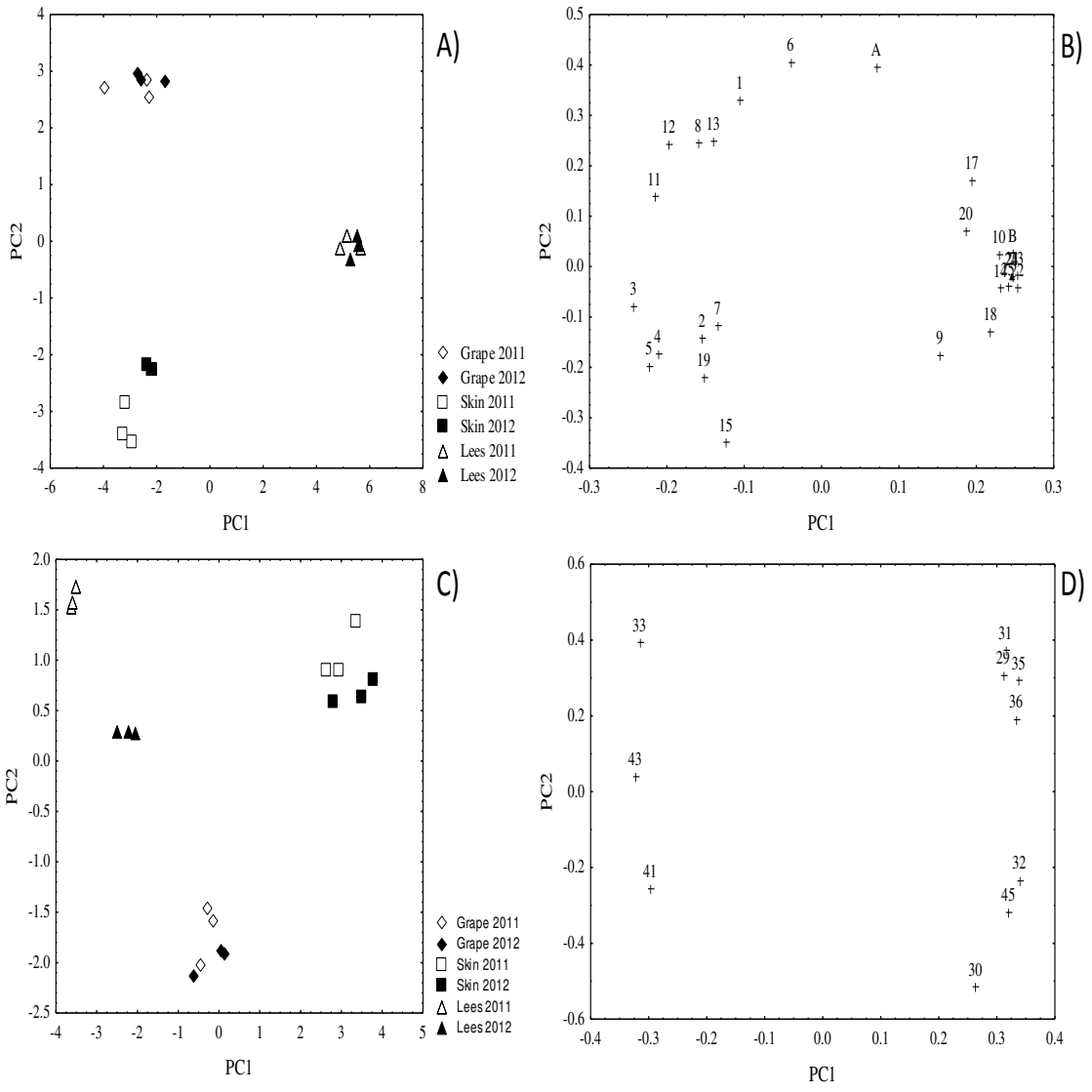

Figure 2.3. Principal Components (PC) analysis applied to the phenolic compound profiles of BRS Violeta samples. Plots on plane PC1 (factor 1) vs. PC2 (factor 2) corresponding to: A) anthocyanin profiles, different sample types (grapes, fermented skins, and wine lees) of two vintages (years 2011 and 2012); B) anthocyanin profiles, most correlated variables (identification of the number referred to Table 2.1); C) flavonols and hydroxycinnamic acid derivatives profiles, different sample types (grapes, fermented skins, and wine lees) of two vintages (years 2011 and 2012); D) flavonols and hydroxycinnamic acid derivatives profiles, most correlated variables (identification of the number referred to Table 2.1).

3.2 Effect of Drying Treatment on Phenolics Content of BRS Violeta Winemaking By-Products

Freeze-drying is considered a gentle drying technique because thermal degradation

is minimized. In fact, a significant of total anthocyanin content in fermented skins of BRS Violeta grape was lost when air-forced flow oven-drying at 50 ºC (D50) was used instead freeze-drying (FD): around 94% of total anthocyanins were lost, from 57.74 g/kg, in FD samples, to only 3.22 g/kg in D50 samples (**Table 2.2**). The loss of anthocyanin content after drying of fresh grape skins from *Vitis vinifera* grape varieties has been reported, the drop in anthocyanins being two-fold higher when oven-drying at 60 ºC for 24 h was used in comparison to freeze-drying (Torres, Díaz-Maroto, Hermosín-Gutiérrez, & Pérez-Coello, 2010). This loss of anthocyanins was accompanied by changes in the anthocyanin profile: most of delphinidin-based 3,5-diglucosides and 3-glucosides (non-methoxylated anthocyanins with double *o*-diphenol substitution pattern in the B-ring of flavonoid skeleton) decreased their molar proportions, whereas malvidin- and peonidin-based anthocyanins (methoxylated anthocyanins without *o*-diphenol substitution pattern in the B-ring of flavonoid skeleton), especially the non-acylated 3,5-diglucosides, increased their molar proportions.

Table 2.2. Molar profiles and total content of phenolic compounds identified in freeze-dried (FD), oven-dried at 50 °C (D50), and spray-dried (SD) samples of fermented skin and lees of BRS Violeta cultivar (only year 2011).

Phenolic compound	**FD skin (molar %)**	**D50 skin (molar %)**	**FD lees (molar %)**	**D50 lees (molar %)**	**SD lees (molar %)**
Anthocyanins and Pyranoanthocyanins					
total (g/kg sample)*	57.74±7.57 ^{A}	3.22±0.15 ^{B}	20.39±0.55 ^{b}	20.17±0.69 ^{b}	24.94±1.34 ^{a}
dp-3,5diglc	15.88±1.16 ^{A}	12.32±0.34 ^{B}	15.10±1.03 ^{a}	12.85±0.07 ^{b}	12.99±0.13 ^{b}
cy-3,5diglc	7.16±0.04 ^{B}	9.03±0.29 ^{A}	5.10±0.09 ^{a}	4.96±0.10 ^{a}	4.47±0.24 ^{b}
pt-3,5diglc	12.91±0.80	11.86±0.41	8.60±0.41	8.47±0.20	7.95±0.06
pn-3,5diglc	8.29±0.69 ^{B}	15.25±0.20 ^{A}	4.67±0.38	4.43±0.16	4.33±0.06

Continuation…

mv-3,5diglc	15.99±0.28 ^{B}	25.24±0.46 ^{A}	9.76±0.13 ^{a}	9.02±0.17 ^{b}	8.27±0.64 ^{b}
dp-3acglc-5glc	0.33±0.01 ^{B}	0.53±0.01 ^{A}	0.44±0.00 ^{a}	0.41±0.01 $^{a\,b}$	0.37±0.03 ^{b}
cy-3acglc-5glc	0.08±0.01 ^{B}	0.11±0.01 ^{A}	0.06±0.00 ^{b}	0.14±0.03 ^{a}	0.15±0.01 ^{a}
pt-3acglc-5glc	0.16±0.01	0.16±0.02	0.11±0.00 ^{b}	0.20±0.02 ^{a}	0.19±0.01 ^{a}
pn-3-acglc-5-glc	0.06±0.01	0.06±0.01	0.11±0.01 ^{a}	0.05±0.00 ^{b}	0.05±0.00 ^{b}
mv-3acglc-5glc	0.05±0.00 ^{B}	0.13±0.01 ^{A}	0.10±0.01 ^{a}	0.06±0.01 ^{b}	0.02±0.00 ^{c}
dp-3glc	4.01±0.26 ^{A}	1.87±0.12 ^{B}	2.88±0.13 ^{a}	2.38±0.08 ^{b}	2.57±0.16 $^{a\,b}$
cy-3glc	0.42±0.02 ^{A}	0.25±0.01 ^{B}	0.24±0.01 ^{a}	0.18±0.02 ^{b}	0.23±0.02 ^{a}
pt-3glc	0.33±0.01 ^{B}	0.53±0.01 ^{A}	0.77±0.01 ^{a}	0.71±0.01 $^{a\,b}$	0.65±0.05 ^{b}
pn-3glc	0.08±0.00 ^{B}	0.10±0.00 ^{A}	0.11±0.00	0.09±0.00	0.11±0.01
mv-3glc	0.23±0.02	0.26±0.01	0.66±0.02 ^{b}	0.79±0.04 ^{a}	0.63±0.03 ^{b}
dp-3cafglc-5glc	0.83±0.05	0.61±0.01	0.27±0.01 ^{a}	0.23±0.02 ^{b}	0.20±0.01 ^{b}
cy-3cafglc-5glc	0.54±0.01 ^{B}	1.48±0.01 ^{A}	ND	ND	ND
dp-3cmglc-5glc	13.56±0.35 ^{A}	4.73±0.05 ^{B}	19.99±2.06	20.77±0.17	20.67±0.17
cy-3cmglc-5glc	3.31±0.09 ^{B}	3.60±0.03 ^{A}	3.79±0.08 ^{b}	3.96±0.03 ^{a}	3.87±0.04 $^{a\,b}$
pt-3cmglc-5glc	4.54±0.69	4.54±0.04	3.71±0.36 ^{b}	6.17±0.23 ^{a}	6.16±0.12 ^{a}
pn-3cmglc-5glc	0.95±0.06 ^{B}	1.17±0.04 ^{A}	1.06±0.02 ^{b}	1.35±0.04 ^{a}	1.34±0.04 ^{a}
mv-3cmglc-5glc	2.19±0.11	2.10±0.08	3.35±0.23	3.46±0.02	3.37±0.07
dp-3cmglc	5.92±0.14 ^{A}	2.23±0.19 ^{B}	13.95±0.87 ^{b}	14.43±0.61 $^{a\,b}$	16.24±0.67 ^{a}
cy-3cmglc	1.33±0.06	1.34±0.05	2.24±0.15	2.30±0.01	2.24±0.05
pt-3cmglc	0.58±0.09 ^{A}	0.30±0.04 ^{B}	1.46±0.06	1.37±0.11	1.54±0.07
pn-3cmglc	0.12±0.01 ^{A}	0.10±0.004 ^{B}	0.84±0.06	0.72±0.08	0.86±0.10
mv-3cmglc	0.15±0.01	0.10±0.01	0.36±0.02	0.34±0.01	0.32±0.02
10-HP-pydp-3glc	ND	ND	0.16±0.00 ^{b}	0.18±0.02 $^{a\,b}$	0.21±0.02 ^{a}
10-HP-pycy-3cmglc	ND	ND	0.06±0.00	ND	ND
10-HP-pypt-3cmglc	ND	ND	0.06±0.00	ND	ND

Flavonols

Continuation…

total (mg/kg sample)**	1387.54±164.09 ^{A}	471.74±21.71 ^{B}	2824.62±122.81 ^{a}	2149.49±131.61 ^{b}	2787.87±226.51 ^{a}
M-3-glcU	2.97±0.04	ND	2.30±0.28	1.92±0.03	2.10±0.11
M-3-glc	63.02±1.18 ^{A}	49.41±0.83 ^{B}	17.24±0.29 ^{a}	18.23±0.47 ^{a}	15.61±0.87 ^{b}
Q-3-glcU	5.10±0.66 ^{B}	9.78±0.48 ^{A}	2.00±0.10	2.06±0.08	2.00±0.14
Q-3-glc	10.11±0.90 ^{B}	26.15±1.64 ^{A}	0.54±0.06	0.49±0.03	0.47±0.05
free M	9.99±1.06 ^{A}	1.64±0.15 ^{B}	65.18±0.36 ^{a}	62.93±0.64 ^{b}	65.09±0.46 ^{a}
L-3-glc	2.06±0.19 ^{B}	7.73±0.73 ^{A}	ND	ND	ND
I-3-glc	2.74±0.16 ^{A}	1.68±0.24 ^{B}	0.87±0.06 ^{b}	1.05±0.04 ^{a}	0.98±0.05 $^{a\,b}$
S-3-glc	1.29±0.17 ^{A}	0.74±0.04 ^{B}	0.17±0.03	0.16±0.02	0.19±0.01
free Q	2.73±0.30	2.87±0.16	10.19±0.31 ^{b}	11.67±0.14 ^{a}	12.13±0.57 ^{a}
free L	ND	ND	0.94±0.10	0.84±0.02	0.85±0.03
free I	ND	ND	0.24±0.01	0.27±0.06	0.21±0.01
free S	ND	ND	0.33±0.06	0.37±0.02	0.37±0.02
Hydroxycinnamic acid derivatives					
total (mg/kg sample)***	872.01±85.71 ^{A}	279.90±54.93 ^{B}	4379.73±140.68 ^{a}	3317.60±379.13 ^{b}	4340.85±118.29 ^{a}
trans-caftaric acid	40.37±0.42 ^{A}	14.40±0.24 ^{B}	50.91±1.96 $^{a\,b}$	46.19±3.67 ^{b}	54.18±3.12 ^{a}
trans-1-glc-caffeic acid	16.67±2.58 ^{B}	42.22±4.74 ^{A}	ND	ND	ND
trans-coutaric acid	10.45±2.22 ^{B}	32.14±2.84 ^{A}	14.97±0.40	14.18±0.54	13.97±2.16
trans-caffeic acid	2.94±0.26	ND	6.98±0.18 ^{b}	9.22±0.90 ^{a}	7.80±0.27 ^{b}
trans-fertaric acid	21.90±1.75 ^{A}	2.46±0.06 ^{B}	3.44±0.15	4.02±0.23	3.39±0.46
cis-fertaric acid	7.66±1.33	8.78±1.79	ND	ND	ND
p-coumaric acid	ND	ND	17.97±1.93 $^{a\,b}$	19.74±1.47 ^{a}	14.92±0.13 ^{b}
ethyl *p*-coumarate	ND	ND	5.74±0.33	6.65±0.64	5.74±0.15

Nomenclature abbreviations: dp, delphinidin; cy, cyanidin; pt, petunidin; pn, peonidin; mv, malvidin; glc, glucoside; diglc, diglucosides; acglc, 6”-acetyl-glucoside; cfglc, 6”-caffeoyl-glucoside; cmglc, 6”-*p*-coumaroyl-glucoside; pydo, pyranodelphinidin; pycy, pyranocyanidin; pypt, pyranopetunidin; 10-HP, 10-(4‴-hydroxy)-phenyl; M, myricetin; Q, quercetin; L, laricitrin; I, isorhamnetin; S, syringetin; glcU, glucuronide. *As malvidin 3,5-diglucosides equivalents (mv-3,5-diglc). **As quercetin-3-glucoside equivalents (Q-3-glc). ***As caftaric acid. ND, not detected. Different letters in the same row means significant differences (ANOVA, $p < 0.05$) among dried skin samples (capital letters) or wine lees samples (low case letters). Molar percentages given as mean value ± standard deviation (n=3).

In contrast, drying treatments did not affected so much the anthocyanin content on lees. Surprisingly, no significant differences were found between the total anthocyanin content of dried lees by freeze-drying (FD) and air-forced flow oven-drying at 50 ºC (D50). Moreover, the spray-drying treatment (SD) showed to significantly higher total anthocyanin content (24.94 g/kg, vs. 20.39 and 20.17 g/kg for FD and D50 treatments, respectively). With regard to anthocyanin profiles, the drying treatment caused some significant differences in many of these compounds. However, it was of little less important for anthocyanins (e.g., the molar proportions of dp-3,5-diglc were 15.10, 12.85, and 12.99% for FD, D50, and SD samples, respectively), and in some cases no significant differences were found (e.g., the molar proportions of dp-3cmglc-5-diglc were 19.99, 20.77, and 20.67% for FD, D50, and SD samples, respectively). Finally, it is remarkable that some of the very minor hydroxyphenyl-pyrananthocyanins found in FD lees samples were not detected in the respective D50 and SD samples.

Even that air-forced flow oven-drying at 50 ºC (D50) is a cheap drying process, the abovementioned results suggest that it is not indicated for drying of BRS Violeta fermented skin. In contrast, BRS Violeta wine lees can be effectively dried using D50 process, and the yield of anthocyanins can be slightly improved by spray-drying, a technique also cheaper than freeze-drying.

The effect of drying treatment on total content of both flavonols and hydroxycinnamic acid derivatives (DAHC) of fermented skins was showed a similar behavior than that observed for anthocyanins (**Table 2.2**): the air-forced flow oven-drying at 50 ºC (D50) caused a reduction of 66% of the total flavonol content and of 68% of the total DAHC content with regard to freeze-drying treatment (FD). The most affected

flavonols were those derived from myricetin and even M-3-glcU was not found in the D50 skin samples. Myricetin has a flavonoid structure with a double *o*-diphenol substitution pattern (B-ring tri-hydroxylated flavonoid) and seemed to be as labile as delphinidin-based anthocyanins (also B-ring tri-hydroxylated flavonoids) towards the D50 drying treatment.

In the case of HCAD, *trans*-caftaric and *trans*-fertaric acids were the compounds that disappear in greater proportions, although another caffeic-based DAHC, *trans*-1-glc-caffeic acid, was apparently not affected.

Wine lees did not affect so much by drying treatment. In contrast to that found for anthocyanins, FD and spray-drying (SD) treatments yielded similar results, higher in 24% than D50 treatment for total contents of both flavonols and HCAD. The reduction in flavonol concentration in D50 wine lees samples was due to a general loss of all kinds of compounds with only slight, but significant, differences. The situation was similar for HACD, although D50 showed lower proportions of caftaric acid and higher proportions of its hydrolysis product, caffeic acid.

Once again, the more expensive FD drying treatment seemed to be more suitable for fermented skin samples, in agreement with reported data (Torres et al., 2010), whereas SD yielded similar results than FD in wine lees samples and even the cheaper D50 treatment could be considered of interest for drying of wine lees samples.

3.3 Grape and Winemaking By-Products of BRS Lorena: Their Contents in Flavonols, Hydroxycinnamic Acid Derivatives (HCAD), and Stilbenes

BRS Lorena is a white grape variety. Therefore, it not contains anthocyanins and their flavonol profile showed the expected lack of B-ring tri-substituted flavonoid

structures, namely, myricetin, laricitrin, and syringetin (Castillo-Muñoz, Gómez-Alonso, García-Romero, & Hermosín-Gutiérrez, 2010, Hermosín-Gutiérrez et al., 2011, Lago-Vanzela et al., 2011b). Only flavonol-3-glycosides were detected in grape samples (**Table 2.3, and Figure 2.4**): the 3-glucosides of kaempferol, quercetin and isorhamnetin; the 3-galactosides and 3-glucuronides of kaempferol and quercetin; the 3-rutinoside of quercetin; and the recently reported 3-rhamnoside of quercetin (García-Romero, Castillo-Muñoz, Mena-Morales, Gómez-Alonso, & Hermosín-Gutiérrez, 2012). In addition, free aglycons (free kaempferol, quercetin, and isorhamnetin) were also detected in non-fermented skins and wine lees samples, usually as the main compounds, especially in the case of wine lees. Quercetin-type was the predominant flavonols in grape samples but their total contribution (95.13% in 2011 and 91.58%) and, especially, the individual percentages showed significant differences between years (**Table 2.3**). As previously reported, the flavonol profile is considered a varietal characteristic of grapes (Hermosín-Gutiérrez et al., 2011) but it has also been reported as associated to the ripening degree of a grape variety, especially if it is not at a complete mature degree (Castillo-Muñoz et al., 2009). Nevertheless, the average flavonol profile shown by BRS Lorena resembles with another hybrid grape variety also developed by the same Brazilian institution (Embrapa Uva e Vinho). It is called BRS Clara (Lago-Vanzela et al., 2011b) and the chromatographic flavonol profile has as major compounds by Q-3-glcU (41.66-58.01%, vs. 47.59% in BRS Clara) and followed in importance by Q-3-glc (29.57-42.61%, vs. 35.64% in BRS Clara). Quercetin-type flavonols are also dominant in the flavonol profiles of non-fermented skins (89.44 and 93.44% in 2011 and 2012) and wine lees (94.94%). Significant differences were found among flavonol profiles of grapes, non-fermented skins and wine lees, but in now it could be explained on the basis of similar arguments given for red grape BRS Violeta: differences in

partition coefficients during the usually short duration pressing step of white wine elaboration, for which it is not common a maceration phase of grape must with non-fermented skins; the occurrence of hydrolysis of flavonol-3-glycosides in non-fermented skins after pressing and storage, and also in white wine until lees separation; the less solubility in white wine of released free flavonol aglycons. As a result of the aforementioned causes, non-fermented skins showed high proportion of free flavonol aglycons, 55.29% in 2011 and 34.06% in 2012, whereas wine lees flavonols were mainly composed of free quercetin (85.05%) together with kaempferol (5.06%).

Regarding hydroxycinnamic acid derivatives (HCAD) composition, the BRS Lorena grape showed a great variability among vintage years, and also among the type of sample considered (**Table 2.3**). As expected grape berries contained hydroxycinnamoyl-tartaric acids, as the only class of phenolic acids. Samples from year 2012 had lower content of HCAD than in the previous year (**Table 2.3**). As well as the behavior content. However, *trans*-coutaric acid accounted for the half of HCAD in 2011 but only for 34.11% in 2012. In contrast, *trans*-caftaric acid accounted for 39.95% in 2011 and an appreciable proportion of GRP (7.37%), its reaction product with gluthation after oxidation, was detected (Cejudo-Bastante, Hermosín-Gutiérrez, & Pérez-Coelho, 2011); in year 2012, the proportion of *trans*-caftaric acid was significantly lower (32.26%) and a great proportion of GRP was found (29.42%). A similar situation occurred in non-fermented skin samples. Thus it is possible to perceive a significant decrease in the total amount of HCAD at 2011 (53.10 mg/kg) vs. 2012 (603.39 mg/kg), especially by the marked decrease in the proportions of trans-coutaric acid and, trans-caftaric acid. Consequently proportions of GRP and trans-fertaric acid were increased. The aforementioned differences could be likely related with

the polyphenoloxidase activity present in grape and, especially, crushed non-fermented skins, which could be responsible of important destruction of the potential content of HCAD. In the case of wine lees, they retained relevant amounts of HCAD (420.31 mg/kg), mainly composed by caffeic acid derivatives (ca. 75%): 24.73% of original *trans*-caftaric acid from grape; 26.38% of its oxidation product (GRP); 17.88% of the hydrolysis product (free caffeic acid); and 5.79% of ethyl ester of caffeic acid. Wine lees also contained free *p*-coumaric acid (only 3.16%), but its ethyl ester was not detected.

Resveratrol-based stilbenes were detected in low and variable amounts in some of the BRS Lorena grape berries and 2012 skins (**Table 2.3**). However, it was not detected in wine lees and non-fermented skins from 2011. The detection of such compounds was possible because of the simplicity of the respective chromatograms in the time frame the eluted. In the case of red grape BRS Violeta, in this frame zone also eluted flavonols and residual anthocyanins making difficult a suitable separation and identification of stilbenes of interest.

Table 2.3. Flavonols, hydroxycinnamic acid derivatives, and stilbenes identified in freeze-dried samples of BRS Lorena grapes, their skins and lees, in years 2011 and 2012: chromatographic and mass spectral characteristics (negative ionization mode).

peak	Phenolic compound	R_t (min)	molecular ion; product ions (*m/z*)	grape 2011 (molar %)	grape 2012 (molar %)	skin 2011 (molar %)	skin 2012 (molar %)	lees 2012 (molar %)
	Flavonols							
	total (mg/kg sample)*			145.42±8.02 $^{a B}$	100.67±5.84 $^{b \beta}$	297.56±17.70 ^{A}	310.66±22.85 $^{\alpha}$	377.60±53.22 $^{\alpha}$
1	Q-3-gal	25.45	463; 301	2.24±0.23 $^{b A}$	3.69±0.46 $^{a \beta}$	0.71±0.04 $^{b B}$	1.75±0.07 $^{a \gamma}$	6.84±0.45 $^{\alpha}$
2	Q-3-glcU	25.93	477; 301	58.01±0.45 $^{a A}$	41.66±0.44 $^{b \alpha}$	14.77±0.62 $^{b B}$	33.01±0.73 $^{a \beta}$	1.36±0.24 $^{\gamma}$
3	Q-3-glc	27.26	463; 301	29.57±0.67 $^{b A}$	42.61±0.25 $^{a \alpha}$	19.63±0.48 $^{b B}$	22.24±0.13 $^{a \beta}$	1.69±0.34 $^{\gamma}$
4	Q-3-rut	27.90	609; 301	4.28±0.26 ^{a}	2.67±0.19 $^{b \beta}$	4.16 ±0.15 ^{a}	3.31±0.15 $^{b \alpha}$	ND
5	K-3-gal	30.57	447; 285	0.77±0.10 ^{b}	1.39±0.16 $^{a \alpha}$	0.81±0.06	0.90±0.03 $^{\beta}$	ND
6	Q-3-rha	32.30	447; 301	1.03±0.06 ^{A}	0.95±0.09 $^{\alpha}$	0.82±0.04 $^{a B}$	0.57±0.06 $^{b \beta}$	ND
A	K-3-glcU	32.30	461; 285	0.52±0.02 $^{a A}$	0.37±0.07 $^{b \alpha}$	0.35±0.02 $^{a B}$	0.25±0.03 $^{b \beta}$	ND
7	K-3-glc	33.41	447; 285	3.07±0.26 $^{b A}$	5.84±0.42 $^{a \alpha}$	2.53±0.12 $^{b B}$	3.21±0.04 $^{a \beta}$	ND
8	I-3-glc	36.48	477; 315	0.52±0.14 $^{b B}$	0.84±0.05 $^{a \alpha}$	0.91±0.04 $^{a A}$	0.42±0.02 $^{b \beta}$	ND
9	free Q	40.21	301	ND	ND	49.35±1.64 ^{a}	32.56±0.70 $^{b \beta}$	85.05±0.83 $^{\alpha}$
10	free K	48.83	285	ND	ND	4.58±0.20 ^{a}	1.25±0.03 $^{b \beta}$	5.06±0.95 $^{\alpha}$
11	free I	52.65	315	ND	ND	1.36±0.13 ^{a}	0.25±0.05 ^{b}	ND
	Hydroxycinnamic acid derivatives							
	total (mg/kg sample)**			372.21±28.76 $^{a A}$	169.85±6.75 $^{b \gamma}$	53.10±4.64 $^{b B}$	603.39±29.54 $^{a \alpha}$	420.31±19.54 $^{\beta}$
12	GRP	3.41	616	7.37±1.70 $^{b B}$	29.42±2.44 $^{a \alpha}$	47.11±0.74 $^{a A}$	2.33±0.06 $^{b \beta}$	26.38±2.88 $^{\alpha}$
13	*trans*-caftaric acid	3.66	311; 179, 149	39.95±1.85 $^{a A}$	32.26±2.64 $^{b \alpha}$	3.01±0.05 $^{b B}$	36.87±0.14 $^{a \alpha}$	24.73±2.74 $^{\beta}$
14	*trans*-coutaric acid	5.13	295; 163, 149	50.13±1.47 $^{a A}$	34.11±0.45 $^{b \beta}$	19.71±0.34 $^{b B}$	58.13±0.14 $^{a \alpha}$	23.47±2.11 $^{\gamma}$
15	*trans*-caffeic acid	5.30	179	ND	ND	ND	ND	17.88±3.22
16	*trans*-fertaric acid	7.36	325; 193, 149	0.68±0.18 $^{b B}$	1.68±0.27 $^{a \beta}$	30.18±0.49 $^{a A}$	0.96±0.03 $^{b \beta}$	4.38±0.47 $^{\alpha}$

Continuation…

17	*cis*-fertaric acid	9.30	325; 193, 149	1.88±0.17 ^{b}	2.53±0.15 $^{a\,\alpha}$	ND	1.71±0.03 $^{\beta}$	ND
18	*p*-coumaric acid	11.07	163	ND	ND	ND	ND	3.16±0.66
19	ethyl caffeate	39.90	207; 179	ND	ND	ND	ND	5.79±0.56
	Stilbenes							
	total (mg/kg sample)***			35.32±0.12 ^{a}	0.89±0.13 $^{b\,\beta}$	ND	16.15±1.47 $^{\alpha}$	ND
20	*trans*-piceid	17.80	389;227	39.24±3.72 ^{a}	48.82±1.79 $^{b\,\alpha}$	ND	44.71±3.43 $^{\alpha}$	ND
21	*trans*-resveratrol	29.00	227	53.42±4.35 ^{a}	41.97±1.34 $^{b\,\alpha}$	ND	48.99±2.69 $^{\beta}$	ND
22	*cis*-piceid	30.60	389;227	7.33±0.95 ^{b}	9.21±0.61 $^{a\,\beta}$	ND	6.31±0.77 $^{\alpha}$	ND

Nomenclature abbreviations: M, myricetin; Q, quercetin; L, laricitrin; I, isoramnetin; S, syringetin; glcU, glucuronide; glc, glucoside; rha, rhamnoside; rut, rutinoside (6''-rhamnosylglucoside); GRP, Grape Reaction Product (2-*S*-glutathionyl-*trans*-caftaric acid). ND, not detected. *As quercetin-3-glucoside equivalents (Q-3-glc). **As caftaric acid. ***As resveratrol equivalents. Different low case letters for the samples of different vintages (2011 and 2012) of each kind of sample (grape, skin, or lees) means significant differences (ANOVA, $p< 0.05$). Different capital or Greek letters for the three samples (grape, fermented skin, and lees) of each vintage (years 2011 or 2012, respectively) means significant differences (ANOVA, $p< 0.05$). Molar percentages given as mean value ± standard deviation (n=3).

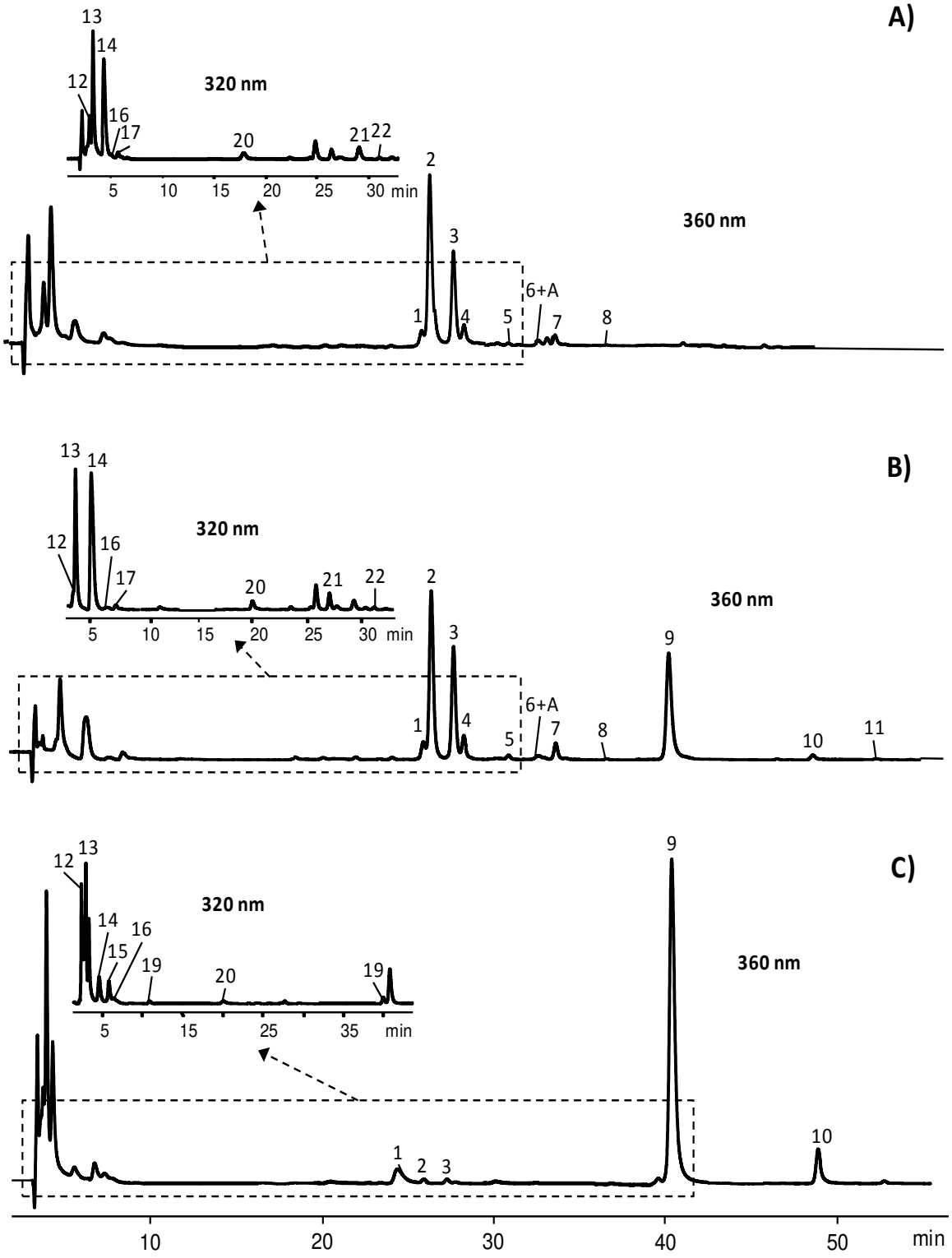

Figure 2.4. DAD-chromatograms corresponding to the profiles of flavonols (detection at 360 nm), hydroxycinnamic acid derivatives and stilbenes (figure enlargements with detection at 320 nm), and identification of the peaks referred to Table 2.2, of BRS Lorena samples: A) grapes; (B) skins, (C) wine lees.

All the above mentioned differences discussed on the basis of ANOVA results for DAHC and stilbene composition of BRS Lorena samples were confirmed by a Principal Component (PC) analysis (**Figure 2.5**), PC-1, PC-2 and PC-3 explaining 60.15 , 27.08 and 11.04% of total variance, respectively. The higher molar percentages of *trans*-fertaric acid and GRP of skin samples of year 2011 contributed to their separation from skin samples of year 2012, characterized by higher proportions of both *trans*-coutaric and *trans*-caftaric acids. Wine lees separated from the rest by percentages of Q-3-gal that doubled those of other samples. Grape samples from different vintages could be separated by differences in molar percentages of Q-3-glcU and Q-glc, as well as GRP.

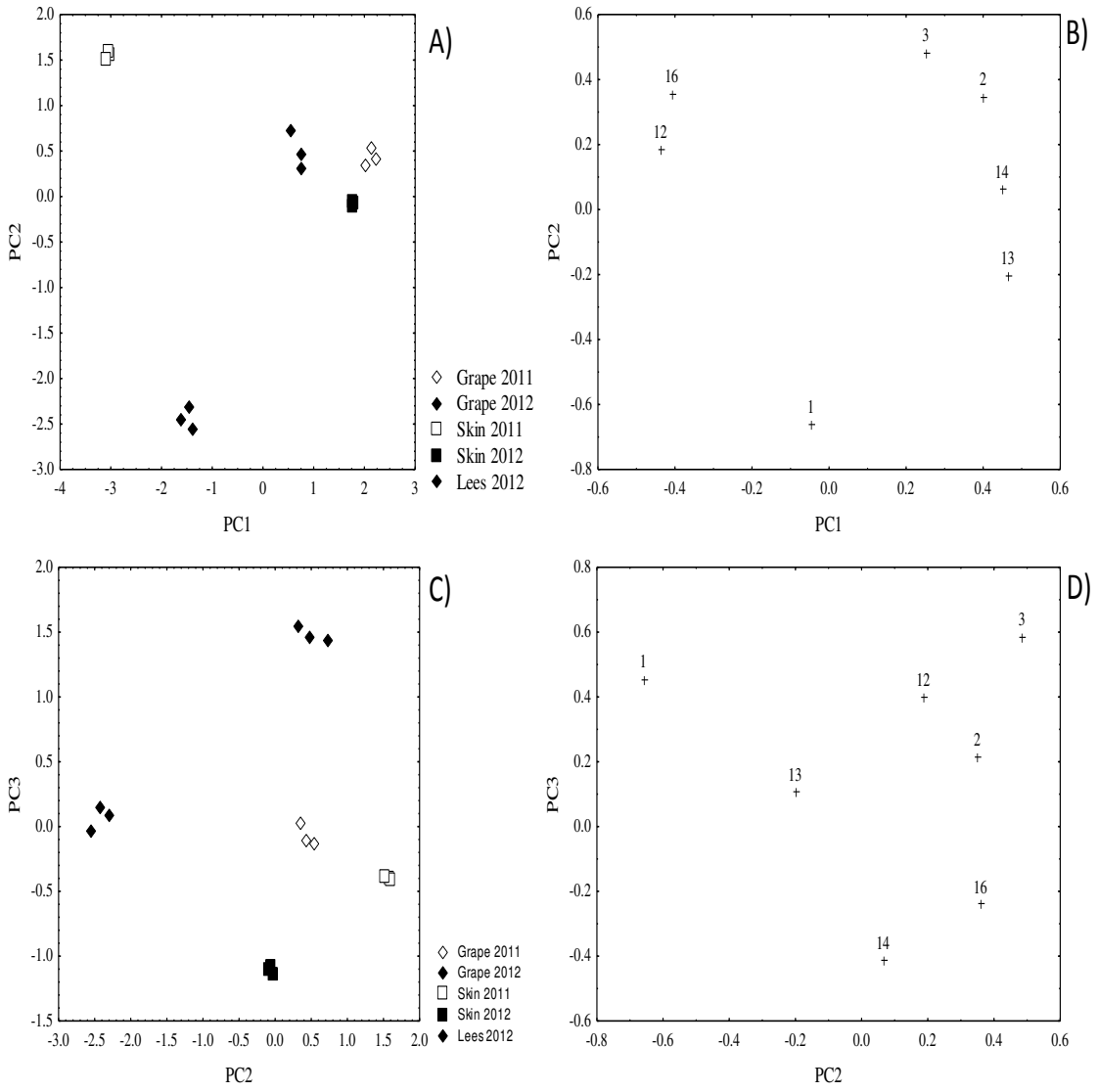

Figure 2.5. Principal Components (PC) analysis applied to the flavonols and hydroxycinnamic acid derivatives profiles of BRS Lorena samples. Plots of: A) different sample types (grapes, skins, and wine lees) of two vintages (years 2011 and 2012; for lees, only year 2011) on plane PC1 (factor 1) vs. PC2 (factor 2); B) most correlated variables with PC1 and PC2 (abbreviations like in Table 2.2); C) different sample types (grapes, skins, and wine lees) of two vintages (years 2011 and 2012; for lees, only year 2011) on plane PC2 (factor 2) vs. PC3 (factor 3); B) most correlated variables with PC2 and PC3 (abbreviations like in Table 2.2).

3.4 Effect of Drying Treatment on Flavonols, Hydroxycinnamic Acid Derivatives (HCAD), and Stilbenes Content of BRS Lorena Winemaking By-Products

Finally, the drying treatment applied to non-fermented skins of BRS Lorena did not change the total content of flavonols and only induced slight changes in their flavonol profile (mainly a higher degree of hydrolysis of quercetin-3-glucosides was observed in D50 treatment), whatever freeze-drying (FD) or air-forced flow oven-drying at 50 ºC (D50) conditions were used (**Table 2.4**). In contrast, the D50 treatment caused the total disappearance of the low content of stilbenes that remained in FD samples.

Table 2.4. Molar profiles and total content of flavonols, hydroxycinnamic acid derivatives, and stilbenes identified in freeze-dried (FD) and oven-dried at 50 °C (D50) samples of skin of BRS Lorena (only year 2011).

Phenolic compound	FD skin (molar %)	D50 skin (molar %)
Flavonols		
total (mg/kg sample)*	297.56±17.70	283.16±11.72
Q-3-gal	0.71±0.04[b]	1.58±0.21[a]
Q-3-glcU	14.77±0.62[a]	10.62±0.47[b]
Q-3-glc	19.63±0.48[a]	16.28±0.71[b]
Q-3-rut	4.16±0.15[a]	3.15±0.06[b]
K-3-gal	0.81±0.06	ND
Q-3-rha	0.82±0.04	0.77±0.03
K-3-glcU	0.35±0.02	0.33±0.01
K-3-glc	2.53±0.12[a]	2.13±0.15[b]
I-3-glc	0.91±0.04[a]	0.82±0.02[b]
free Q	49.35±1.64[b]	58.38±1.24[a]
free K	4.58±0.20	4.51±0.13
free I	1.36±0.13	1.42±0.06
Hydroxycinnamic acid derivatives		
total (mg/kg sample)**	53.10±4.64	59.05±4.50
GRP	47.11±0.74	50.29±2.42
trans-caftaric acid	3.01±0.05	3.21±0.15
trans-coutaric acid	19.71±0.34[b]	21.01±0.49[a]
trans-caffeic acid	ND	ND
trans-fertaric acid	30.18±0.49[a]	25.49±2.29[b]
cis-fertaric acid	ND	ND
p-coumaric acid	ND	ND
ethyl caffeate	ND	ND
Stilbenes		
total (mg/kg sample)***	35.27±0.12	ND
trans-piceid	39.24±3.72	ND
trans-resveratrol	53.42±4.35	ND
cis-piceid	7.33±0.95	ND

Nomenclature abbreviations: M, myricetin; Q, quercetin; L, laricitrin; I, isorhamnetin; S, syringetin; glcU, glucuronide; glc, glucoside; rha, rhamnoside; rut, rutinoside (6''-rhamnosylglucoside); GRP, Grape Reaction Product (2-*S*-glutathionyl-*trans*-caftaric acid); CAFT, caftaric acid; caff, caffeic acid; COUT, coutaric acid; *p*-coum, *p*-coumaric acid; FERT, fertaric acid. ND, no detected. *As quercetin-3-glucoside equivalents (Q-3-glc). **As caftaric acid. ***As resveratrol equivalents. Different letters in the same row means significant differences (ANOVA, $p<0.05$). Molar percentages given as mean value ± standard deviation (n=3).

4 CONCLUSIONS

The anthocyanins found in grapes and winemaking by-products (skin and lees) of BRS Violeta are mainly delphinidin and malvidin, as 3,5-diglucosides and 3-cmglc-5glc derivatives. Analysis by HPLC-DAD-ESI-MS/MS allowed the identification of over 23 anthocyanins and 3 pyranoanthocyanins, the latter compounds developed during the fermentation occurring in the presence lees. The presence of hydroxycinnamic acid and flavonols were also reported in this paper in entire grape berries before processing and also in by-products of winemaking, in this case skin and lees. The grape and winemaking by-products BRS Lorena had 12 flavonols, 8 hydroxycinnamic acid derivatives and 3 stilbenes in the polyphenolic composition.. From the values determined in the BRS Violeta and BRS Lorena, it is concluded winemaking by-products exhibit important amounts of these bioactive compounds (especially in the case of BRS Violeta) as a potential functional component to be used in industrial applications.

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6 REFERENCES

Abe, L. T., Mota, R. V., Lajolo, F. M., & Genovese, M. I. (2007). Compostos fenólicos e capacidade antioxidante de cultivares de uvas *Vitis labrusca* L. e *Vitis vinifera* L. *Ciência e Tecnologia de Alimentos* [online], *27*, 394-400.

Amico, V., Napoli, E.M., Renda, A., Ruberto, G., Spatafora, C., & Tringali, C. (2004). Constituents of grape pomace from the Sicilian cultivar 'Nerello Mascalese'. *Food Chemistry*, *88*, 599–607.

Blanco-Vega, D., López-Bellido, F. J., Alía-Robledo, J. M., & Hermosín-Gutiérrez, I. (2011). HPLC-DAD-ESI-MS/MS characterization of pyranoanthocyanins pigments formed in model wine. *Journal of Agricultural and Food Chemistry*, *59*, 9523-9531.

Boulton, R. (2001). The copigmentation of anthocyanins and its role in the color of red wine: A critical review. *American Journal of Enology and Viticulture*, *52*, 67-87.

Camargo, U. A., Maia, J. D. G., & Nachtigal, J. C. (2005). BRS Violeta: Nova cultivar de uva para suco e vinho de mesa. Embrapa Uva e Vinho. Comunicado Técnico, 63. Available in: http://www.cnpuv.embrapa.br/publica/comunicado/cot063.pdf.

Camargo, U. A., & Guerra, C. C. (2001). BRS Lorena, Cultivar para a elaboração de vinhos aromáticos. Embrapa Uva e Vinho. Comunicado Técnico, 39.

Castillo-Muñoz, N., Fernández-González, M., Gómez-Alonso, S., García-Romero, E., & Hermosín-Gutiérrez, I. (2009). Red-Color Related Phenolic Composition of Garnacha Tintorera (*Vitis vinifera* L.) Grapes and Red Wines. *Journal Agriculture Food Chemistry, 57,* 7883–7891.

Castillo-Muñoz, N., Gómez-Alonso, S., García-Romero, E., & Hermosín-Gutiérrez, I. (2010). Flavonol profiles of *Vitis vinifera* white grape cultivars. *Journal Food Composition and Analysis, 23*, 699–705.

Cejudo-Bastante, M. J., Hermosín-Gutiérrez, I., & Pérez-Coello, M. S. (2011). Micro-oxygenation and oak chip treatments of red wines: Effects on colour-related phenolics, volatile composition and sensory characteristics. Part I: Petit Verdot wines. *Food Chemistry, 124*, 727–737.

Gallego, M. A. G., García-Carpintero, E. G., Sánchez-Palomo, E., Viñas, M. A. G., & Hermosín-Gutiérrez, I. (2012). Oenological potential, phenolic composition, chromatic characteristics and antioxidant activity of red single-cultivar wines from Castilla-La Mancha. *Food Research International, 48*, 7–15,.

García-Romero, E., Castillo-Muñoz, N., Mena-Morales, A., Gómez-Alonso, S., & Hermosín-Gutiérrez, I. (2012). Flavonol 3-glycosides of minority Spanish *Vitis vinifera* white grape cultivars. *Macrowine 2012 - Macrovision of viticulture, wine-making and markets*. Bordeaux, France, June 18-21.

Hermosín-Gutiérrez, I, Castillo-Muñoz, N., Gómez-Alonso, S., & García-Romero, E. (2011). Chapter 8. Flavonol profiles for grape and wine authentication. In S. E. Ebeler, G. R. Takeoka, & P. Winterhalter, *Progress in authentication of food and wine* (pp. 113-129). Washington, DC: American Chemical Society, ACS Symposium Series.

Kammerer, D., Claus, A., Carle, R., & Schieber, A. (2004). Polyphenol Screening of Pomace from Red and White Grape Varieties (*Vitis vinifera* L.) by HPLC-DAD-MS/MS. *Journal of Agricultural and Food Chemistry, 52*, 4360−4367.

Lago-Vanzela, E.S., Da-Silva, R., Gomes, E., García-Romero, E., & Hermosín-Gutiérrez, I. (2011a). Phenolic Composition of the Edible Parts (Flesh and Skin) of Bordô Grape (*Vitis labrusca*) Using HPLC−DAD−ESI-MS/MS. *Journal of Agricultural and Food Chemistry*, *59*, 13136−13146.

Lago-Vanzela, E.S., Da-Silva, R., Gomes, E., García-Romero, E., & Hermosín-Gutiérrez, I. (2011b). Phenolic Composition of the Brazilian Seedless Table Grape Varieties BRS Clara and BRS Morena. *Journal of Agricultural and Food Chemistry*, *59*, 8314–8323.

Maragkoudakis, P.A., Nardi, T., Bovo, B., D'Andrea, M., Howell, K. S., Giacomini, A., & Corich, V. (2013). Biodiversity, dynamics and ecology of bacterial community during grape marc storage for the production of grappa. International Journal of Food Microbiol*ogy, 162*, 143-151.

Makris, D.P., Boskou, G., & Andrikopoulos, N.K. (2007). Polyphenolic content and in vitro antioxidant characteristics of wine industry and other agri-food solid waste extracts. *Journal of Food Composition and Analysis, 20*, 125–132.

Morata, A., Gómez-Cordovés, M. C., Colomo, B., & Suárez, J. A. (2005). Cell Wall anthocyanin adsorption by different *Saccharomyces* strains during the fermentation of *Vitis vinifera* L. cv Graciano grapes. *European Food Research and Technology*, *220*, 341-346.

Nixdorf, S. L., & Hermosín-Gutiérrez, I. (2010). Brazilian red wines made from the hybrid grape cultivar Isabel: Phenolic composition and antioxidant capacity. *Analytica Chimica Acta, 659*, 208–215.

Paradelo, R., Moldes, A. B., & Barral, M. T. (2010). Utilization of a factorial design to study the composting of hydrolyzed grape marc and vinification lees. *Journal of Agricultural and Food Chemistry*, *58,* 3085–3092.

Pérez-Serradilla, J. A., & Luque de Castro, M. D. (2008). Role of lees in wine production: A review. *Food Chemistry*, *111*, 447-456.

Pérez-Serradilla, J. A., & Luque de Castro, M. D. (2011). Microwave-assisted extraction of phenolic compounds from wine lees and spray-drying of the extract. *Food Chemistry*, *124*, 1652-1659.

Poudel, P. R., Mochioka, R., Beppu, K., & Kataoka, I. (2009). Influence of temperature on berry composition of interspecific hybrid wine grape 'Kadainou R-1' (*Vitis ficifolia* var. ganebu x *V. vinifera* 'Muscat of Alexandria'). *Journal of Japanese Society of Horticultural Sciences*, *78*, 169–174.

Rebello, L. P. G., Lago-Vanzela, E. S., Barcia, M. T., Ramos, A. M., Stringheta, P. C., Da-Silva, R., Castillo-Muñoz, N., Gómez-Alonso, S., & Hermosín-Gutiérrez, I. (2013). Phenolic composition of the berry parts of hybrid grape cultivar BRS Violeta (BRS Rubea × IAC 1398-21) using HPLC–DAD–ESI-MS/MS. *Food Research International, 54*, 354–366.

Sant'Anna, V., Brandelli, A., Marczak, L. D. F., & Tessaro, I. C. (2012). Kinetic modeling of total polyphenol extraction from grape marc and characterization of the extracts. *Separation and Purification Technology*, *100*, 82–87.

Shojaee-Aliabadi, S., Hosseini, S. M., Tiwari, B., Hashemi, M., Fadavi, G., & Khaksar, R. (2013). Polyphenols content and antioxidant activity of Ghure (unripe grape) marc extract: influence of extraction time, temperature and solvent type. *International Journal of Food Science and Technology, 48*, 412–418.

Torres, C., Díaz-Maroto, M. C., Hermosín-Gutiérrez, I., & Pérez-Coello, M. S. (2010). Effect of freeze-drying and oven-drying on volatiles and phenolics composition of grape skin. *Analytica Chimica Acta, 660*, 177–182

ARTIGO 3

Occurrence of Low Molecular Phenolics in *Vitis vinifera* Red Grape Cultivars and Their Winemaking By-Products from São Paulo (Brazil).

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Occurrence of Low Molecular Phenolics in *Vitis vinifera* Red Grape Cultivars and Their Winemaking By-Products from São Paulo (Brazil).

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ABSTRACT

The content of low molecular weight phenolic compounds present in winemaking by-products (skins from grape marc and wine lees) of red wine made from V. vinifera grape cultivars Cabernet sauvignon and Cabernet franc in the state of São Paulo (Brazil) has been examined. The study was extended to two consecutive years and showed that winemaking by-products still contained high concentrations of interesting functional and bioactive phenolic compounds. In addition to original grape phenolics, the winemaking by-products contained new phenolics originated by the action of enzymatic (yeast mediated) and chemical reactions during winemaking, including formation of anthocyanin-derived pigments and hydrolysis products from flavonol glycosides and tartaric esters of hydroxycinnamic acids. A total of 19 anthocyanins, 9 pyranoanthocyanins, 18 flavonols, 7 hydroxycinnamic acid derivatives, and 3 resveratrol-based stilbenes were identified. Wine lees have been revealed as an interesting source of flavonol aglycones and pyranoanthocyanins. The best sample preparation technique prior the extraction of phenolic compounds, with regards to the maintenance of phenolic compounds profiles and concentrations, was freeze-drying; however, in the case of lees, spray-drying showed to be also effective in the preservation of phenolic compounds and is a less expensive technique. Oven-drying at 50 ºC should be avoided if there is interest in using winemaking by-products for further purposes, since phenolic compounds concentration were hardly reduced by thermal degradation

Keywords: *Vitis vinifera,* grape marc, lees, anthocyanins, flavonols, hydroxycinnamic acid, stilbenes.

1 INTRODUCTION

São Paulo (southeastern Brazil), is currently a region with increased activity in the production of wine. The wine industry produces a large amount of by-products and residues. All these residues contain biodegradable organic matter, and its disposal creates serious environmental problems (Rebello, et al., 2013, Mendes, Prozil, Evtuguin, & Lopes, 2012).

Grape marc (a set of skin and seed) is the most abundant residues of wine making, which is generated after concomitant fermentation maceration of the grapes in red wine production. After fermentation of the must, a decanting wine process occurs, where the supernatant is separated from the lees. The lees are composed of fine particles of grape residue and yeasts (Maragkoudakis et al., 2013, Paradelo, Mould, Barral, 2010, Cortés, Rodríguez, Salgado, & Domínguez, 2011).

Over the past few years, the by-products of wine have attracted attention as possible sources of phenolic compounds, because of these compounds in the grape are transferred to the wine. However, the majority of the phenolic compounds remains in the residue, since they are present in the solid parts of the grape. Thus, the recovery of phenols from industrial residues is gaining considerable attention, especially because of the antioxidant properties attributed to these compounds. These compounds also play other activities such as anti-inflammatory, anticancer and antimutagenic activity (Cheng, Bekhit, McConnell, Mros, & Zhao, 2012, Rubilar, Pinelo, Shene, Sineiro, & Nuñez, 2007, Shrikhande, 2000).

The use of bioactive compounds for various purposes in the food industry, cosmetics and pharmaceuticals from residue is good for the environment, in addition to low the cost and efficiency. However, in many cases there is a significant lack of studies of the

viability of such residue, and as a result, its use is still under investigation (Babbar, Oberoi, Uppal, & Patil, 2011).

The objectives of this study was to perform a characterization of the different classes of phenolic compounds present in winemaking by-products (skin from grape marc and lees) obtained during the preparation of red wine from grapes Cabernet sauvignon and Cabernet franc, both *Vitis vinifera*, generated by a winery in the State of São Paulo/Brazil for two consecutive years. In addition, the effect of three drying techniques (drying-oven at 50 °C, spray-drying, and freeze-drying) on the content of phenolic compounds was evaluated.

2 MATERIAL AND METHODS

2.1 Chemicals

All solvents were of HPLC quality and all chemicals of analytical grade (> 99 %). Water was of ultrapure (Milli-Q quality). The following commercial standards were used for quantification: quercetin 3-gucoside (Extrasynthese, Genay, France), *trans*-caftaric acid (Phytolab, Vestenbergsgreuth, Germany), and *trans*-resveratrol (Sigma, Madrid, Spain). Other standards used for identification were: malvidin 3-glucoside, caffeic acid, *p*-coumaric acid, *trans*-piceid (Phytolab, Vestenbergsgreuth, Germany); cyanidin 3-glucoside, kaempferol, quercetin, isorhamnetin, myricetin, syringetin, and the 3-glucosides of kaempferol, quercetin, isorhamnetin, and syringetin (Extrasynthese, Genay, France); myricetin 3-glucoside, quercetin 3-glucuronide, and laricitrin 3-glucoside were previously isolated from Petit Verdot grape skins (Castillo-Muñoz, Fernández-Gonzalez, Gómez-

Alonso, García-Romero, & Hermosín-Gutiérrez, 2009). The *trans* isomers of resveratrol and its 3-glucoside (piceid) were transformed into their respective *cis* isomers by UV-irradiation (366 nm light during 5 minutes in quartz vials) of 25% MeOH solutions of the *trans* isomers.

2.2 Samples

Samples of grape berries used for elaboration of wine and their resulting winemaking by-products (skins and lees) of two *Vitis vinifera* varieties (Cabernet sauvignon and Cabernet franc), collected in triplicate during the harvest of 2011 and 2012 (with the exception of the skin Cabernet franc in the year 2011, which was lost in the industry) from a winery located at the municipal district of São Roque (São Paulo, Brazil), which lies at 23º 31'44" S and 47º 08'06" W, and 771 m above sea level (referred to datum WGS84, World Geodetic System 1984) and which has a subtropical climate (maximum, 23.1 °C; minimum, 15.5 °C). Grape berry samples were frozen at −20 °C for 24 h, cut in two halves, re-frozen at −20 °C for 24 h, and further freeze-dried for 48 h. Samples of fermented grape marc and lees from both grape varieties were frozen at −20 °C for 24 h and then freeze-dried for 48 h. After that, skins were manually separated from dried grape marc samples. Dried samples of skins from grape marc and lees were also obtained by drying-oven at 50 °C under forced air flow. Finally, a third drying treatment was applied to lees: spray-drying. After drying, the samples of entire grapes and skins were crushed and homogenized. All dried samples were stored at −18 °C until analyses.

2.3 Extraction of Phenolic Compounds

Dried samples in amounts of 2.5, 0.25, and 0.25 g (grapes, skins and lees, respectively) were extracted with 50 mL (grape and lees) or 75 mL (skins) of a mixture of

methanol, water, and formic acid (50:48.5:1.5 v/v), with the help of an ultrasonic bar for 2 min and were then centrifuged at 5000*g* at 5 ºC for 5 min. A second and third extractions of the resulting pellets yielded nearly 99% of the sample phenolic content, as confirmed by HPLC of successive extractions (up to five). The combined supernatants were stored at −18 °C until use. The samples extracts (grape: 1ml, skin and lees: 5mL) was dried in a rotary evaporator (35 °C) and re-dissolved in 0.5, 0.5 and 1 mL (grape, skin and lees, respectively) of 0.1 M HCl , filtered (0.20 μm, polyester membrane, Chromafil PET 20/25, Macherey-Nagel, Düren, Germany) and directly injected the HPLC system for anthocyanin determination.

For analysis of non-anthocyanin phenolics, a cleaning step using ECX SPE cartridges (40 μm, 500 mg, 6 mL; Scharlab, Sentmenat, Barcelona, Spain) for removing anthocynins was applied, following a previously described method (Rebello et al., 2013). The eluates were dried in a rotary evaporator (35 °C) and re-dissolved in 0.5 mL of 20% methanol in water and directly injected in the HPLC equipment for analyzing flavonols, hydroxycinnamic acid derivatives and stilbenes.

2.4 Identification and Quantification of Phenolic Compounds by HPLC-DAD-ESI-MSn

2.4.1 Analysis of Anthocyanins and Derived Compounds

HPLC separation, identification, and quantification of anthocyanins and pyranoanthocyanins were performed on an Agilent 1100 Series system (Agilent, Germany), equipped with DAD (G1315B) and a LC/MSD Trap VL (G2445C VL) electrospray ionization mass spectrometry (ESI-MS^n) system, and coupled to an Agilent ChemStation (version B.01.03) data-processing station. The mass spectra data were processed with the

Agilent LC/MS Trap software (version 5.3). 10 μL of samples extracts were injected on a reversed-phase column Zorbax Eclipse XDB-C18 (2.1 × 150 mm; 3.5 μm particle; Agilent, Germany), thermostatized at 40 °C. The solvents system were based on mixtures of water, acetonitrile, and formic acid (88.5:3:8.5, v/v/v, solvent A; 41.5:50:8.5, v/v/v, solvent B), and the flow rate was 0.19 mL/min. The linear gradient for solvent B was as follows: 0 min, 6%; 10 min, 30%; 30 min, 50%; 34 min, 100%; 36 min, 100%; 42 min, 6%. For identification, ESI-MS^n was used setting the following parameters: positive ionization mode; dry gas, N_2, 11 mL/min; drying temperature, 350 °C; nebulizer, 65 psi; capillary, -2500 V; capillary exit offset, 70 V; skimmer 1, 20 V; skimmer 2, 6 V; compound stability, 100%; scan range, 50-1200 m/z. For quantification, DAD-chromatograms were extracted at 520 nm and their total concentrations were expressed as equivalents of malvidin-3-diglucoside (g/kg of dry weight sample).

2.4.2 Analysis of Non-Anthocyanin Phenolic Compounds

HPLC separation, identification, and quantification of non-anthocyanin phenolic compounds were performed on the same chromatographic system formerly described for anthocyanins. In this case, 20 μL of anthocyanin-free extract fractions were injected on a reversed-phase column Zorbax Eclipse XDB-C18 (2.1 × 150 mm; 3.5 μm particle; Agilent, Germany), thermostatized at 40 °C. The solvents were as follows: solvent A (acetonitrile/water/formic acid, 3:88.5:8.5, v/v/v), solvent B (acetonitrile/water/formic acid, 50:41.5:8.5, v/v/v), and solvent C (methanol/water/formic acid, 90:1.5:8.5, v/v/v). The flow rate was 0.19 mL/min. The linear solvents gradient was as follows: 0 min, 98% A, and 2% B; 8 min, 96% A, and 4% B; 37min, 70% A, 17% B, and 13% C; 51 min, 50% A, 30% B, and 20% C; 51.5 min, 30% A, 40% B, and 30% C; 56 min, 50% B, and 50% C; 57 min,

50% B, and 50% C; 64 min, 98%A, and 2% B. For quantification, DAD chromatograms were extracted at 360 nm (flavonols), and 320 nm (hydroxycinnamic acid derivatives and resveratrol forms), and their concentrations were expressed as in mg per kg of dried samples equivalents of quercetin 3-glucoside (flavonols), *trans*-caftaric acid (hydroxycinnamic acid), and *trans*-resveratrol (resveratrol forms).

2.4.3 Statistics

The ANOVA Tukey test (STATISTIC 7.0, $p < 0.05$) was used for mean value comparison. In addition, Principal Component Analysis (Pirouette 3.11.) was applied to the data matrix in order to highlight similarities and/or differences within samples in a determined set of data. Data was previously pre-escalated before submitting them to statistics analysis.

3 RESULTS AND DISCUSSION

3.1 Anthocyanins and Derived Compounds in Grapes and their Winemaking By-Products

The profile and content of anthocyanins in freeze-dried grapes, fermented skin and lees of Cabernet sauvignon and Cabernet franc, for the years of 2011 and 2012, and the characteristics of the chromatographic spectrum (molecular ion product generated by ESI-MS/MS and retention time) together with the molar profile are shown in **Tables and Figures 3.1 and 3.2.**

A total of 19 anthocyanins and 9 pyranoanthocyanins were tentatively identified, although not always all of them were detected in all samples. The identification of each

anthocyanin was tentatively based on the comparison of spectroscopic data, especially MS/MS those of spectra, with those obtained from standard or previously reported (Nixdorf & Hermosín-Gutiérrez, 2010, Lago-Vanzela, Da-Silva, Gomes, García-Romero, & Hermosín-Gutiérrez, 2011a).

The usual structures of the anthocyanidins delphinidin (dp), cyanidin (cy), petunidin (pt), peonidin (pn) and malvidin (mv) were identified on the basis of their respective product ions at *m/z* 303, 287, 317, 301 and 331 generated after fragmentation (MS/MS spectra) of suspected anthocynins. In contrast, perlagonidin-based anthocyanins were not found in the samples, as noted by the absence of any signal at *m/z* 271 attributable to their product ions.

Table 3.1. Anthocyanins and pyranoanthocyanins identified in freeze-dried samples of Cabernet franc, grape, their fermented skins and lees, in years 2011 and 2012: chromatographic and mass spectral characteristics (positive ionization mode); molar percentages given as mean value ± standard deviation (n=3). Peak numbers as in Figure 3.1.

peak	assignation*	R_t (min)	molecular ion; product ions (*m/z*)	grape 2011 (molar %)	grape 2012 (molar %)	skin 2012 (molar %)	less 2011 (molar %)	lees 2012 (molar %)
1	dp-3glc	6.67	465; 303	6.29 ± 0.19^{aA}	$6.15\pm0.012^{a\alpha}$	$3.36\pm0.14^{\beta}$	1.10 ± 0.03^{bB}	$1.20\pm0.03^{a\gamma}$
2	cy-3glc	9.56	449; 287	1.66 ± 0.00^{a}	$1.20\pm0.11^{b\alpha}$	$0.30\pm0,04^{\beta}$	ND	ND
3	pt-3glc	11.48	479; 317	5.15 ± 0.08^{bA}	$5.92\pm0.07^{a\alpha}$	$5.57\pm0.20^{\alpha}$	3.07 ± 0.15^{bB}	$4.24\pm0.18^{a\beta}$
4	pn-3glc	13.21	463; 301	9.64 ± 0.04^{A}	$9.55\pm0.33^{\alpha}$	$5.19\pm0.22^{\beta}$	2.50 ± 0.06^{aB}	$1.61\pm0.05^{b\gamma}$
5	mv-3glc	14.36	493; 331	39.14 ± 0.24^{B}	$37.67\pm1.21^{\gamma}$	$49.91\pm2.11^{\beta}$	56.27 ± 0.63^{bA}	$62.19\pm0.93^{a\alpha}$
6	dp-3acglc	14.90	507; 303	1.42 ± 0.09^{A}	$1.43\pm0.09^{\alpha}$	$0.71\pm0.05^{\beta}$	1.02 ± 0.03^{B}	$0.90\pm0.16^{\beta}$
7	cy-3acglc	16.61	491; 287	0.19±0.01	$0.21\pm0.02^{\beta}$	$0.35\pm0.02^{\alpha}$	ND	ND
8	pt-3acglc	17.59	521; 317	1.45 ± 0.07^{A}	$1.58\pm0.14^{\alpha}$	$1.32\pm0.19^{\alpha}$	0.79 ± 0.04^{aB}	$0.67\pm0.02^{b\beta}$
9	pn-3acglc	19.75	505; 301	2.99 ± 0.07^{A}	$3.32\pm0.27^{\gamma}$	$2.61\pm0.16^{\beta}$	1.46 ± 0.02^{aB}	$1.16\pm0.03^{b\alpha}$
10	mv-3acglc	20.41	535; 331	18.94 ± 0.41^{aA}	$16.87\pm1.02^{b\alpha}$	$15.86\pm1.64^{\alpha}$	15.58 ± 0.26^{aB}	$10.40\pm0.19^{b\beta}$
11	dp-3cmglc	19.33	611; 303	0.50 ± 0.01^{b}	$0.70\pm0.05^{a\alpha}$	$0.52\pm0.07^{\beta}$	ND	$0.58\pm0.01^{\alpha\beta}$
12	cy-3cmglc	21.50	595; 287	0.18 ± 0.01^{a}	0.13 ± 0.01^{b}	$0.15\pm0.01^{\alpha}$	ND	ND
13	pt-3cmglc	22.41	625; 317	0.73 ± 0.02^{bA}	$0.93\pm0.08^{a\alpha}$	$0.46\pm0.04^{\gamma}$	0.27 ± 0.06^{aB}	$0.73\pm0.04^{b\beta}$
14	mv-3-*cis*-cmglc	23.10	639; 331	0.32±0.02	$0.29\pm0.01^{\beta}$	$0.24\pm0.02^{\beta}$	0.32 ± 0.00^{b}	$0.39\pm0.04^{a\alpha}$
15	pn-3cmglc	25.11	609; 301	2.37 ± 0.07^{A}	$2.44\pm0.10^{\alpha}$	$1.21\pm0.09^{\gamma}$	0.84 ± 0.03^{bB}	$1.85\pm0.09^{a\beta}$
16	mv-3-*trans*-cmglc	26.06	639; 331	8.95 ± 0.25^{aA}	$10.97\pm0.33^{b\alpha}$	$5.35\pm0.20^{\beta}$	5.37 ± 0.14^{bB}	$10.16\pm0.55^{a\alpha}$
17	pt-3cflgc	19.10	641; 317	ND	ND	0.20±0.01	ND	ND
18	pn-3cfglc	21.10	625; 301	ND	ND	0.88±0.05	ND	ND
19	mv-3 cfglc	22.14	655; 331	0.08 ± 0.00^{bB}	$0.64\pm0.08^{a\beta}$	$4.27\pm0.41^{\alpha}$	0.37 ± 0.05^{A}	$0.33\pm0.01^{\beta}$
20	10-carboxy-pymv-3glc	15.78	561; 399	ND	ND	0.47±0.02	5.10 ± 0.22^{a}	0.51 ± 0.02^{b}

Continuation…

21	pymv-3glc	16.56	517; 355	ND	ND	$0.66\pm0.05^{\alpha}$	0.21 ± 0.02^{b}	$0.29\pm0.01^{a\beta}$
22	10-carboxy-pypn-3glc	18.70	531; 369	ND	ND	ND	ND	0.33±0.07
23	10-carboxy-pymv-3acglc	16.96	603; 399	ND	ND	0.20±0.03	1.53±0.08	ND
24	pymv-3acglc	18.08	559; 355	ND	ND	0.20±0.04	0.10±0.01	ND
25	10-carboxy-pymv-3cmglc	20.08	707; 399	ND	ND	ND	0.53 ± 0.01^{a}	0.12 ± 0.04^{b}
26	10-DHP-pymv-3glc	27.26	625; 463	ND	ND	ND	0.24±0.01	0.27±0.02
27	10-HP-pymv-3glc	30.19	609; 447	ND	ND	ND	2.36 ± 0.10^{a}	1.54 ± 0.07^{b}
28	10-HP-pymv-3acglc	32.93	651; 447	ND	ND	ND	1.00 ± 0.10^{a}	0.54 ± 0.07^{b}
Total (g /kg sample)**				2.14 ± 0.19^{aA}	$1.78\pm0.04^{b\gamma}$	$3.61\pm0.10^{\alpha}$	1.45 ± 0.05^{bB}	$2.26\pm0.22^{a\beta}$

Nomenclature abbreviations: dp, delphinidin; cy, cyanidin; pt, petunidin; pn, peonidin; mv, malvidin; glc, glucoside; acglc, 6”-acetyl-glucoside; cfglc, 6”-caffeoyl-glucoside; cmglc, 6”-*p*-coumaroyl-glucoside; pymv, pyranomalvidin; pycy, pypn, pyranopeonidin; 10-DHP, 10-(3‴,4‴-hydroxy)-phenyl; 10-HP, 10-(4‴-hydroxy)-phenyl. 10-carboxy-pymv-3glc and pymv-3-glc are also known as vitisins A and B, respectively.

** As malvidin 3-diglucoside equivalents (mv-3diglc). ND, not detected. Different low case letters for the samples of different vintages (2011 and 2012) of each kind of sample (grape, skin, or lees) means significant differences (ANOVA, $p< 0.05$). Different capital or Greek letters for the three samples (grape, fermented skin, and lees) of each vintage (years 2011 or 2012, respectively) means significant differences (ANOVA, $p< 0.05$).

Table 3.2. Anthocyanins and pyranoanthocyanins identified in freeze-dried samples of Cabernet sauvignon, grape, their fermented skins and lees, in years 2011 and 2012: chromatographic and mass spectral characteristics (positive ionization mode); Peak numbers as in Figure 3.2.

peak	assignation*	R_t (min)	molecular ion; product ions (*m/z*)	grape 2011 (molar %)	grape 2012 (molar %)	skin 2011 (molar %)	skin 2012 (molar %)	less 2011 (molar %)	lees 2012 (molar %)
1	dp-3glc	6.67	465; 303	6.11 ± 0.26^{aA}	$2.40\pm0.32^{b\alpha}$	2.92 ± 0.29^{B}	$3.22\pm0.68^{\alpha}$	1.56 ± 0.16^{aC}	$1.08\pm0.05^{b\beta}$
2	cy-3glc	9.56	449; 287	2.05 ± 0.12^{aA}	$0.41\pm0.12^{b\alpha}$	0.56 ± 0.12^{aB}	$0.35\pm0.03^{b\alpha}$	0.15 ± 0.02^{C}	$0.13\pm0.01^{\beta}$
3	pt-3glc	11.48	479; 317	4.06 ± 0.17^{A}	$3.91\pm0.56^{\alpha}$	3.16 ± 0.33^{B}	$3.85\pm0.76^{\alpha}$	3.28 ± 0.37^{aB}	$2.28\pm0.26^{b\beta}$
4	pn-3glc	13.21	463; 301	9.79 ± 0.39^{aA}	$5.26\pm0.17^{b\alpha}$	4.06 ± 0.12^{aB}	$2.37\pm0.22^{b\beta}$	1.86 ± 0.20^{aC}	$0.69\pm0.01^{b\gamma}$
5	mv-3glc	14.36	493; 331	39.22 ± 1.74^{bC}	$53.86\pm0.24^{a\gamma}$	57.19 ± 0.53^{bB}	$62.54\pm2.52^{a\beta}$	63.54 ± 3.33^{A}	$66.23\pm0.13^{\alpha}$
6	dp-3acglc	14.90	507; 303	1.26 ± 0.11^{aA}	$0.63\pm0.06^{b\beta}$	0.63 ± 0.08^{B}	$0.46\pm0.12^{\beta}$	0.64 ± 0.07^{bB}	$1.22\pm0.08^{a\alpha}$
7	cy-3acglc	16.61	491; 287	0.24 ± 0.05^{b}	0.44 ± 0.06^{a}	ND	ND	ND	ND
8	pt-3acglc	17.59	521; 317	1.16 ± 0.12^{aA}	$0.76\pm0.05^{b\alpha}$	0.69 ± 0.08^{aB}	$0.42\pm0.13^{b\beta}$	0.87 ± 0.11^{aB}	$0.54\pm0.02^{b\beta}$
9	pn-3acglc	19.75	505; 301	2.93 ± 0.18^{aA}	$1.29\pm0.07^{b\alpha}$	1.08 ± 0.07^{aB}	$0.50\pm0.15^{b\beta}$	0.87 ± 0.12^{bB}	$1.39\pm0.11^{a\alpha}$
10	mv-3acglc	20.41	535; 331	22.87 ± 1.05^{A}	$24.02\pm0.28^{\alpha}$	16.52 ± 0.75^{aB}	$9.72\pm0.10^{b\gamma}$	13.06 ± 1.02^{aC}	$11.01\pm0.04^{b\beta}$
11	dp-3cmglc	19.33	611; 303	0.24 ± 0.02	0.36 ± 0.12	0.18 ± 0.04^{b}	0.29 ± 0.01^{a}	0.25 ± 0.03^{b}	0.49 ± 0.09^{a}
12	cy-3cmglc	21.50	595; 287	0.13 ± 0.01	ND	0.17 ± 0.05	ND	ND	0.07 ± 0.01
13	pt-3cmglc	22.41	625; 317	0.40 ± 0.02^{aA}	$0.24\pm0.03^{b\beta}$	0.18 ± 0.04^{bB}	$0.33\pm0.03^{a\alpha}$	0.36 ± 0.01^{A}	$0.37\pm0.03^{\alpha}$
14	mv-3-*cis*-cmglc	23.10	639; 331	0.34 ± 0.03^{aA}	$0.16\pm0.01^{b\beta}$	0.25 ± 0.03^{B}	$0.25\pm0.02^{\beta}$	0.34 ± 0.04^{bA}	$0.55\pm0.07^{a\alpha}$
15	pn-3cmglc	25.11	609; 301	1.83 ± 0.14^{aA}	$0.55\pm0.05^{b\gamma}$	0.70 ± 0.06^{B}	$0.81\pm0.08^{\beta}$	0.77 ± 0.13^{bB}	$1.08\pm0.03^{a\alpha}$
16	mv-3-*trans*-cmglc	26.06	639; 331	7.37 ± 0.14^{aA}	$4.90\pm0.57^{b\beta}$	5.57 ± 0.58^{aB}	$4.45\pm0.26^{b\beta}$	5.73 ± 0.50^{bB}	$9.09\pm0.06^{a\alpha}$
19	mv-3 cfglc	22.14	655; 331	ND	$0.77\pm0.07^{\beta}$	1.39 ± 0.11^{bA}	$1.96\pm0.04^{a\alpha}$	0.34 ± 0.03^{B}	$0.39\pm0.05^{\gamma}$
20	10-carboxy-pymv-3glc	15.78	561; 399	ND	ND	2.73 ± 0.12^{A}	$2.57\pm0.62^{\alpha}$	1.54 ± 0.16^{aB}	$0.45\pm0.02^{b\beta}$
21	pymv-3glc	16.56	517; 355	ND	ND	0.73 ± 0.06^{bA}	$3.64\pm0.25^{a\alpha}$	0.27 ± 0.02^{B}	$0.27\pm0.02^{\beta}$
22	10-carboxy-pypn-3glc	18.70	531; 369	ND	ND	ND	ND	0.10 ± 0.02^{b}	0.81 ± 0.15^{a}
23	10-carboxy-pymv-3acglc	16.96	603; 399	ND	ND	0.93 ± 0.18^{A}	$1.00\pm0.13^{\alpha}$	0.44 ± 0.05^{aB}	$0.14\pm0.00^{b\beta}$

Continuation…

24	pymv-3acglc	18.08	559; 355	ND	ND	0.32 ± 0.03^{bA}	1.09 ± 0.09^{a}	0.10 ± 0.02^{B}	ND
25	10-carboxy-pymv-3cmglc	20.08	707; 399	ND	ND	0.18 ± 0.03^{B}	0.20 ± 0.01	0.24 ± 0.02^{A}	ND
26	10-DHP-pymv-3glc	27.26	625; 463	ND	ND	ND	ND	0.40 ± 0.05^{a}	0.20 ± 0.01^{b}
27	10-HP-pymv-3glc	30.19	609; 447	ND	ND	ND	ND	2.46 ± 0.28^{a}	1.23 ± 0.15^{b}
28	10-HP-pymv-3acglc	32.93	651; 447	ND	ND	ND	ND	0.84 ± 0.06^{a}	0.30 ± 0.03^{b}
Total (g /kg sample)**				1.78 ± 0.20^{aB}	$1.11\pm0.20^{b\beta}$	2.59 ± 0.36^{aA}	$0.58\pm0.04^{b\gamma}$	1.47 ± 0.06^{bB}	$2.22\pm0.02^{a\alpha}$

Nomenclature abbreviations: dp, delphinidin; cy, cyanidin; pt, petunidin; pn, peonidin; mv, malvidin; glc, glucoside; acglc, 6”-acetyl-glucoside; cfglc, 6”-caffeoyl-glucoside; cmglc, 6”-*p*-coumaroyl-glucoside; pymv, pypn, pyranopeonidin; 10-DHP, 10-(3‴,4‴-hydroxy)-phenyl; 10-HP, 10-(4‴-hydroxy)-phenyl. 10-carboxy-pymv-3glc and pymv-3-glc are also known as vitisins A and B, respectively.

** As malvidin 3-glucoside equivalents (mv-3glc). ND, not detected. Different low case letters for the samples of different vintages (2011 and 2012) of each kind of sample (grape, skin, or lees) means significant differences (ANOVA, $p< 0.05$). Different capital or Greek letters for the three samples (grape, fermented skin, and lees) of each vintage (years 2011 or 2012, respectively) means significant differences (ANOVA, $p< 0.05$). Molar percentages given as mean value ± standard deviation (n=3).

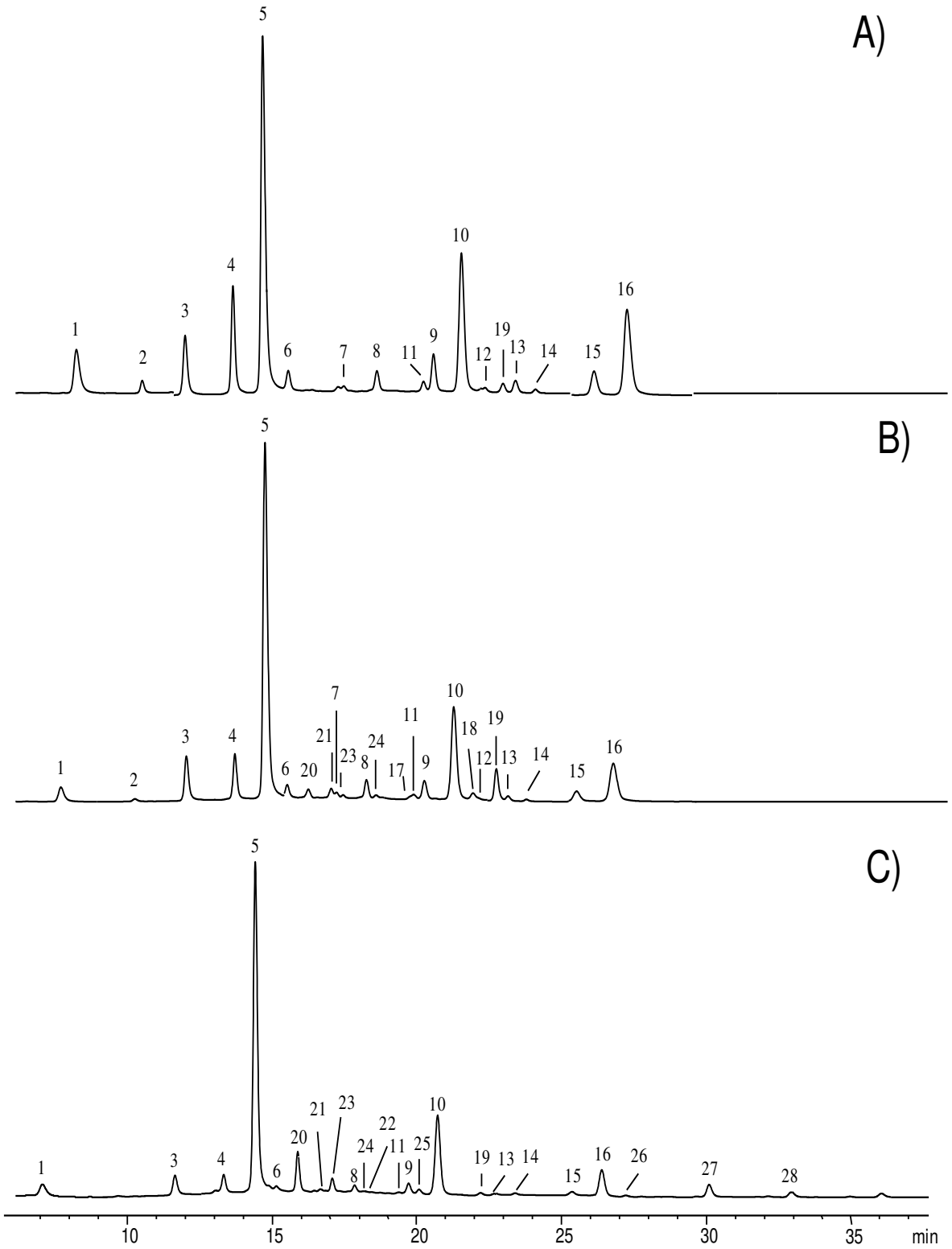

Figure 3.1. DAD-chromatograms corresponding to the anthocyanin profiles (detection at 520 nm) of Cabernet franc samples: A) grapes; (B) fermented skins, (C) wine lees. Identification of the peaks referred to Table 3.1.

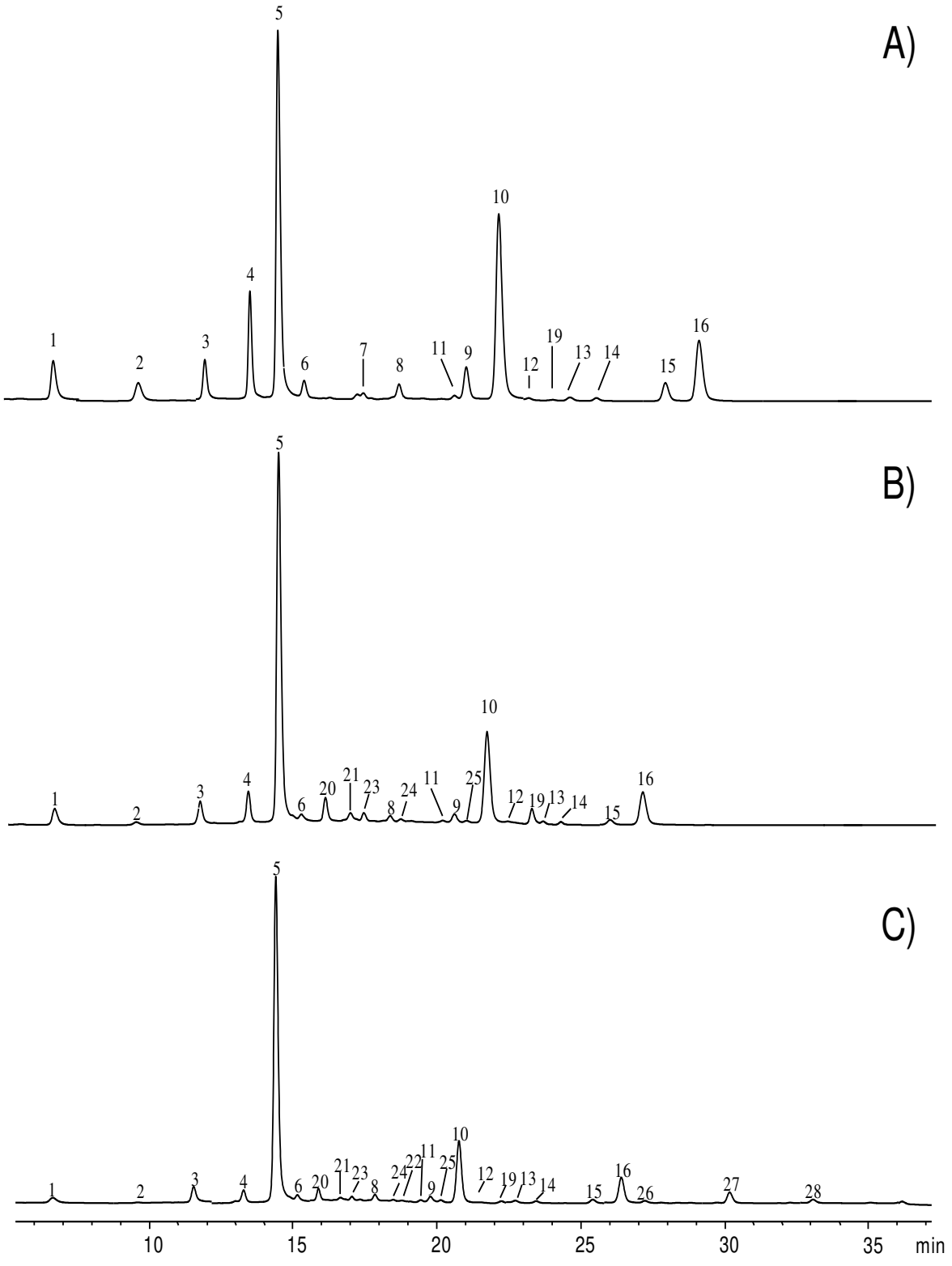

Figure 3.2. DAD-chromatograms corresponding to the anthocyanin profiles (detection at 520 nm) of Cabernet sauvignon samples: A) grapes; (B) fermented skins, (C) wine lees. Identification of the peaks referred to Table 3.2.

The usual structures of anthocyanidins delphinidin (dp), cyanidin (cy), petunidin (pt), peonidin (pn) and malvidin (mv) were identified with the product ion m/z 303, 287, 317, 301 and 331, respectively. However, perlagonidin was not found in the samples, as noted by the absence of the signal m/z 271 product ion.

The profile of anthocyanins (molar percentage for each individual anthocyanin; **Tables 3.1 and 3.2; Figures 3.1 and 3.2**) for both grape cultivars contained non-acylated and acylated 3-glucosides, which accounted in the following decreasing order: 3-glucosides (3-glc), 3-(6”-acetyl)-glucosides (3-acglc), 3-(6’’-*p*-coumaroyl)-glucosides (3-cmglc), and 3-(6”-caffeoyl)-glucosides (3-cfglc). The presence of only 3-glucoside derivatives confirmed the nature of *Vitis vinifera* grapes cultivars (Nixford et al., 2010). The assignment of the 3-glucoside moiety was based on the fragmentation patterns observed in the spectra, because the entire glucoside rest was released and only one product ion corresponding to anthocyanidin was observed, regardless of whether glucose was acylated or not (Nixdorf et al., 2010, Vanzela-Lago, Da-Silva, Gomes, García-Romero, & Hermosín-Gutiérrez, 2011b).

The major individual anthocyanins in grapes were, in average, mv-3-glc (49-57%), mv-3-acglc (around 16%), and mv-3-*trans*-cmglc (6-8%), whereas the caffeoyl derivative (mv-3-cfglc) was found in concentrations below 2%. The others anthocyanins were found in the two grapes analyzed, and also in both winemaking by-products (skin fermented and lees), with the exception of cy-3-cmglc that was not detected in the samples of lees form Cabernet franc. Another peculiarity was that the fermented skin of Cabernet franc (year 2012) showed high levels of mv-3-cfglc (4.27%) and other minor caffeoyl derivatives were also detected: pt-3-cfglc (0.20%) and pn-3-cfglc (0.88%), which were not detected in any of

the other samples of grape, fermented skin and lees. Finally, the minor cy-3-acglc was detected only in both grape samples from two years (average, 0.20-0.34 %) and also in fermented skin of Cabernet franc (0.35%) of the year 2012.

The anthocyanin profiles found in our study were in agreement with literature reported data for Cabernet Sauvignon and similar grape cultivars like Cabernet mitos, Lemberger, Spatburgunder, Schwarzriesling, Trollinger (Ferradino, Carra, Rolle, Schneider, & Schubert, 2012, Ryan & Revilla, 2003, Kammerer, Claus, Schieber, & Reinhold, 2005, Kammerer, Claus, Carle, & Schieber, 2004). It is widely know that the profile of anthocyanins of a variety is closely linked to their genetic inheritance, although environmental factors may have some influence on this profile (Poudel, Mochioka, Beppu, & Kataoka, 2009). However, the differences in the profile between samples of anthocyanins may be due to differences in partition coefficients solids/liquids and solubility in the wine that modulate the transfer of the anthocyanin from the grape to the wine.

Pyranoanthocyanins are formed from anthocyanidin 3-glucosides, and can be easily identified by their characteristic UV-vis and MS^n spectra (Nixford et al., 2010, Blanco-Vega, López-Bellido, Alía-Robledo, & Hermosín-Gutiérrez, 2011). In red wine made from *Vitis vinifera* grape cultivars, vitisin-type pyranoanthocyanins are formed in the initial steps of fermentation, mostly derived from the reaction of pyruvic acid and acetaldehyde with anthocyanins, which are two yeast intermediate metabolites (Blanco-Vega et al., 2011). Amico, Napoli, Renda, Ruberto, Spatafora and Tringali (2004) reported the presence of pyranoanthocyanins in winemaking by-products of the grape Sicilian cultivar Nerello Mascalese. The following vitisin-type pyranoanthocyanins were found in the samples under study (**Tables 3.1** and **3.2; Figures 3.1** and **3.2**): Vitisin A (10-carboxy-pymv-3-glc) and its

acetyl and *p*-coumaroyl derivatives; the A-type vitisin derived from pn-3-glc (10-carboxy-pypn-3-glc); and vitisin B (pymv-3-glc) and its acetyl derivative. The A-type vitisin from pn-3-glc was only found in lees samples. All the aforementioned vitisin-type pyranoanthocyanins accounted for low molar percentages and reflected the molar percentages found for their respective anthocyanin precursors. However, in the case of Cabernet franc they accounted for higher proportions in fermented skins whereas in the case of Cabernet Sauvignon they did in the lees.

Hydroxyphenyl-pyranoanthocyanins are another type of pyranoanthocyanins, which result from the reaction of anthocyanidin 3-glucoside with hydroxycinnamic acids or its decarboxylation products (Blanco-Vega et al., 2011). Maybe because of their low polarity, they were only found in lees samples and the detected structures were assigned as: 10-(4”-hydroxyphenyl)-pyranomalvidin-3-glucoside (10-HP-pymv-3-glc) and its acetyl derivative (10-HP-pymc-3-acglc), derived from *p*-coumaric acid; and 10-(3”,4”-dihydroxyphenyl)-pyranomalvidin-3-glucoside (10-DHP-pymv-3-glc), derived from caffeic acid. According to Morata, Gómez-Cordovés, Colomo and Suárez (2005) these compounds may be formed during the winemaking process and they may be absorbed by the lees. These hydroxyphenyl-pyranoanthocyanins were formed in total lower amounts than vitisin-type pyranoanthocyanins, but their only presence in lees made they usually accounted for higher amounts in this type of winemaking by-product, with the only exception of lees of Cabernet franc from 2011.

The total anthocyanin content in grapes of Cabernet sauvignon and Cabernet franc varied statistically among the years studied (2.14 and 1.78 g/kg dry sample, for year 2011; 1.78 and 1.11 g/kg dry sample, for year 2012), but they were similar when compared to

each other. However, only in the year 2012 the grape Cabernet franc was statistically different ($p<0.05$) from grape Cabernet sauvignon, as well as the content in fermented skin of Cabernet franc, which was greater than that of Cabernet sauvignon. The lees of Cabernet sauvignon and Cabernet franc showed no significant difference ($p<0.05$) in the total anthocyanin concentration (g/kg dry sample) when analyzed together for each year, but they were different for years 2011 and 2012. Multiple factors, such as climate, degree of maturity, berry's size, grape variety, and the applied technology to winemaking may be the cause of variations in the profile and content of anthocyanins in different years (Kammerer et al., 2005). Koundouras et al. (2009) investigated the importance of irrigation and root stocks on the concentration of phenolic compounds in Cabernet sauvignon grapes. According to this experiment, limiting water caused a substantial increase in the concentration of anthocyanins in grape skins and malvidin 3-glucoside was affected by water supply.

The transfer of anthocyanins from the grapes to wine is very limited, usually below 40% (Boulton, 2001), thus, most of anthocyanins from grape remain in the winemaking by-products, and even those transferred to wine can partially be adsorbed by lees (Morata et al., 2005). The fermented skin separated from the grape marcs retained large quantities of anthocyanins, despite the grapes initially showed less total anthocyanins than fermented skin because of the sugars present in the dry matter; the content of anthocyanins was lower in the skins of Cabernet sauvignon in year 2012 (0.58 g/kg) than in the corresponding grape used for winemaking. This last result is most likely related to the fact that the transference of anthocyanins in winemaking is not a simple process of partition and other physico-chemical processes are suggested to modulate such transference (Boulton, 2001). The

reported total anthocyanin content for fermented skins of diverse red grape cultivars (Lemberger, Spätburgunder, Schwarzriesling, and Trollinger: 91.24, 9.78, 9.76, 5.25, 3.74 g/kg dry matter, respectively) (Kammerer et al., 2004) were higher than those found in this study, with the exception of the fermented skin of Cabernet franc of year 2012 (3.61g/kg). The same authors also found variations in anthocyanins content between the years analyzed for Cabernet mitos (variation of 38%), Spätburgunder (44%) and Trollinger (57%). Rockenbach, Gonzaga, Rizelio, Gonçalves, Genovese and Fett (2011) analyzed the fermented skins of Cabernet sauvignon and found total anthocyanin content higher than that of the present study (9.34 g/kg dry matter, as cyanidin 3-glucoside; 7.74 g/kg dry matter, calculated as malvidin 3-glucoside).

Application of Principal Component (PC) analysis to the data matrix composed by the composition of anthocyanins of all freeze-dried samples (grapes, skin and lees, of both cultivars and both years) allows us to better visualize the main similarities and differences between the samples and their relations with the composition of anthocyanins. The samples of lees separated from grape samples along the PC-1 axis (explaining 42.51% of the total variance; **Figure 3.3A**) on the basis of the occurrence of pyranoanthocyanins in lees but not in grapes (**Figure 3.3B**) and also because the main anthocyanins, mv-3-glc, accounted for higher proportions in lees (**Table 3.1**). Moreover, lees samples separated form skin samples (which also contain pyranoanthocyanins) along the PC-2 axis (explaining 19.97% of the total variance; **Figure 3.3A**) on the basis of the predominant type of such anthocyanin-derived pigments: vitisin-type in skin samples and hydroxyphenyl-pyranoanthocyanins in lees samples (**Figure 3.3B**). Skin samples were also separated from their respective grape samples (same grape variety and year) because of the lack of pyranoanthocyanins in the

latter. However, the whole set of grape samples was not totally separated from the whole set of skin samples along PC-2 axis because of the overlapping of grape samples of Cabernet sauvignon of year 2012 with skin samples of year 2011, as a consequence of the high proportion of mv-3-glc found that was quite similar in both samples (53.86 and 57.19%, respectively; **Table 3.2**).

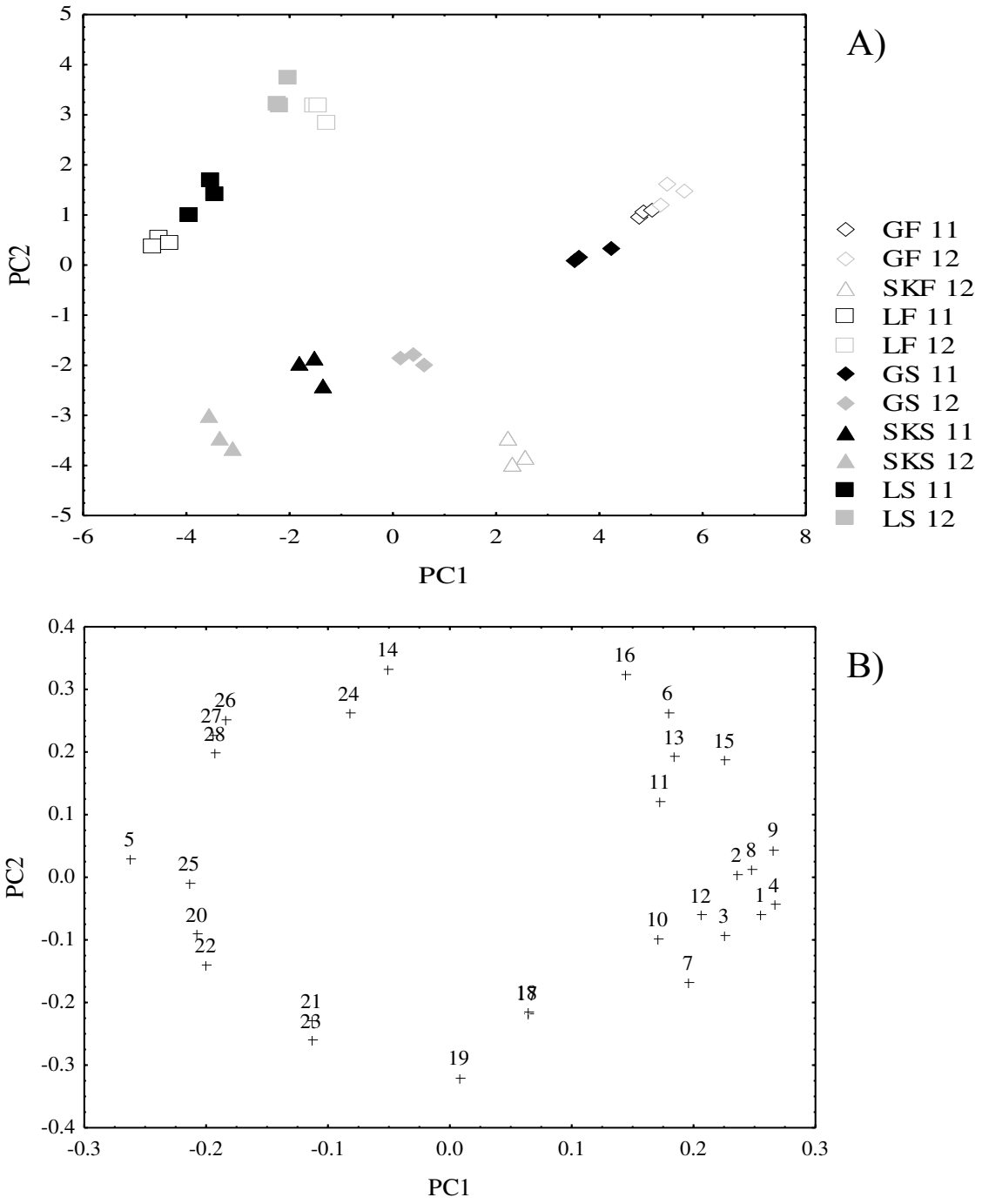

Figure 3.3. Principal Components Analysis (PCA) applied to the anthocyanins profiles of Cabernet franc (F) and Cabernet sauvignon (S) . Plots on plane PC1 (factor 1) and PC2 (factor 2) corresponding to: A) anthocyanins profiles, different sample types (grapes, fermented skins, and wine lees) of two vintages (years 2011 and 2012); B) anthocyanins profiles, most correlated variables (identification of the number referred to Table 3.1. G: grape; SK: skin; L: lees; 11: year 2011 and 12: year 2012.

3.2 Flavonols, Hydroxycinnamic Acid Derivatives (HCAD) and stilbenes in Grapes and their Winemaking By-Products

In this study, 18 flavonols were detected in grapes of cultivars Cabernet franc and Cabernet sauvignon and their winemaking by-products (fermented skin and lees), and are presented in **Table 3.3 and 3.4**, respectively. The profile of the flavonols is considered a characteristic of the grape variety. Thus, the identified compounds are derived from the six structures of flavonoids commonly reported for *Vitis vinifera* and not vinifera grapes (**Figures 3.4 and 3.5**) (Hermosín-Gutiérrez, Castillo-Muñoz, Gómez-Alonso, & García-Romero, 2011, Lago-Vanzela et al., 2011a): the B-ring mono-substituted flavonoid, kaempferol (K; *m/z* 285); the B-ring di-substituted, quercetin (Q; *m/z* 301) and isorhamnetin (I; *m/z* 315); and the-B-ring-trisubstituted, myricetin (M; *m/z* 317), laricitrin (L; *m/z* 331) and syringetin (S; *m/z* 345).

Table 3.3. Flavonols, hydroxycinnamic acid derivatives, and stilbenes identified in freeze-dried samples of Cabernet franc grapes, their fermented skins and lees, in years 2011 and 2012: chromatographic and mass spectral characteristics (negative ionization mode); Peak numbers as in Figure 3.4.

peak	assignation*	R_t (min)	molecular ion; product ions (*m/z*)	grape 2011 (molar %)	grape 2012 (molar %)	skin 2012 (molar %)	less 2011 (molar %)	less 2012 (molar %)
1	M-3-glcU	16,89	493; 317	1.88 ± 0.20^{a}	$1.35\pm0.15^{b\alpha}$	$1.15\pm0.01^{\alpha}$	ND	$0.52\pm0.08^{\beta}$
2	M-3-gal	17,45	479; 317	2.23 ± 0.12	$2.10\pm0.17^{\alpha}$	$1.23\pm0.09^{\beta}$	ND	$0.35\pm0.01^{\gamma}$
3	M-3-glc	18.59	479; 317	14.68 ± 0.33^{a}	$9.96\pm0.36^{b\alpha}$	$4.42\pm0.38^{\beta}$	ND	$0.82\pm0.02^{\gamma}$
4	Q-3-gal	25.17	463; 301	3.61 ± 0.13^{bA}	$6.42\pm0.45^{a\alpha}$	$4.17\pm0.09^{\beta}$	1.70 ± 0.26^{bB}	$7.01\pm0.40^{a\alpha}$
5	Q-3-glcU	25.67	477; 301	25.61 ± 0.94^{aA}	$23.50\pm0.66^{b\beta}$	$26.97\pm0.21^{\alpha}$	2.29 ± 0.34^{bB}	$4.15\pm0.35^{a\gamma}$
6	Q-3-glc	27.08	463; 301	26.99 ± 0.87^{bA}	$29.63\pm0.49^{a\alpha}$	$19.02\pm0.41^{\beta}$	1.51 ± 0.07^{bB}	$2.15\pm0.12^{a\gamma}$
7	Q-3-rut	27.74	609; 301	2.03 ± 0.18^{b}	$2.62\pm0.09^{a\alpha}$	$0.76\pm0.03^{\beta}$	ND	ND
8	Free M	29.89	317	0.92 ± 0.42^{B}	$0.55\pm0.05^{\gamma}$	$0.99\pm0.07^{\beta}$	12.96 ± 1.80^{A}	$11.50\pm0.21^{\alpha}$
9	L-3-glc	30.35	493; 331	4.08 ± 1.43	$5.19\pm0.30^{\beta}$	$6.63\pm0.09^{\alpha}$	ND	$1.71\pm0.10^{\gamma}$
10	Q-3-rha	32.55	447; 301	0.68 ± 0.09^{a}	0.50 ± 0.05^{b}	0.56 ± 0.01	ND	ND
11	K-3-glc	32.30	447; 285	2.25 ± 0.24^{bA}	$4.87\pm0.55^{a\alpha}$	$1.62\pm0.14^{\beta}$	1.52 ± 0.05^{aB}	$0.25\pm0.34^{b\gamma}$
12	I-3-glc	36.83	477; 315	6.14 ± 0.17^{bA}	$8.29\pm0.20^{a\alpha}$	$7.27\pm0.18^{\beta}$	0.45 ± 0.05^{bB}	$0.75\pm0.02^{a\gamma}$
13	S-3-glc	38.81	507; 345	5.06 ± 0.21^{a}	$4.29\pm0.17^{b\beta}$	$8.04\pm0.12^{\alpha}$	5.26 ± 0.06^{a}	$2.99\pm0.07^{b\gamma}$
14	free Q	40.59	301	1.40 ± 0.38^{aB}	$0.74\pm0.07^{b\gamma}$	$10.26\pm1.06^{\beta}$	55.73 ± 1.33^{aA}	$52.46\pm0.58^{b\gamma}$
15	Free L	44.20	331	1.82 ± 0.33^{B}	ND	$0.18\pm0.01^{\beta}$	4.27 ± 0.15^{aA}	$2.85\pm0.11^{b\alpha}$
16	free K	49.50	285	0.28 ± 0.14^{B}	ND	$1.90\pm0.23^{\beta}$	5.05 ± 0.31^{A}	$5.44\pm0.23^{\alpha}$
17	free I	53.44	315	0.21 ± 0.03^{B}	ND	$4.58\pm0.35^{\beta}$	8.26 ± 0.61^{aA}	$6.62\pm0.24^{b\alpha}$
18	Free S	55.15	345	0.12 ± 0.04^{B}	ND	$0.27\pm0.03^{\beta}$	1.00 ± 0.14^{aA}	$0.44\pm0.05^{b\alpha}$
total (mg/kg sample)**				212.01 ± 12.74^{bB}	$350.90\pm15.22^{a\gamma}$	$776.60\pm25.90^{\beta}$	1237.86 ± 97.81^{A}	$1310.53\pm172.86^{\alpha}$
19	*trans*-caftaric acid	3.59	311; 179, 149	71.80 ± 0.35^{aA}	$66.47\pm2.61^{b\alpha}$	$69.05\pm1.41^{\alpha}$	16.06 ± 0.11^{bB}	$39.52\pm0.64^{a\beta}$
20	*trans*-coutaric acid	5.03	295; 163, 149	24.13 ± 0.34^{aA}	$18.17\pm1.45^{b\alpha}$	$11.90\pm0.91^{\gamma}$	8.91 ± 0.72^{bB}	$14.82\pm0.41^{a\beta}$
21	*trans*-caffeic acid	6.41	179	ND	ND	ND	66.10 ± 0.29^{a}	22.10 ± 0.72^{b}
22	*trans*-fertaric acid	6.70	325; 193, 149	1.15 ± 0.00^{bB}	$4.95\pm0.99^{a\beta}$	$19.05\pm2.01^{\alpha}$	1.94 ± 0.10^{A}	$2.00\pm0.08^{\beta}$

Continuation…

23	*cis*-fertaric acid	7.22	325; 193, 149	2.92±0.02^{b}	10.40±1.61^{a}	ND	ND	ND
24	*p*-coumaric acid	11.07	163	ND	ND	ND	6.99±0.78^{b}	14.94±0.42^{a}
25	ethyl caffeate	40.00	207; 179	ND	ND	ND	ND	6.62±0.11
total (mg/kg sample)* **				243.27±19.14^{aB}	56.14±7.95$^{b\beta}$	70.55±2.99$^{\beta}$	754.38±63.96^{A}	1015.25±179.33$^{\alpha}$
26	*trans*-piceid	19.04	389;227	94.49±0.59	ND	ND	ND	ND
27	*trans*-resveratrol	28.80	227	ND	ND	ND	ND	ND
28	*cis*-piceid	30.7	389;227	5.51±0.59	ND	ND	ND	ND
total (mg/kg sample)** **				29.88±1.94				

* Nomenclature abbreviations: M, myricetin; Q, quercetin; L, laricitrin; I, isoramnetin; S, syringetin; K, kaempferol; glcU, glucuronide; glc, glucoside; rha, rhamnoside; gal, galactoside; rut, rutinoside (6''-rhamnosylglucoside). ND, not detected. ** As caftaric acid. *** As quercetin-3-glucoside equivalents (Q-3-glc). **** As resveratrol equivalents. Different low case letters for the samples of different vintages (2011 and 2012) of each kind of sample (grape, skin, or lees) means significant differences (ANOVA, $p < 0.05$). Different capital or Greek letters for the three samples (grape, fermented skin, and lees) of each vintage (years 2011 or 2012, respectively) means significant differences (ANOVA, $p < 0.05$). Molar percentages given as mean value ± standard deviation (n=3).

Table 3.4. Flavonols, hydroxycinnamic acid derivatives, and stilbenes identified in freeze-dried samples of Cabernet sauvignon grapes, their fermented skins and lees, in years 2011 and 2012: chromatographic and mass spectral characteristics (negative ionization mode); Peak numbers as in Figure 3.5.

peak	assignation*	R_t (min)	molecular ion; product ions (*m/z*)	grape 2011 (molar %)	grape 2012 (molar %)	skin 2011 (molar %)	Skin 2012 (molar %)	less 2011 (molar %)	less 2012 (molar %)
1	M-3-glcU	16,89	493; 317	1.53 ± 0.16^{bA}	$2.02\pm0.32^{a\alpha}$	0.96 ± 0.03^{bB}	$1.35\pm0.04^{a\beta}$	0.27 ± 0.05^{bC}	$0.89\pm0.09^{a\gamma}$
2	M-3-gal	17,45	479; 317	1.03 ± 0.16^{bA}	$1.73\pm0.08^{a\alpha}$	0.51 ± 0.06^{bB}	$0.99\pm0.12^{a\beta}$	0.13 ± 0.02^{C}	$0.14\pm0.01^{\gamma}$
3	M-3-glc	18.59	479; 317	13.26 ± 0.66^{aA}	$6.56\pm0.12^{b\alpha}$	7.30 ± 0.02^{aB}	$4.05\pm0.35^{b\beta}$	0.83 ± 0.06^{bC}	$2.78\pm0.04^{a\gamma}$
4	Q-3-gal	25.17	463; 301	2.88 ± 0.25^{A}	$3.42\pm0.38^{\alpha}$	1.17 ± 0.18^{B}	$1.03\pm0.05^{\beta}$	0.06 ± 0.02^{bC}	$0.29\pm0.04^{a\gamma}$
5	Q-3-glcU	25.67	477; 301	32.76 ± 1.01^{aA}	$27.28\pm0.06^{\alpha b}$	13.96 ± 0.37^{bB}	$23.69\pm0.35^{a\beta}$	1.32 ± 0.17^{bC}	$4.19\pm0.26^{a\gamma}$
6	Q-3-glc	27.08	463; 301	27.02 ± 0.44^{aA}	$19.66\pm0.26^{b\alpha}$	11.09 ± 0.20^{aB}	$9.29\pm0.34^{b\beta}$	1.74 ± 0.19^{bC}	$2.38\pm0.08^{a\gamma}$
7	Q-3-rut	27.74	609; 301	2.38 ± 0.14^{aA}	$1.77\pm0.01^{b\alpha}$	0.65 ± 0.12^{aB}	$0.28\pm0.05^{b\beta}$	ND	ND
8	Free M	29.89	317	0.47 ± 0.07^{aC}	$0.28\pm0.01^{b\gamma}$	5.62 ± 0.54^{aB}	$2.62\pm0.52^{b\beta}$	18.99 ± 0.74^{bA}	$27.81\pm0.46^{a\alpha}$
9	L-3-glc	30.35	493; 331	3.21 ± 0.29^{bB}	$5.63\pm0.18^{a\beta}$	4.49 ± 0.35^{bA}	$7.55\pm0.14^{a\alpha}$	ND	ND
10	Q-3-rha	32.55	447; 301	1.29 ± 0.10^{b}	$1.64\pm0.13^{a\beta}$	1.25 ± 0.16^{b}	$2.67\pm0.29^{a\alpha}$	ND	$0.61\pm0.03^{\gamma}$
11	K-3-glc	32.30	447; 285	1.96 ± 0.13^{bA}	$2.79\pm0.01^{a\alpha}$	0.54 ± 0.06^{bC}	$0.78\pm0.01^{a\beta}$	1.49 ± 0.10^{aB}	$0.11\pm0.01^{b\gamma}$
12	I-3-glc	36.83	477; 315	5.52 ± 0.11^{bA}	$10.25\pm0.14^{a\alpha}$	2.99 ± 0.11^{bB}	$4.20\pm0.06^{a\beta}$	0.35 ± 0.04^{bC}	$0.48\pm0.01^{a\gamma}$
13	S-3-glc	38.81	507; 345	6.70 ± 0.28^{bB}	$17.25\pm0.64^{a\beta}$	7.59 ± 0.05^{bA}	$24.36\pm0.83^{a\alpha}$	3.14 ± 0.20^{C}	$3.93\pm0.07^{\gamma}$
14	free Q	40.59	301	ND	ND	30.67 ± 0.39^{aB}	$8.33\pm0.55^{b\beta}$	53.79 ± 1.28^{aA}	$43.51\pm0.58^{b\alpha}$
15	Free L	44.20	331	ND	ND	1.33 ± 0.08^{B}	$1.73\pm0.26^{\beta}$	3.97 ± 0.28^{aA}	$2.87\pm0.33^{b\alpha}$
16	free K	49.50	285	ND	ND	2.75 ± 0.12^{aB}	$0.88\pm0.16^{b\beta}$	4.45 ± 0.35^{A}	$4.11\pm0.09^{\alpha}$
17	free I	53.44	315	ND	ND	5.79 ± 0.20^{B}	5.35 ± 0.34	8.36 ± 0.26^{aA}	5.32 ± 0.19^{b}
18	Free S	55.15	345	ND	ND	1.35 ± 0.15^{a}	$0.86\pm0.12^{b\alpha}$	1.12 ± 0.21^{a}	$0.59\pm0.02^{b\beta}$
total (mg/kg sample)**				177.92 ± 15.11^{aC}	$103.14\pm3.23^{b\gamma}$	808.10 ± 60.30^{aB}	$262.85\pm7.70^{b\beta}$	1487.50 ± 154.84^{aA}	$1198.65\pm1.63^{b\alpha}$
19	*trans*-caftaric acid	3.59	311; 179, 149	68.16 ± 0.88^{Ba}	$75.72\pm0.44^{a\alpha}$	47.27 ± 0.19^{bB}	$53.74\pm1.80^{a\beta}$	28.88 ± 1.16^{bC}	$47.57\pm0.60^{a\gamma}$
20	*trans*-coutaric acid	5.03	295; 163, 149	28.25 ± 1.08^{aA}	$12.71\pm0.93^{b\beta}$	8.82 ± 0.54^{bC}	$12.52\pm0.66^{a\beta}$	14.77 ± 0.53^{bB}	$30.12\pm1.08^{a\alpha}$
21	*trans*-caffeic acid	6.41	179	ND	ND	21.57 ± 0.27^{bB}	$26.66\pm2.86^{a\alpha}$	47.44 ± 0.75^{aA}	$12.95\pm1.37^{b\beta}$
22	*trans*-fertaric acid	6.70	325; 193, 149	0.59 ± 0.05^{bC}	$11.57\pm1.32^{a\alpha}$	2.63 ± 0.23^{bA}	$7.09\pm0.60^{a\beta}$	1.54 ± 0.19^{B}	$1.65\pm0.30^{\gamma}$

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23	*cis*-fertaric acid	7.22	325; 193, 149	3.01±0.26^{B}	ND	19.71±0.87^{A}	ND	ND	ND
24	*p*-coumaric acid	11.07	163	ND	ND	ND	ND	7.38±1.34	7.72±0.17
25	ethyl caffeate	40.00	207; 179	ND	ND	ND	ND	ND	ND
total (mg/kg sample)* **				199.80±5.60^{aB}	14.21±1.80$^{b\beta}$	66.11±2.27^{aB}	22.98±6.70$^{b\beta}$	572.38±116.65^{A}	448.50±10.14$^{\alpha}$
26	*trans*-piceid	19.04	389;227	41.47±3.20^{aB}	8.71±0.43$^{b\beta}$	ND	ND	62.84±0.79^{aA}	30.19±1.01$^{b\alpha}$
27	*trans*-resveratrol	28.80	227	53.60±3.45^{bA}	85.73±22.43$^{a\alpha}$	ND	ND	33.12±0.88^{bB}	68.13±1.12$^{a\beta}$
28	*cis*-piceid	30.7	389;227	4.93±0.26^{A}	5.56±2.93$^{\alpha}$	ND	ND	4.04±0.49^{aB}	1.68±0.18$^{b\beta}$
total (mg/kg sample)** **				63.87±3.68^{a}	6.21±0.68$^{b\beta}$			63.33±14.53^{b}	184.41±15.25$^{a\alpha}$

Nomenclature abbreviations: M, myricetin; Q, quercetin; L, laricitrin; I, isoramnetin; S, syringetin; K, kaempferol; glcU, glucuronide; glc, glucoside; rha, rhamnoside; gal, galactoside; rut, rutinoside (6''-rhamnosylglucoside). ND, not detected. ** As caftaric acid. *** As quercetin-3-glucoside equivalents (Q-3-glc). **** As resveratrol equivalents. Different low case letters for the samples of different vintages (2011 and 2012) of each kind of sample (grape, skin, or lees) means significant differences (ANOVA, $p < 0.05$). Different capital or Greek letters for the three samples (grape, fermented skin, and lees) of each vintage (years 2011 or 2012, respectively) means significant differences (ANOVA, $p < 0.05$). Molar percentages given as mean value ± standard deviation (n=3).

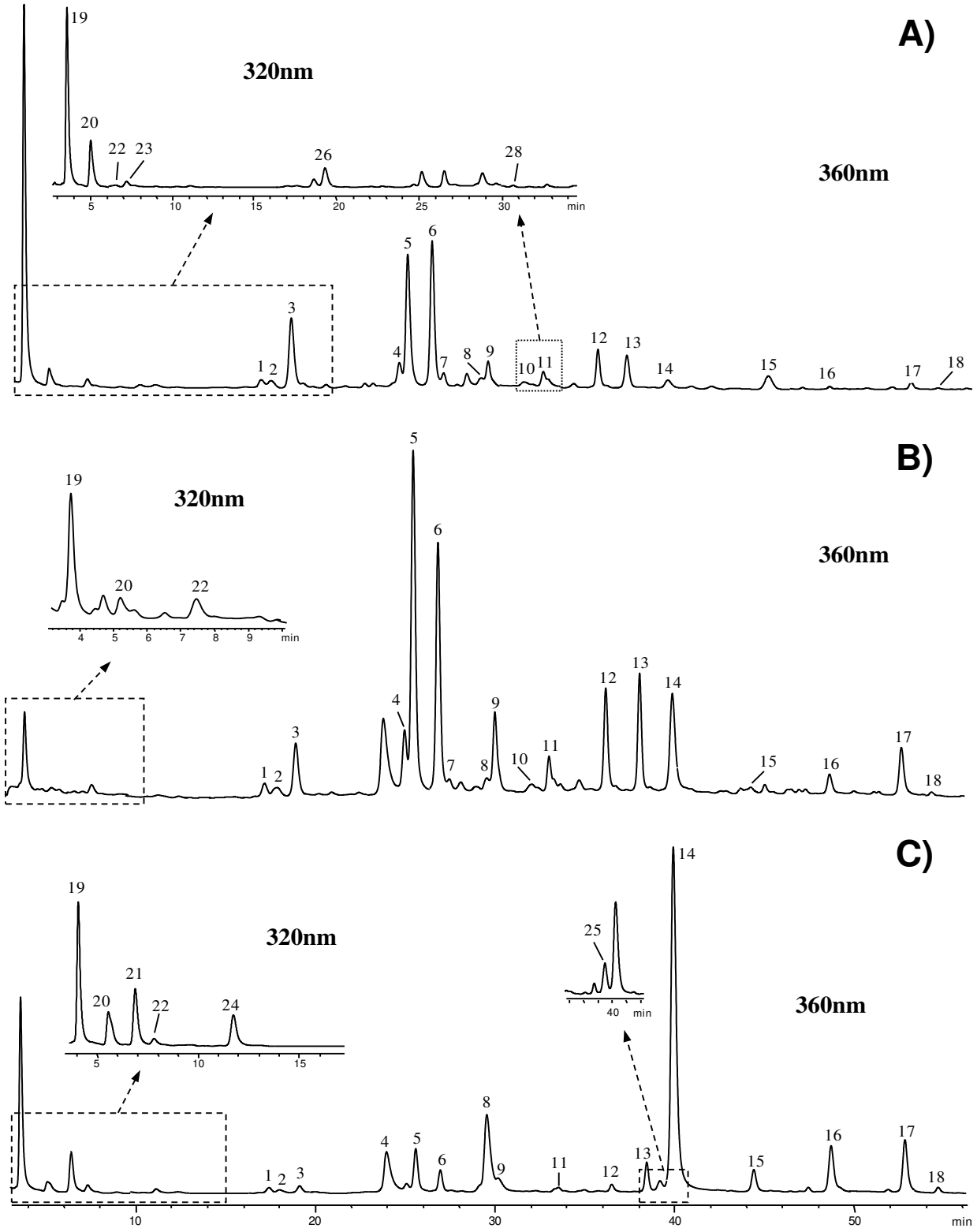

Figure 3.4. DAD-chromatograms corresponding to the profiles of flavonols (detection at 360 nm), hydroxycinnamic acid derivatives and stilbenes (figure enlargements with detection at 320 nm) of Cabernet franc samples: A) grapes; (B) fermented skins, (C) wine lees. Identification of the peaks referred to Table 3.3.

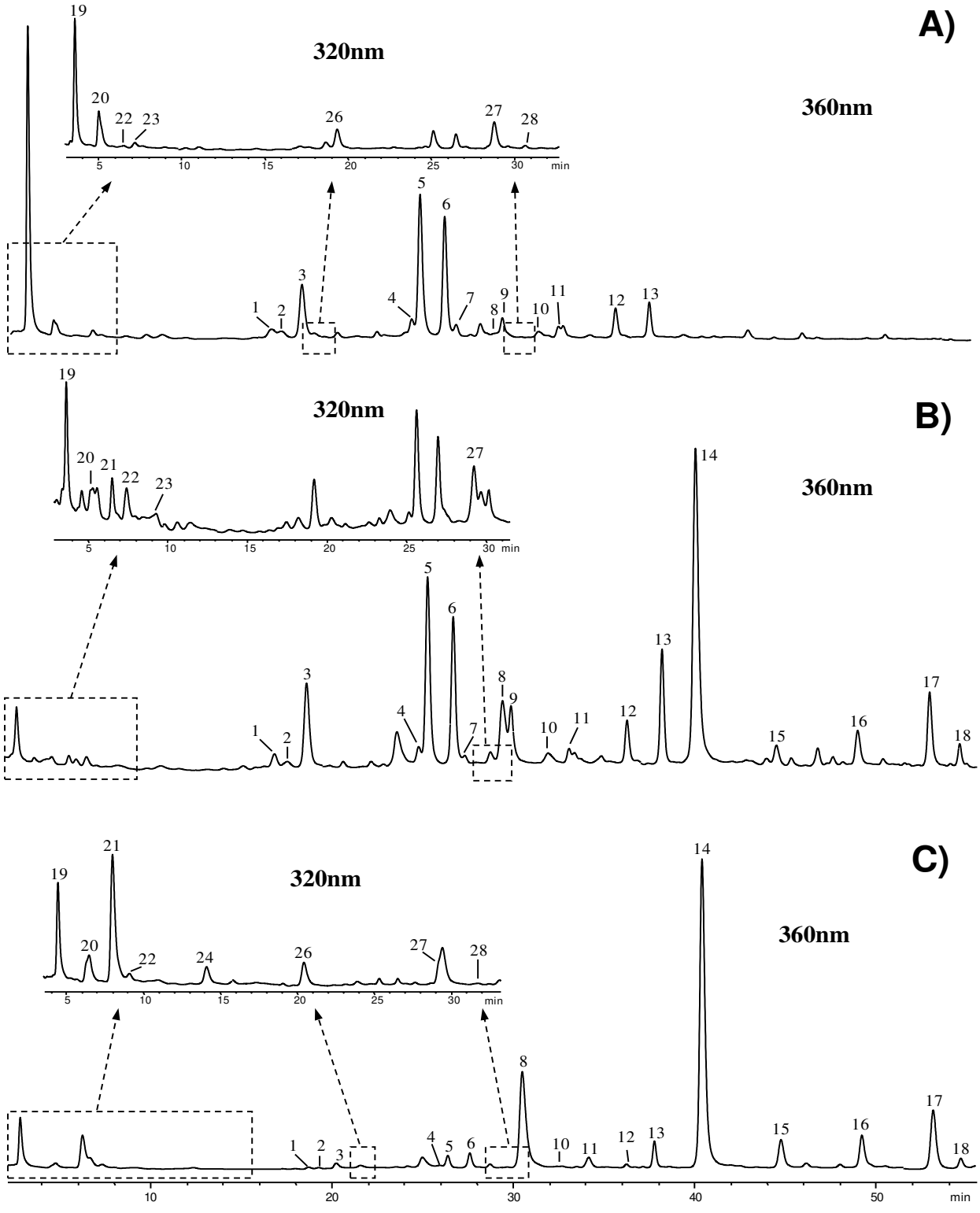

Figure 3.5. DAD-chromatograms corresponding to the profiles of flavonols (detection at 360 nm), hydroxycinnamic acid derivatives and stilbenes (figure enlargements with detection at 320 nm) of Cabernet sauvignon samples: A) grapes; (B) fermented skins, (C) wine lees. Identification of the peaks referred to Table 3.4.

Grape flavonols were mainly detected as the 3-glucosides (3-glc) of all the above mentioned structures, together with the 3-glucuronides (3-glcU) and the 3-galactosides (3-gal) of quercetin and myricetin, and the 3-rutnoside (3-rut) and 3-rhamnoside (3-rhm) of quercetin. In the grapes of both cultivars free M was found (<1%) and, in the case of Cabernet franc, the other five free aglycones were also found (Q, L, K, I, S; all of them less than 2%). Generally, grape samples do not contain flavonols as free aglycones, however, when present, it is considered an artifact of the extraction method under acid conditions, which sometimes happens (Hermosín-Gutiérrez et al., 2011). The same flavonol glycosides were found in both by-products (fermented skins and lees), together with higher proportions of free aglycones, especially in lees (83% for Cabernet franc and 87% for Cabernet sauvignon), very likely due to hydrolysis of flavonol 3-glycosides during vinification (Castillo-Muñoz, Gómez-Alonso, García-Romero, & Hermosín-Gutiérrez, 2007, Hermosín-Gutiérrez et al., 2011). Q-3-rutinoside was missing in the lees of both grape cultivars whereas L-3-glucoside was not found in Cabernet sauvignon and Q-3-rhamnoside was absent in Cabernet franc lees samples. Flavonols based on quercetin were predominant in all samples: average of 51% for free Q in lees; averages of 16% for free Q, 22% for Q-3-glcU and 13% for Q-3-glc in fermented skins; and averages of 27% for Q-3-glcU and 34% for Q-3-glc in grapes.

Ferradino et al. (2012) examined the fresh skins of Cabernet sauvignon cultivated in Italy and found 34.3% Q-3-glc, 25.8% M-3-glc, 25% Q-3-glcU, and 14.5% K-3-glc. Slier, Neira, Solís, Marín, Da-Silva and Laureano (2010) also identified M-3-glc and Q-3-glc in fresh skins of Cabernet sauvignon from Chile, as well as Q-3-gal, K-3-gal, K-3-glc, and I-3-glc. Similar profile was observed in fresh skins of Spanish Cabernet sauvignon (Castillo-Muñoz et al., 2007): 6% M-3-glcU, 20.9% M-3-glc, 23.3% Q-3-glcU, 26.7% Q-3-glc, 6.5% L-3-glc, 3.8% K-3-glc, 4.5% I-3-

glc, and 8.9% S-3-glc. Rockenbach et al. (2011) noted the presence of derivatives of quercetin and kaempferol in fermented skin of Primitivo, Sangiovese, Pinot noir, Negro amaro, Cabernet sauvignon and Isabel cultivars from Brazilian winery by-products. Spanish winery by-products from Cabernet sauvignon showed a similar flavonol profile, however, myricetin-3-glucoside and isorhametin-3-glucoside cultivar were also detected (Rubilar et al., 2007).

Variations of flavonol composition between harvests can be explained by Poudal (2009) and Krammerer et al. (2005) due to several factors, as already mentioned elsewhere. In our study, the molar percentages of flavonols showed variations when comparing the years 2011 and 2012, with the exception of Q-3-gal, M-3-gal, S-3-glc, free L, free K, and free I in samples of Cabernet sauvignon and M-3-gal, L-3-glc, free M, and free K in samples of Cabernet franc which did not showed statistical differences ($p < 0.05$). Regarding the content of total flavonols, there were also statistical variations between the years analyzed in both samples (grape, fermented skin and lees) of the two cultivars (**Table 3.3 and 3.4**). The same was observed when compared grapes and skins of Cabernet franc with Cabernet sauvignon each year, where Cabernet franc showed predominantly higher concentrations. The lees of the two cultivars were not statistically different at $p<0.05$ when compared in the same year. However, one cannot suggest the causes of variations, because the biosynthesis of these compounds is affected by many factors and may be different depending on the class of phenolic compounds. The lees of both cultivars were found as important sources of flavonols showing higher amounts than in the grapes and fermented skin: average of 1274.20 mg/kg (Cabernet franc) and 1347.10 mg/kg (Cabernet sauvignon) in the lees; average of 776.60 mg/kg (Cabernet franc) and 535.47 mg/kg (Cabernet sauvignon) in the fermented skins; and average of 281.50 mg/kg (Cabernet franc) and 140.53 mg/kg (Cabernet sauvignon) in the grapes.

Hydroxycinnamic acid derivatives (HCAD) were identified in the samples based on comparison of spectroscopic data, especially MS/MS spectra shown in **Tables 3.3 and 3.4.** The hydroxycinnamoyl-tartaric acids were detected in all samples. Two HCAD predominated, namely, caftaric acid (from caffeic acid) in an average of 53% and coutaric acid (from *p*-coumaric acid) in an average of 17%. Low concentrations of a third HCAD, the *trans* isomer of fertaric acid (from ferulic acid) in an average of 5%; its *cis* isomer was also detected in the grapes of Cabernet franc and in grape and fermented skin of Cabernet sauvignon (only year 2011). Hydroxycinnamoyl-tartaric acids might undergo hydrolysis during the winemaking process thus releasing free hydroxycinnamic acids, which can further react with the ethanol to form ethyl esters. Therefore, caffeic acid and *p*-coumaric acid were found in the lees of both samples. In the fermented skin, the caffeic acid was detected only for Cabernet sauvignon and ethyl caffeate was present in the lees Cabernet franc (year 2012). The HCAD profile in fresh skins of Cabernet sauvignon was previously reported by Ferradino et al. (2012), showing the presence of *trans*-caftaric acid, *cis*-*p*-coutaric acid, *trans*-*p*-coutaric acid, and *trans*-fertaric acid. In winemaking by-products from Cabernet mitos, caffeic, *p*-coumaric, ferulic, caftaric, coutaric, and fertaric acids were detected (Krammerer et al., 2005). The lees of both cultivars showed quantitatively and statistically ($p<0.05$) higher values of HCAD compared with the grapes and fermented skin, with average values of 884.82 mg/kg (Cabernet franc) and 510.44 mg/kg (Cabernet sauvignon), versus 149.71 mg/kg (Cabernet franc) and 107.0 mg/kg (Cabernet sauvignon) in the grapes and 63.35 mg/kg (Cabernet franc) and 44.55 mg/kg (Cabernet sauvignon) in the fermented skin.

The presence of *trans*-resveratrol was expected. Several studies have reported that fresh skins contains about 50-100 μg/g of resveratrol, while concentration in red wine varies between 1.5 and 3.0 mg/L. Several studies on the bioactive properties of resveratrol and its derivatives

have recently demonstrated its potential as antimutagenic, antioxidant, anti-inflammatory and anti-proliferative agent (Rockenbach et al., 2011). In this study, the detection of stilbenes was possible because of the simplicity of the chromatogram in the elution interval. However, this area is the same where the flavonols and residual anthocyanins are eluted, making it difficult to separate and identify the appropriate stilbenes of interest. The latter may be one reason for the observed lack of *trans*-resveratrol in grapes, fermented skin and lees of Cabernet franc (**Table 3.3**). In the grapes and lees of Cabernet sauvignon *trans*-resveratrol was detected, in addition to *trans*-piceid and *cis*-piceid (**Table 3.4**). It is suggested that part of resveratrol may have been transferred to the wine and part further absorbed on the lees, thus, the absence of resveratrol in the fermented skins could be explained. Quantitatively, the grape Cabernet franc of year 2011 showed a resveratrol content of 29.88 mg/kg, whereas the grape Cabernet sauvignon showed a content of 63.87 and 6.21 mg/kg for years 2011 and 2012, respectively. The lees of Cabernet sauvignon (year 2011) showed no significant difference ($p<0.05$) compared with the grape in the same year, by contrast, in year 2012 the lees exhibited a concentration greater than grape (184.41 vs. 6.21 mg/kg).

All differences above mentioned, based on ANOVA results for the composition of flavonols, stilbenes and HCAD, were better visualized by principal component (PC) analysis (**Figure 3.6**), where PC1 (factor 1) and PC2 (factor 2) explained 54.62 and 14.46% of total variance, respectively. The results showed in **Figure 3.6A** clearly represented the differences between the samples of grape, fermented skin and lees along the PC-1 axis: lees separated mainly by their higher proportions of hydrolysis products, mainly free flavonol aglycones and free hydroxycinnamic acids; on the opposite site, grape samples characterizaed by the highest proportions of non-hydrolyzed flavonols, HCAD and stilbenes (**Figure 3.6B**). Fermented skin

samples just presented an intermediate situation, and was additionally separated from lees samples and most of the grape samples (except those of Cabernet sauvignon from year 2012) along the PC-2 axis: fermented skin accounted for lower proportions of some minor compounds, like some flavonols and HACD (S-3-glc, free S, L-3-glc, Q-3-rha, and *trans*-fertaric acid).

The **Figure 3.6C** shows the distinction between the Cabernet sauvignon and Cabernet franc cultivars, which PC3 explained 8.98% of the total variance, while the PC5 contributed 4.80% of the total variance explained. The main HCAD that influenced in this separation were *trans*-coutaric acid, *trans*-fertaric, and ethyl cafeoato, the flavonol was quercetin-3-galactoside, and the stilbene was *trans*-resveratrol (**Figura 3.6D**).

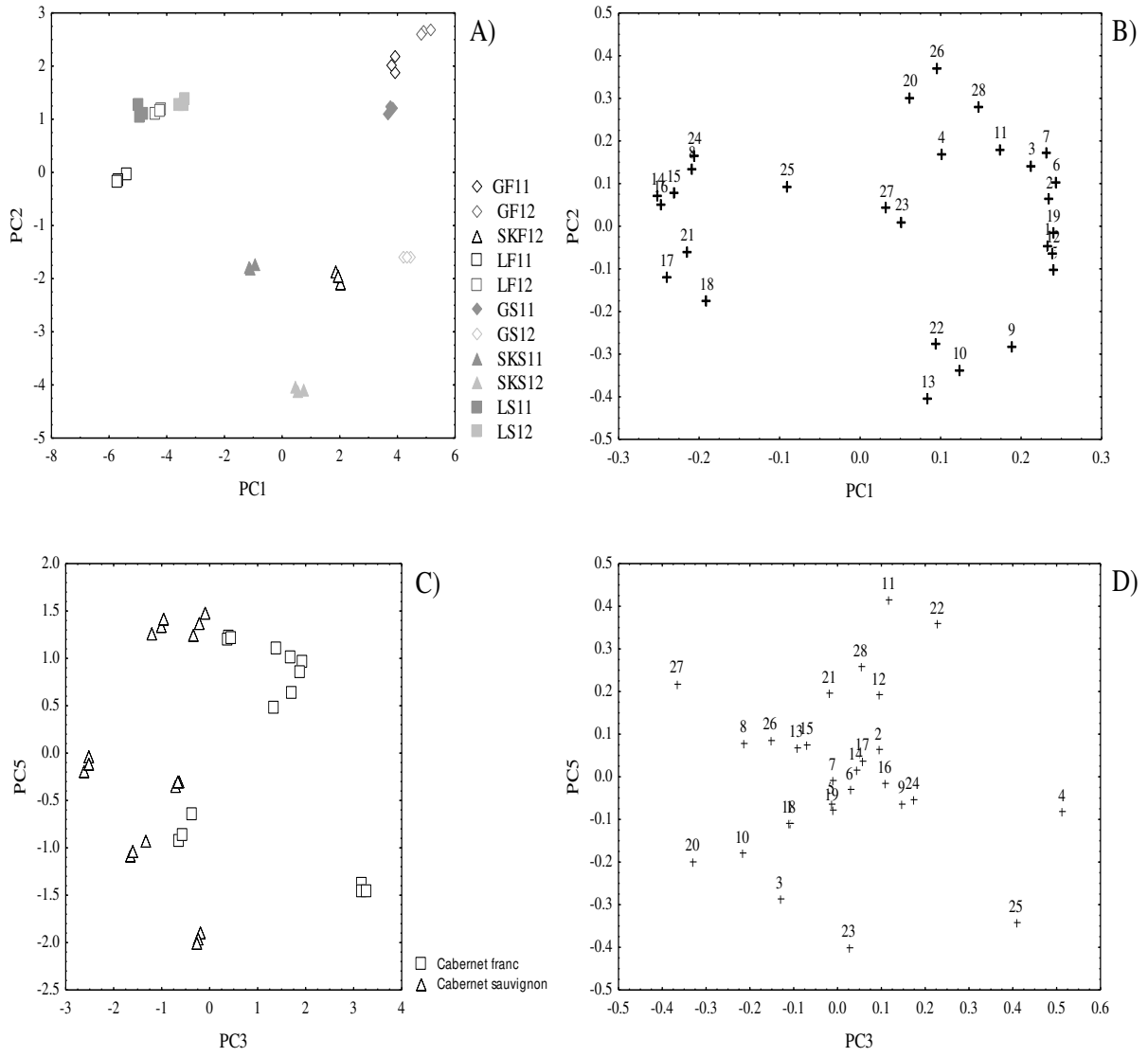

Figure 3.6. Principal Components Analysis (PCA) applied to the phenolic compound profiles of Cabernet franc (F) and Cabernet sauvignon (S). Plots on plane PC1 (factor 1) , PC2 (factor 2), PC3 (factor 3), and PC5 (factor5) corresponding to: A) phenolic compound profiles, different sample types (grapes, fermented skins, and wine lees) of two vintages (years 2011 and 2012); B) phenolic compound profiles, most correlated variables (identification of the number referred to Table 3.3); C) profiles of Cabernet franc and Cabernet sauvignon; D) flavonols and hydroxycinnamic acid derivatives profiles, most correlated variables (identification of the number referred to Table 3.3). *G: grape; SK: skin; L: lees; 11: year 2011 and 12: year 2012.

3.3 Effect of Drying Treatment on Anthocyanins, Flavonols, Hydroxycinnamic Derivatives and Stilbenes Content of Cabernet sauvignon and Cabernet franc Winemaking By-Products

The freeze-drying (FD) technique was used to achieve minimal thermal degradation of phenolic compounds present in analyzed samples (**Table 3.5**). The fermented skin of Cabernet sauvingon dried at 50 °C (D50) only contained 0.94 g/kg of total anthocyanins, thus representing a loss of around 47 % when compared with freeze-drying (FD) technique (**Figure 3.7**). This loss of anthocyanins was accompanied by changes in the profile of anthocyanins, where there was an increase in the molar percentage of pyranoanthocyanins and a decrease in the molar proportions of other anthocyanins, except for mv-3-glc, mv-3-acglc, dp-3-acglc, pn-3-acglc, and mv-3-*cis* cmglc, that remained statistically unchanged ($p<0.05$) when dried at 50 °C (D50).

The D50 lees contained 0.49 g/kg (Cabernet sauvignon) and 0.41 g/kg (Cabernet franc) of total anthocyanins, with a significant loss of 67 and 72%, respectively, with regards to FD samples. In contrast, the drying treatment by spray-drying (SD) did not affect the total anthocyanin content of the lees compared with FD. The drying treatments evaluated (D50 and SD) caused significant differences in the profiles of anthocyanins and derived compounds. Some of these pigments maintained almost unchanged their respective molar percentages (mv-3-glc, mv-3-*cis* cmglc, pn-3-cmglc, and pycy-3-glc for the lees of Cabernet sauvignon; pn-3-cmglc for the lees of Cabernet franc). In the lees subjected to D50 a significant increase in the content of pyranoanthocyanins was observed, and was also noticed the absence of some anthocyanins in the lees from D50 and SD treatments, which were detected in the lees treated by FD.

The D50 and SD techniques caused a negative effect in the total content of flavonols, hydroxycinnamic acid derivatives (HCAD), and stilbenes, which was of greater extent than those

observed for total anthocyanins content (**Figure 3.7**). In the fermented skin of Cabernet sauvignon, the flavonol (343.44 mg/kg) and HCAD (18.18 mg/kg of HCAD) total contents in D50 samples represented 57 and 98% reduction, respectively, when compared with FD (**Table 3.4**). The molar percentages of flavonols Q-3-rut, Q-3-rha, free I, and free S were not affected by D50 treatment. Regarding HCAD, a total loss of *cis*-fertaric acid was observed, together with 10-fold increase of the molar percentage of *trans*-fertaric acid. The others HCAD apparently were not affected.

The FD and SD lees showed similar flavonol profiles (**Figure 3.7**). However, the SD lees of Cabernet sauvignon (total flavonols, 1111.13 mg/kg), and Cabernet franc (total flavonols, 712.89 mg/kg) showed a loss of 25 and 42% in their respective total content of flavonols. The decrease in the concentration of flavonols in samples of D50 lees was 78% for Cabernet sauvignon (total flavonols, 324.20 mg/kg) and 90% for Cabernet franc (total flavonols, 121.56 mg/kg), mainly due to losses of free quercetin and free myricetin. The behavior of HACD was similar, with losses ranging between 55 and 91% in the total content of HACD. For the D50 lees, the final concentration was 54.37 and 77.80 mg/kg for Cabernet sauvignon and Cabernet franc, respectively. In the case of SD lees, the final concentrations were 260.46 and 183.56 mg/kg for the Cabernet sauvignon and Cabernet franc, respectively. These are due to the significant loss of *trans*-caffeic in the lees treated by D50 and losses of all other types of HACD in the lees treated by SD. It is noticeable that for Cabernet sauvignon lees there was a total loss of stilbenes.

Thus, freeze-dryng (FD) technique seems to be the most suitable to preserve the phenolic compounds and, subsequently, to process winemaking by-product samples. However, the spray-drying (SD) technique appears to be the adequate for preservation of the anthocyanins, a less expensive alternative to freeze-drying. On the basis of the results previously discussed, oven-

drying at 50 °C under forced air flow (D50), even being a non-expensive process, is suggested as a not indicated process for drying of winemaking by-products.

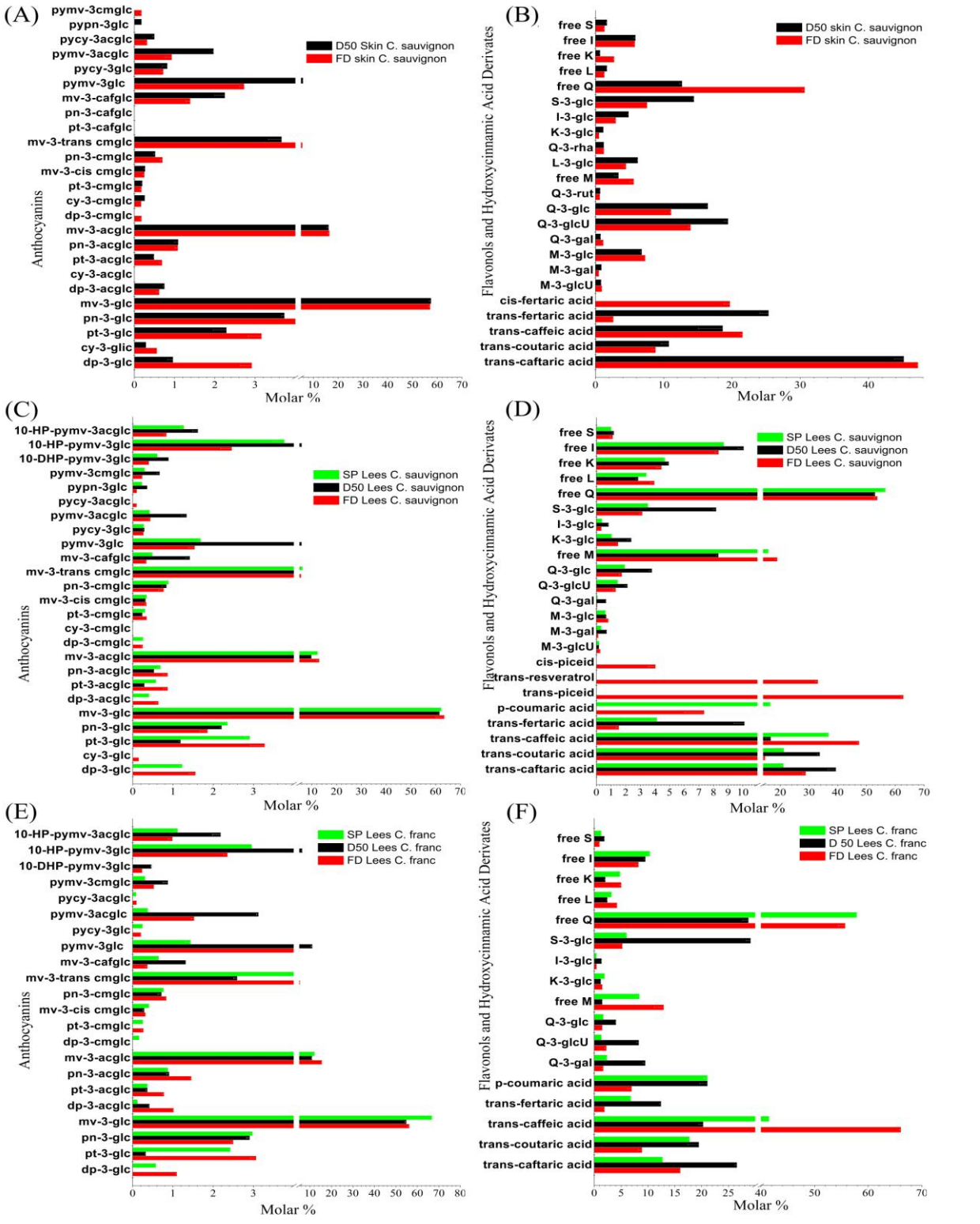

Figure 3.7. Molar profiles (%) of anthocyanins, flavonols and hydroxycinnamic acid derivatives identified in freeze-drying (FD), oven-dried at 50 °C (D50), and spray-dried (SP) samples of fermented skin and lees of Cabernet sauvignon (A, B, C, and D) and Cabernet franc cultivar (E and F) (only year 2011). Abbreviations like in Table 3.1 and 3.3.

4 CONCLUSIONS

Grapes and the winemaking by-products of *Vitis vinifera* cultivars contained on average 19 anthocyanins and 9 pyranoanthocyanins, predominantly anthocyanidin 3-glucosides. The presence of flavonols, hydroxycinnamic acids and stilbenes was detected in samples of grapes and in the by-products of Cabernet sauvingon and Cabernet franc cultivars. However, due to variations between sample years, further studies focused on the winemaking process and the vintage effect are needed in order to understand the parameters controlling the phenolic composition of winemaking by-products and, maybe, find the way to make standardized by-products for further good use. The total concentration of bioactive phenolic compounds found in these by-products was significant, especially when freeze-dried technique was applied. Thus, the winemaking by-products could become promissory sources of high-functional compounds.

5 ACKNOWLEDGMENTS

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6 REFERENCES

Amico, V., Napoli, E.M., Renda, A., Ruberto, G., Spatafora, C., & Tringali, C. (2004). Constituents of grape pomace from the Sicilian cultivar ‘Nerello Mascalese’. *Food Chemistry*, *88*, 599–607.

Babbar, N., Oberoi, H. S., Uppal, D. S., & Patil, R. T. (2011). Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. *Food Research International*, *44,* 391–396.

Blanco-Vega, D., López-Bellido, F. J., Alía-Robledo, J. M., & Hermosín-Gutiérrez, I. (2011). HPLC-DAD-ESI-MS/MS characterization of pyranoanthocyanins pigments formed in model wine. *Journal of Agricultural and Food Chemistry*, *59*, 9523-9531.

Boulton, R. (2001). The copigmentation of anthocyanins and its role in the color of red wine: A critical review. *American Journal of Enology and Viticulture*, *52*, 67-87.

Castillo-Muñoz, N., Fernández-Gonzalez, M., Gómez-Alonso, S., García-Romero, E., & Hermosín-Gutiérrez, I. (2009). Red-Color Related Phenolic Composition of Garnacha Tintorera (Vitis vinifera L.) Grapes and Red Wines. *Journal Agriculture Food Chemistr., 57,* 7883–7891.

Castillo-Muñoz, N., Gómez-Alonso, S., García-Romero, E., & Hermosín- Gutiérrez, I. (2007). Flavonol Profiles of Vitis vinifera Red Grapes and Their Single-Cultivar Wines. *Journal Agriculture Food Chemistry, 55*, 992−1002.

Cheng, V. J., Bekhit, A. E. A., McConnell, M., Mros, S., & Zhao, J. (2012). Effect of extraction solvent, waste fraction and grape variety on the antimicrobial and antioxidant activities of extracts from wine residue from cool climate. *Food Chemistry, 134,* 474–482.

Cortés, S., Rodríguez, R., Salgado, J. M., & Domínguez, J. M. (2011). Comparative study between Italian and Spanish grape marc spirits in terms of major volatile compounds. *Food Control, 22*, 673-680.

Ferrandino, A., Carra, A., Rolle, L., Schneider, A., & Schubert, A. (2012). Profiling of Hydroxycinnamoyl Tartrates and Acylated Anthocyanins in the Skin of 34 Vitis vinifera Genotypes. *Journal of Agriculture and Food Chemistry, 60*, 4931−4945.

Hermosín-Gutiérrez, I, Castillo-Muñoz, N., Gómez-Alonso, S., & García-Romero, E. (2011). Chapter 8. Flavonol profiles for grape and wine authentication. In S. E. Ebeler, G. R. Takeoka, & P. Winterhalter, Progress in authentication of food and wine (pp. 113-129). Washington, DC: American Chemical Society, ACS Symposium Series.

Kammerer, D., Claus, A., Carle, R., & Schieber, A. (2004). Polyphenol Screening of Pomace from Red and White Grape Varieties (*Vitis vinifera* L.) by HPLC-DAD-MS/MS. *Journal of Agriculture and Food Chemistry*, *52*, p. 4360−4367.

Kammerer, D., Claus, A., Schieber, A., & Reinhold C. (2005). A Novel Process for the Recovery of Polyphenols from Grape (*Vitis vinifera* L.) Pomace. *Journal of Food Science*, *70*, 2, 157-163.

Koundouras, S., Hatzidimitriou, E., Karamolegkou, M., Dimopoulou, E., Kallithraka, S., Tsialtas, J.T., Zioziou, E., Nikolaou, N., & Kotseridis, Y. (2009). Irrigation and root stock effects on the phenoliccon centration an daroma potential of *Vitis vinifera* L. cv. Cabernet Sauvignon grapes. *Journal of Agricultural and Food Chemistry, 57*, 7805-7813.

Lago-Vanzela, E.S., Da-Silva, R., Gomes, E., García-Romero, E., & Hermosín-Gutierrez, I. (2011a). Phenolic Composition of the Edible Parts (Flesh and Skin) of Bordô Grape (*Vitis labrusca*) Using HPLC−DAD−ESI-MS/MS. *Journal of Agricultural and Food Chemistry*, *59*, 13136−13146.

Lago-Vanzela, E.S., Da-Silva, R., Gomes, E., García-Romero, E., & Hermosín-Gutierrez, I. (2011b). Phenolic Composition of the Brazilian Seedless Table Grape Varieties BRS Clara and BRS Morena. *Journal of Agricultural and Food Chemistry*, *59*, 8314–8323.

Maragkoudakis, P.A., Nardi, T., Bovo, B., D'Andrea, M., Howell, K. S., Giacomini, A., & Corich, V. (2013). Biodiversity, dynamics and ecology of bacterial community during grape marc storage for the production of grappa. *International Journal of Food Microbiology, 162*, 2, 143-151.

Mendes, J. A. S., Prozil, S. O., Evtuguin, D. V., & Lopes, L. P. C. (2013). Towards comprehensive utilization of winemaking residues: Characterization of grape skins from red grape pomaces of variety Touriga Nacional. *Industrial Crops and Products, 43,* 25–32.

Morata, A., Gómez-Cordovés, M. C., Colomo, B., & Suárez, J. A. (2005). Cell Wall anthocyanin adsorption by different *Saccharomyces* strains during the fermentation of *Vitis vinifera* L. cv Graciano grapes. *European Food Research and Technology*, *220*, 341-346.

Nixdorf, S. L. & Hermosín-Gutiérrez, I. (2010). Brazilian red wines made from the hybrid grape cultivar Isabel: Phenolic composition and antioxidant capacity. *Analytica Chimica Acta, 659*, 208–215.

Paradelo, R., Moldes, A. B., & Barral, M. T. (2010). Utilization of a Factorial Design To Study the Composting of Hydrolyzed Grape Marc and Vinification Lees. *Journal of Agricultural and Food Chemistry*, *58*, 3085–3092.

Poudel, P. R., Mochioka, R., Beppu, K., & Kataoka, I. (2009). Influence of temperature on berry composition of interspecific hybrid wine grape 'Kadainou R-1' (*Vitis ficifolia* var. ganebu x *V. vinifera* 'Muscat of Alexandria'). *Journal of Japanese Society of Horticultural Sciences*, *78*, 169–174.

Rebello, L. P. G., Lago-Vanzela, E. S., Barcia, M. T., Ramos, A. M., Stringheta, P. C., Da-Silva, R., Castillo-Muñoz, N., Gómez-Alonso, S., & Hermosín-Gutiérrez, I. (2013). Phenolic composition of the berry parts of hybrid grape cultivar BRS Violeta (BRS Rubea × IAC 1398-21) using HPLC–DAD–ESI-MS/MS. *Food Research International, 54*, 354–366.

Rockenbach, I. I., Gonzaga, L. V., Rizelio, V. M., Gonçalves, A. E. S. S., Genovese, M. I., & Fett, R. (2011). Phenolic compounds and antioxidant activity of seed and skin extracts of red grape (Vitis vinifera and Vitis labrusca) pomace from Brazilian winemaking. *Food Research International, 44,* 897–901.

Rubilar, M., Pinelo, M., Shene, C., Sineiro, J., & Nuñez, M. J. (2007). Separation and HPLC-MS Identification of Phenolic Antioxidants from Agricultural Residues: Almond Hulls and Grape Pomace. *Journal of Agricultural and Food Chemistry*, *55,* 10101–10109.

Ryan, J. & Revilla, E. (2003). Anthocyanin Composition of Cabernet Sauvignon and Tempranillo Grapes at Different Stages of Ripening. *Journal of Agricultural and Food Chemistry, 51,* 3372-3378.

Shrikhande, A. J. (2000). Wine by-products with health benefits. *Food Research International, 33,* 469-474.

Slier, E. O., Neira, A. P., Solís, R. L., Marín, F. Z., Da-Silva, J. M. R., & Laureano, O. (2010). Comparative Study of the Phenolic Composition of Seeds and Skins from Carménère and Cabernet Sauvignon Grape Varieties (*Vitis vinifera* L.) during Ripening. *Journal of Agricultural and Food Chemistry, 58*, 3591–3599

CONCLUSÕES GERAIS

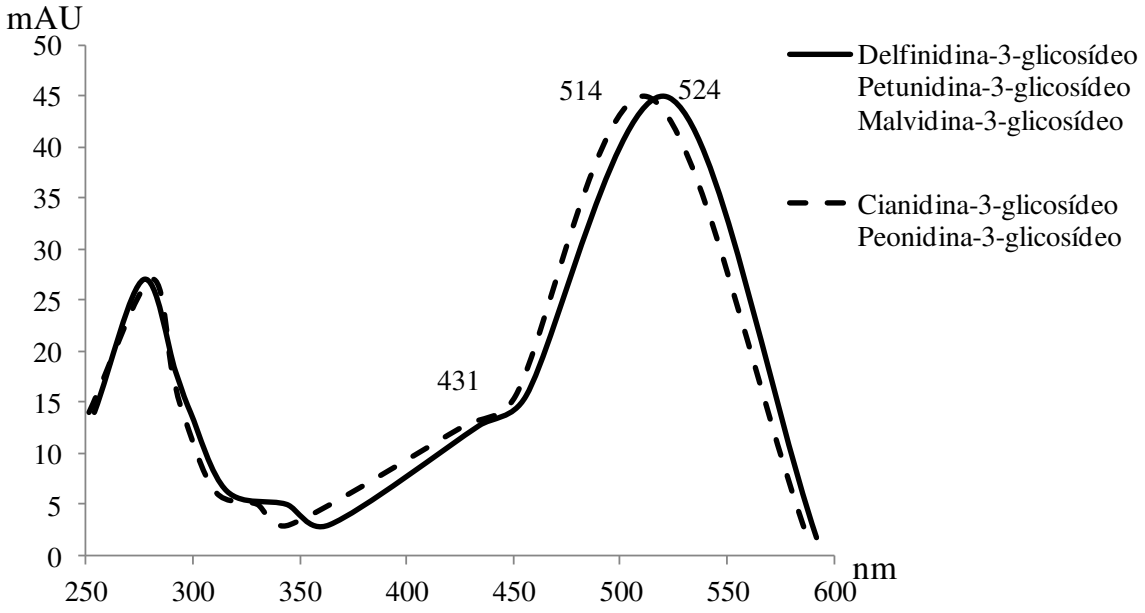
Através deste trabalho, conclui-se que os resíduos vinícolas analisados apresentaram quantidades consideráveis de fitoquímicos com possíveis propriedades promotoras de saúde, com destaque para os resíduos da cultivar BRS Violeta, hibrida, que apresentou as maiores concentrações de compostos fenólicos e antocianinas totais.

Dentre os resíduos analisados, as sementes de todas as cultivares se destacaram quanto à concentração de compostos fenólicos totais para todas as cultivares (BRS Violeta, BRS Lorena, Cabernet sauvignon e Cabernet franc), mostrando consequentemente as maiores atividades antioxidantes.

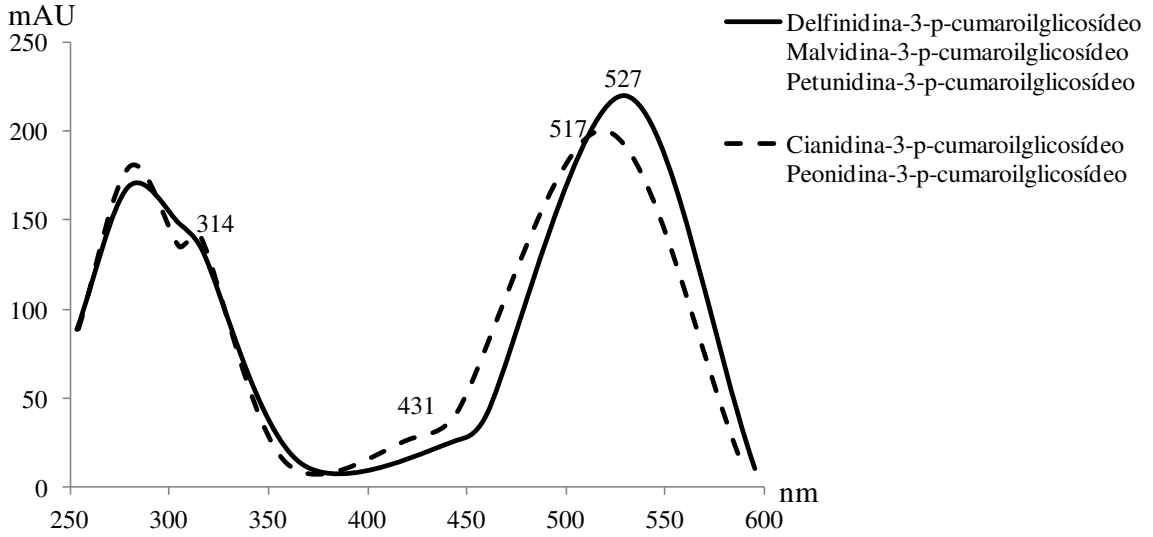
Assim, nos subprodutos da vinificação estudados se mostraram fontes promissoras de compostos funcionais (compostos fenólicos) estáveis com importante capacidade antioxidante.

APÊNDICES

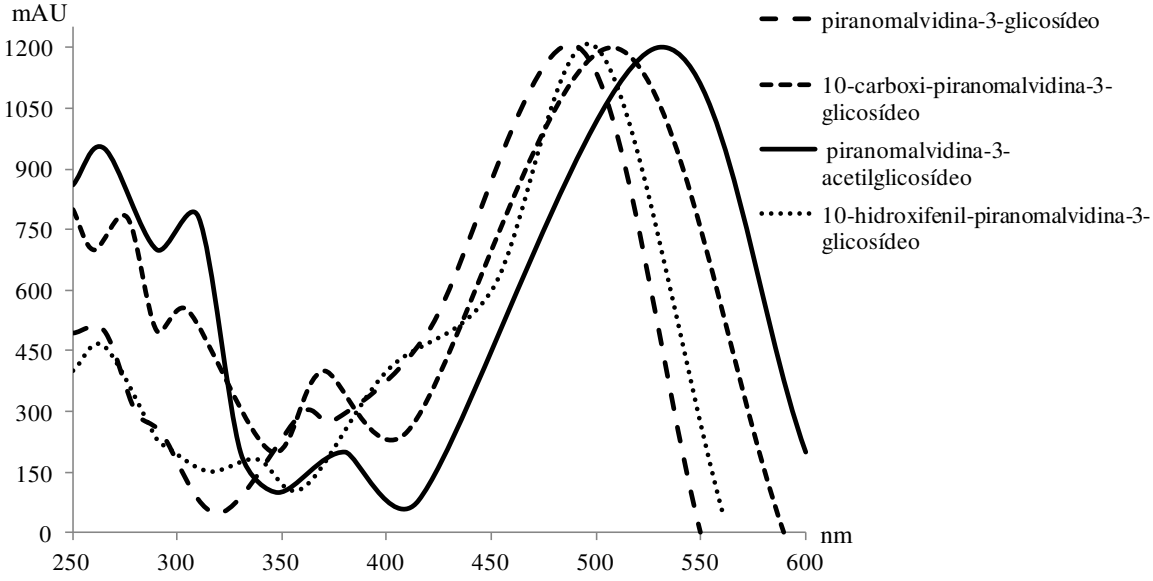
Apêndice 1. Espectros de DAD-UV-visível das antocianinas disubstituída (Cianidina e Peonidina) e trisubstituída (Delfinidina, Petunidina e Malvidina).



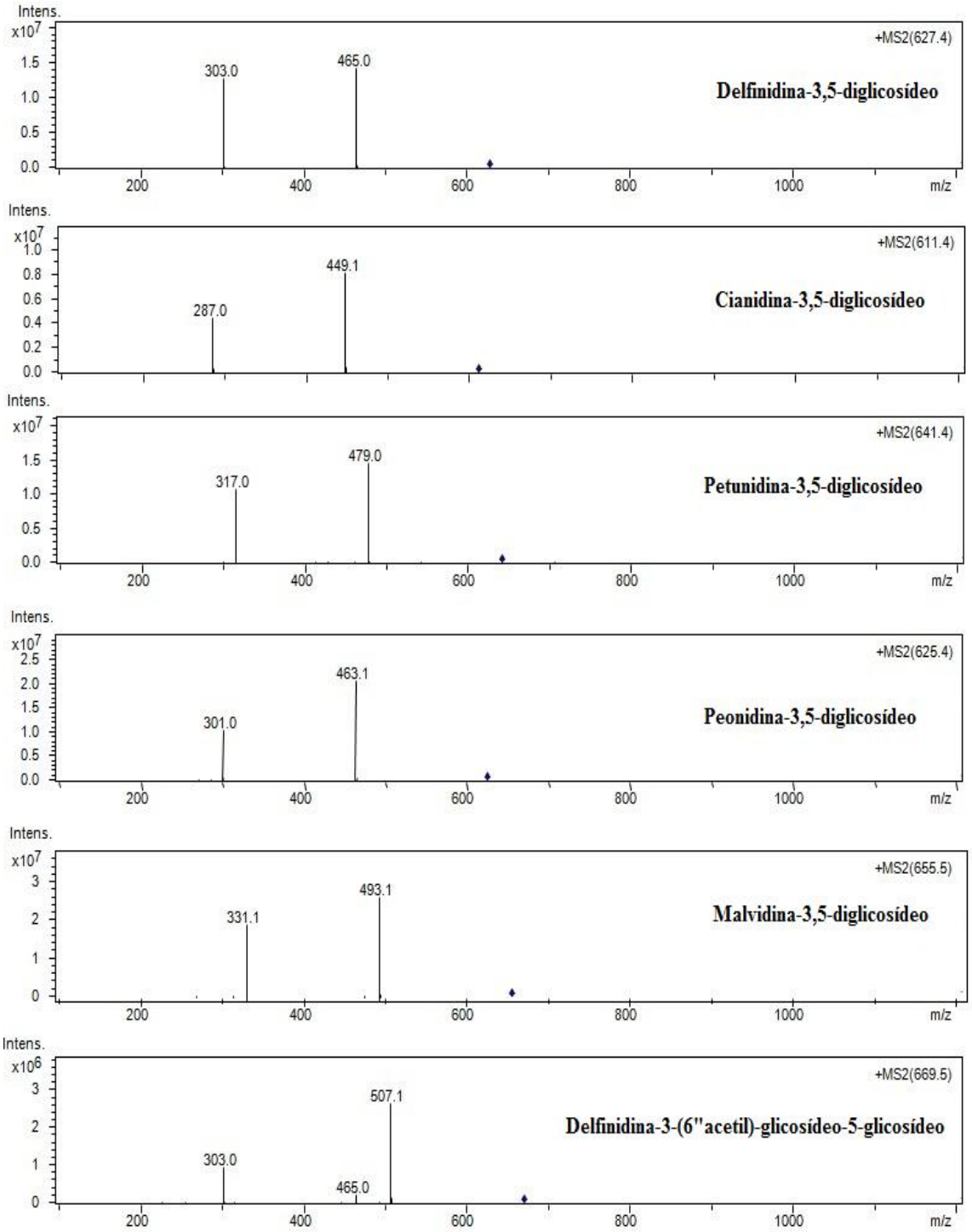
Apêndice 2. Comparação entre os espectros DAD-UV-visível das diferentes antocianinas aciladas disubstituída (Cianidina e Peonidina) e trisubstituída (Delfinidina, Petunidina e Malvidina).

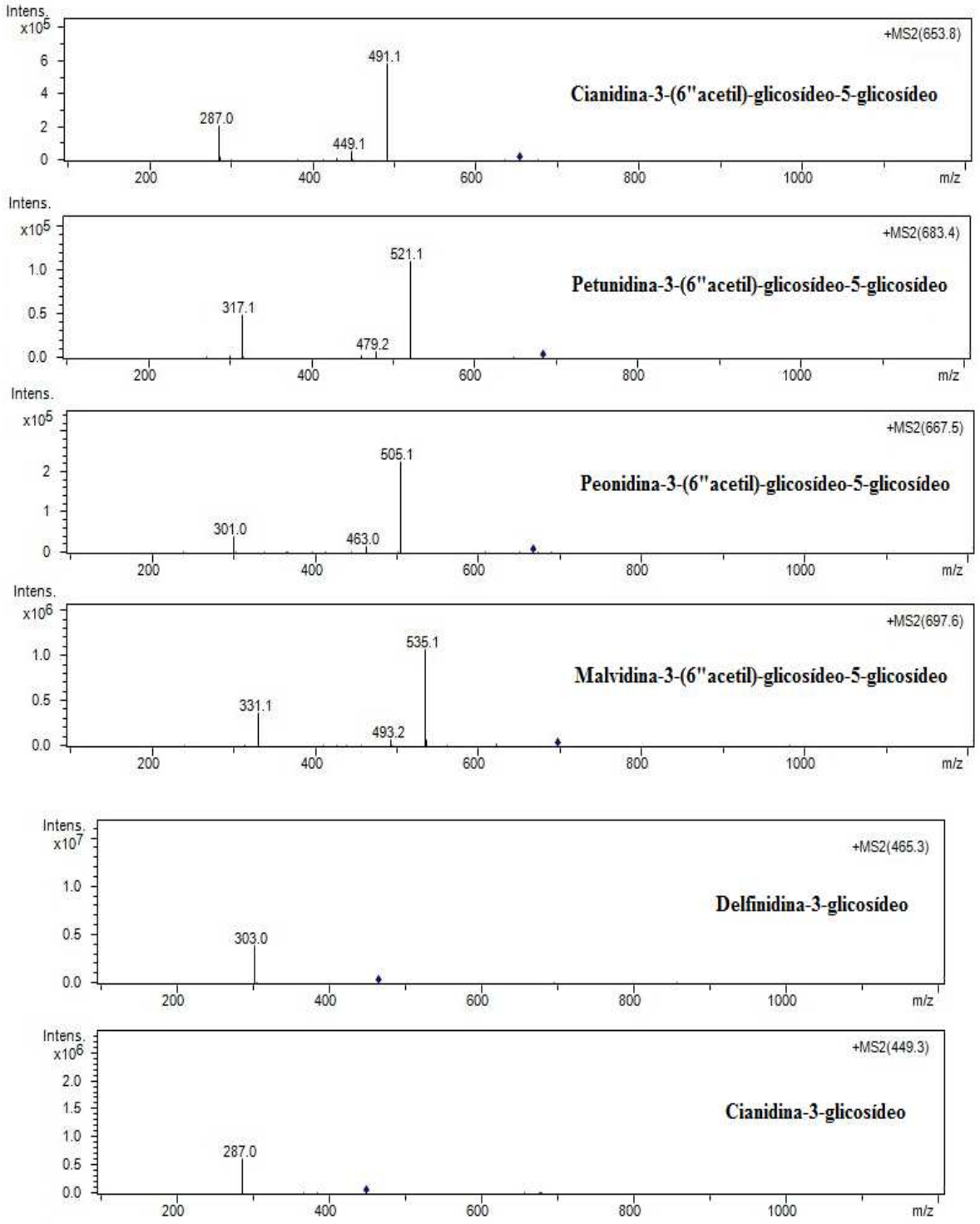


Apêndice 3. Espectros DAD-UV-visível das diferentes piranoantocianinas.



Apêndice 4. Espectros de massas (ESI-MS/MS) das diferentes antocianinas encontradas nas amostras de uvas e subprodutos do processo da vinificação.




Intens.
x10^5
+MS2(653.8)
491.1
287.0
449.1
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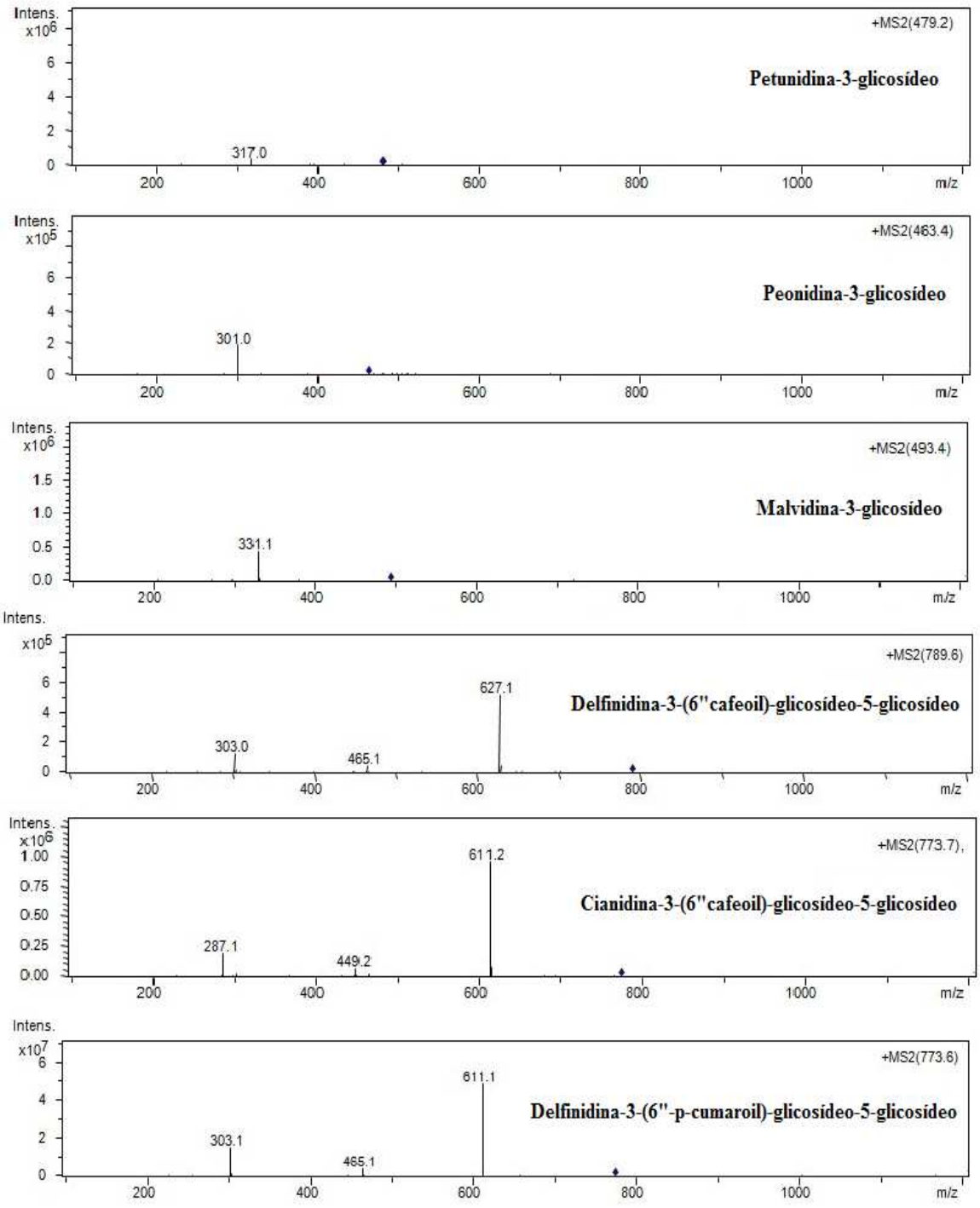
m/z
Intens.
x10^5
+MS2(683.4)
521.1
317.1
479.2
Petunidina-3-(6"acetil)-glicosídeo-5-glicosídeo
m/z
Intens.
x10^5
+MS2(667.5)
505.1
301.0
463.0
Peonidina-3-(6"acetil)-glicosídeo-5-glicosídeo
m/z
Intens.
x10^6
+MS2(697.6)
535.1
331.1
493.2
Malvidina-3-(6"acetil)-glicosídeo-5-glicosídeo
m/z
Intens.
x10^7
+MS2(465.3)
303.0
Delfinidina-3-glicosídeo
m/z
Intens.
x10^6
+MS2(449.3)
287.0
Cianidina-3-glicosídeo
m/z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Petunidina-3-glicosídeo
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Intens.
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301.0
Intens.
x10^6
+MS2(493.4)
Malvidina-3-glicosídeo
331.1
Intens.
x10^5
+MS2(789.6)
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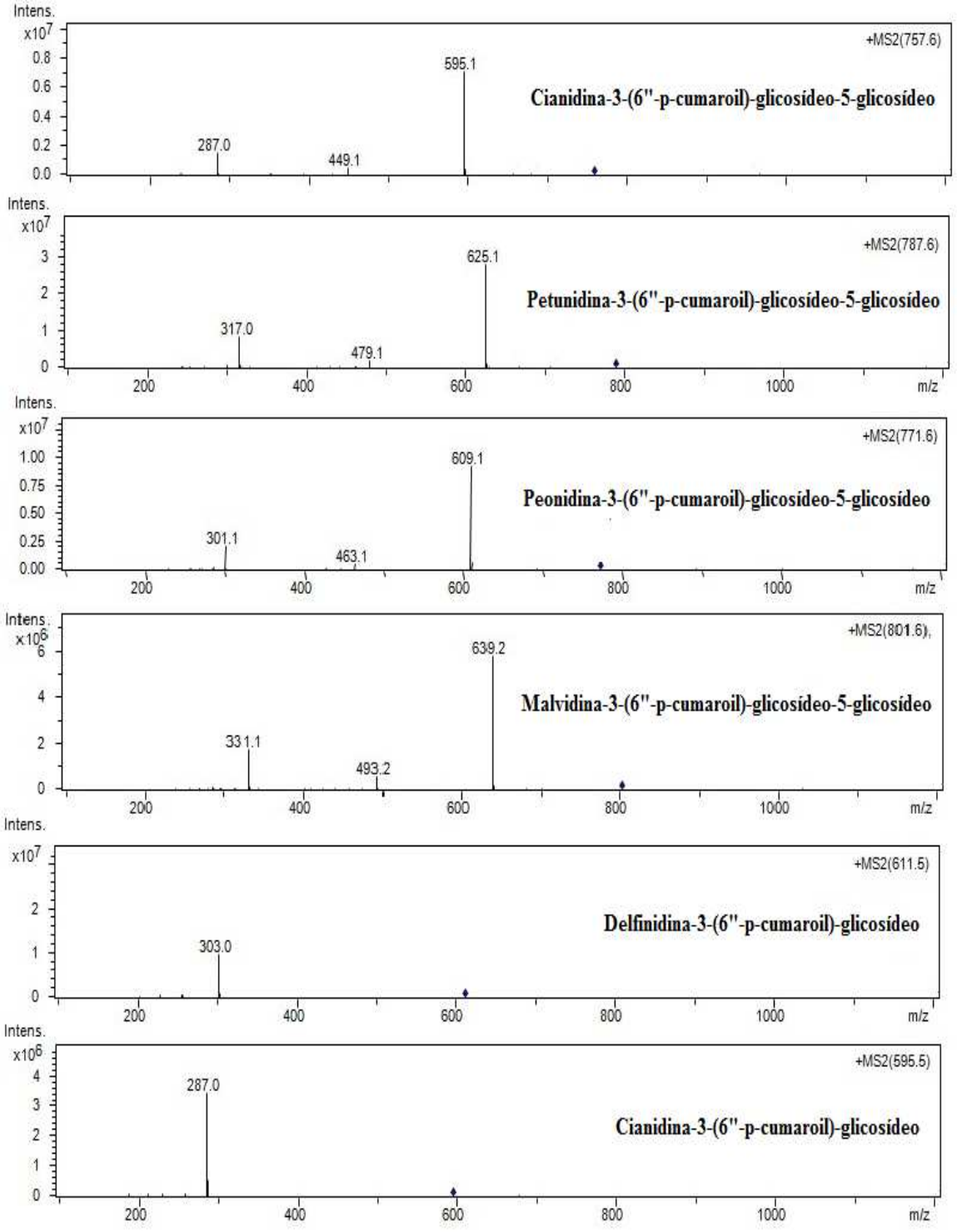
627.1
303.0
465.1
Intens.
x10^6
+MS2(773.7),
Cianidina-3-(6"cafeoil)-glicosídeo-5-glicosídeo
611.2
287.1
449.2
Intens.
x10^7
+MS2(773.6)
Delfinidina-3-(6"-p-cumaroil)-glicosídeo-5-glicosídeo
611.1
303.1
465.1
m/z


Intens.
x10^7
+MS2(757.6)
595.1
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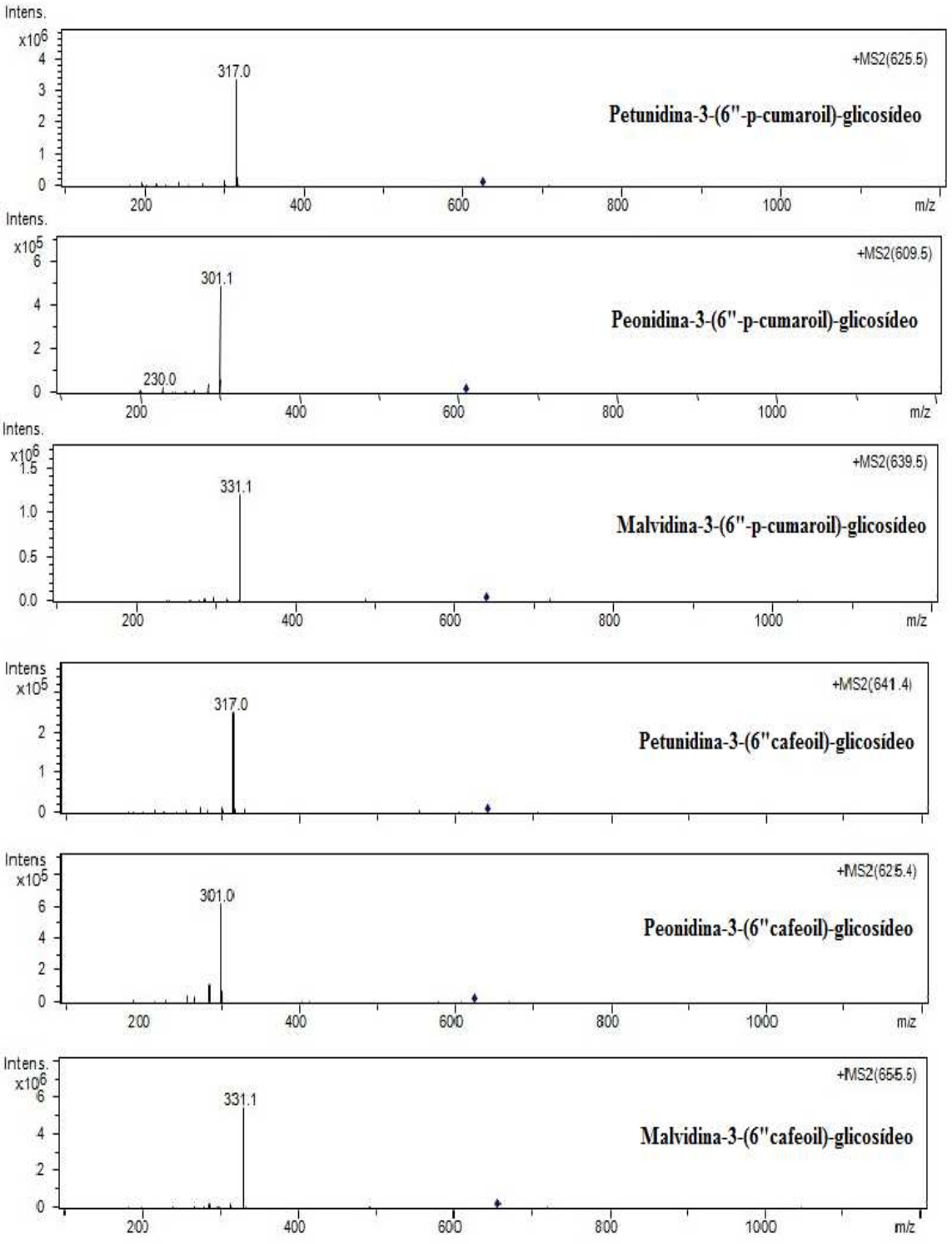
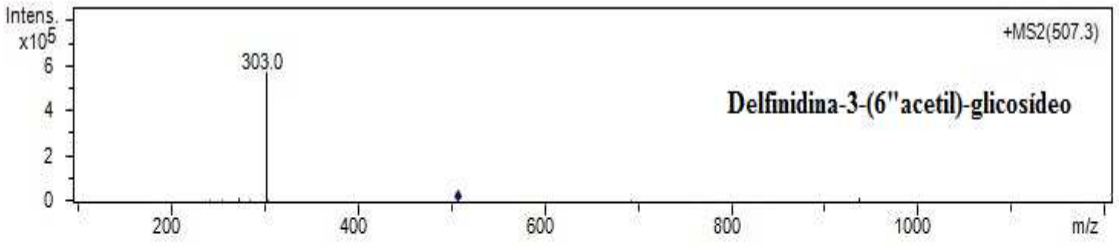
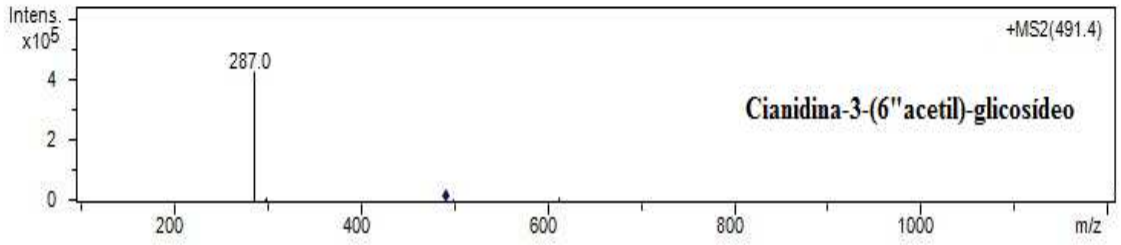
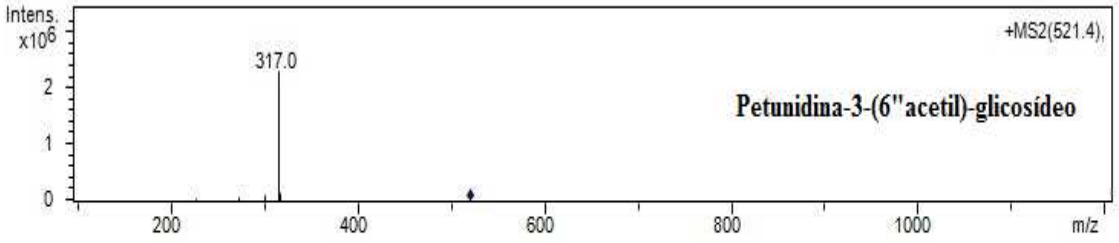
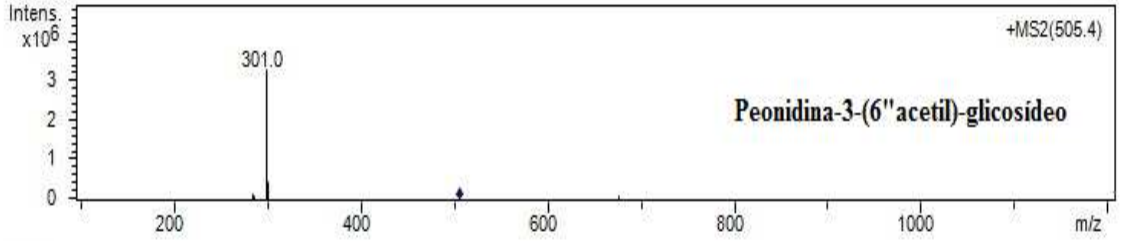
287.0
449.1
Intens.
x10^7
+MS2(787.6)
625.1
Petunidina-3-(6''-p-cumaroil)-glicosídeo-5-glicosídeo
317.0
479.1
m/z
Intens.
x10^7
+MS2(771.6)
609.1
Peonidina-3-(6''-p-cumaroil)-glicosídeo-5-glicosídeo
301.1
463.1
m/z
Intens.
x10^6
+MS2(801.6),
639.2
Malvidina-3-(6''-p-cumaroil)-glicosídeo-5-glicosídeo
331.1
493.2
m/z
Intens.
x10^7
+MS2(611.5)
Delfinidina-3-(6''-p-cumaroil)-glicosídeo
303.0
m/z
Intens.
x10^6
+MS2(595.5)
287.0
Cianidina-3-(6''-p-cumaroil)-glicosídeo
m/z

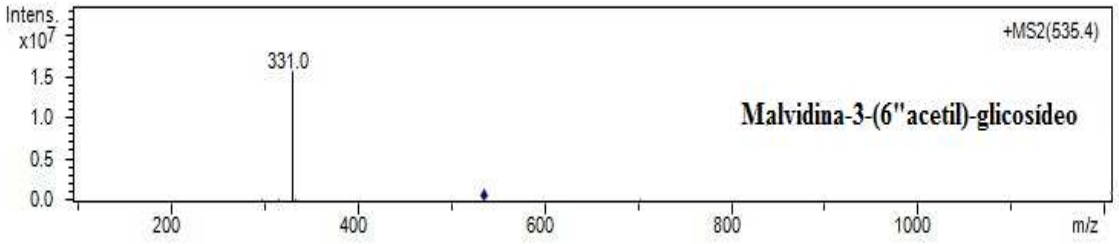

Intens.
x10^6
317.0
+MS2(625.5)
Petunidina-3-(6"-p-cumaroil)-glicosídeo
m/z
Intens.
x10^5
301.1
230.0
+MS2(609.5)
Peonidina-3-(6"-p-cumaroil)-glicosídeo
m/z
Intens.
x10^6
331.1
+MS2(639.5)
Malvidina-3-(6"-p-cumaroil)-glicosídeo
m/z
Intens
x10^5
317.0
+MS2(641.4)
Petunidina-3-(6"cafeoil)-glicosídeo
Intens
x10^5
301.0
+MS2(625.4)
Peonidina-3-(6"cafeoil)-glicosídeo
m/z
Intens.
x10^6
331.1
+MS2(655.5)
Malvidina-3-(6"cafeoil)-glicosídeo
m/z

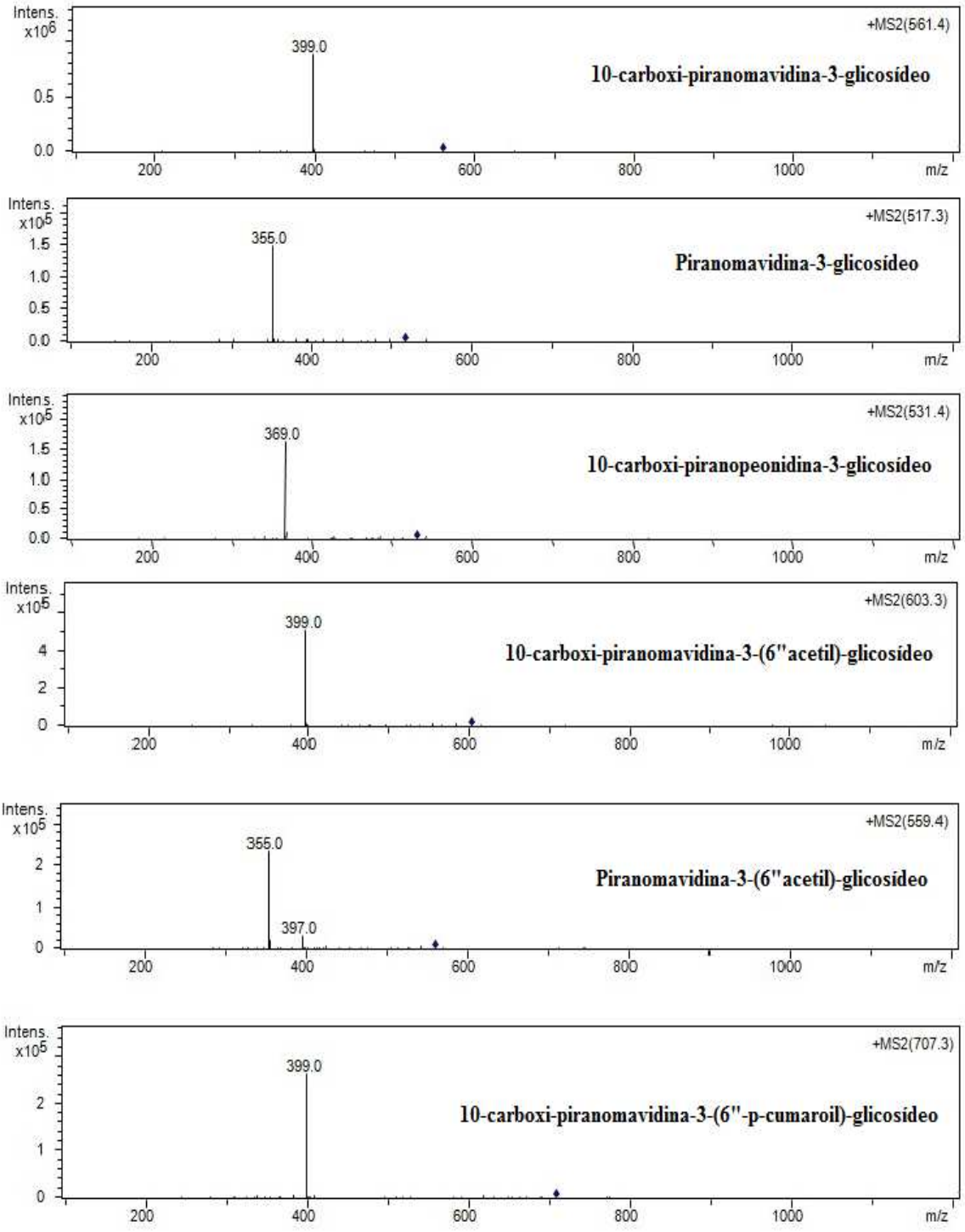

Intens.
x10^5
6
4
2
0
303.0
+MS2(507.3)
Delfinidina-3-(6"acetil)-glicosídeo
200
400
600
800
1000
m/z

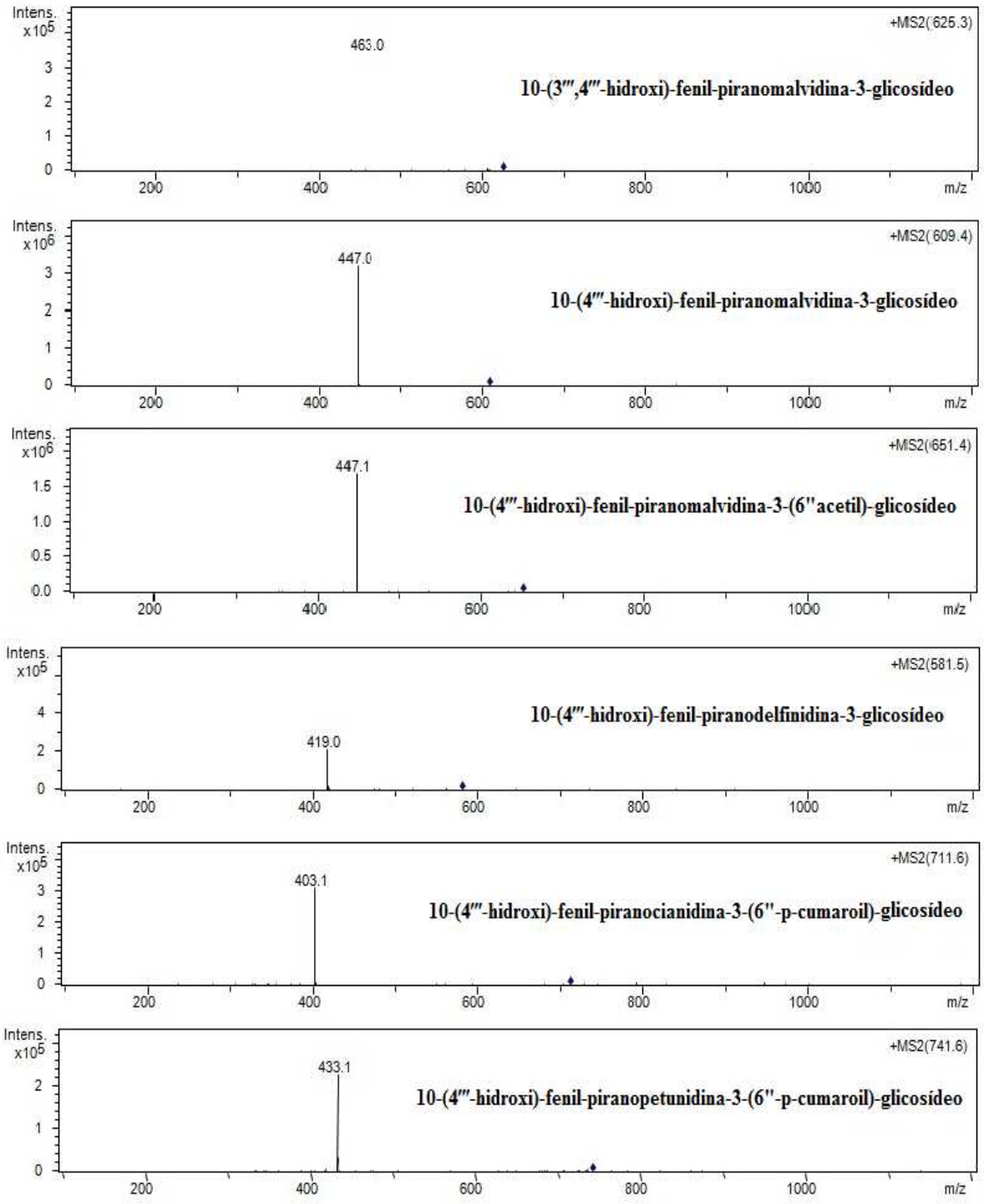

Intens.
x10^5
4
2
0
287.0
+MS2(491.4)
Cianidina-3-(6"acetil)-glicosídeo
200
400
600
800
1000
m/z


Intens.
x10^6
2
1
0
317.0
+MS2(521.4),
Petunidina-3-(6"acetil)-glicosídeo
200
400
600
800
1000
m/z

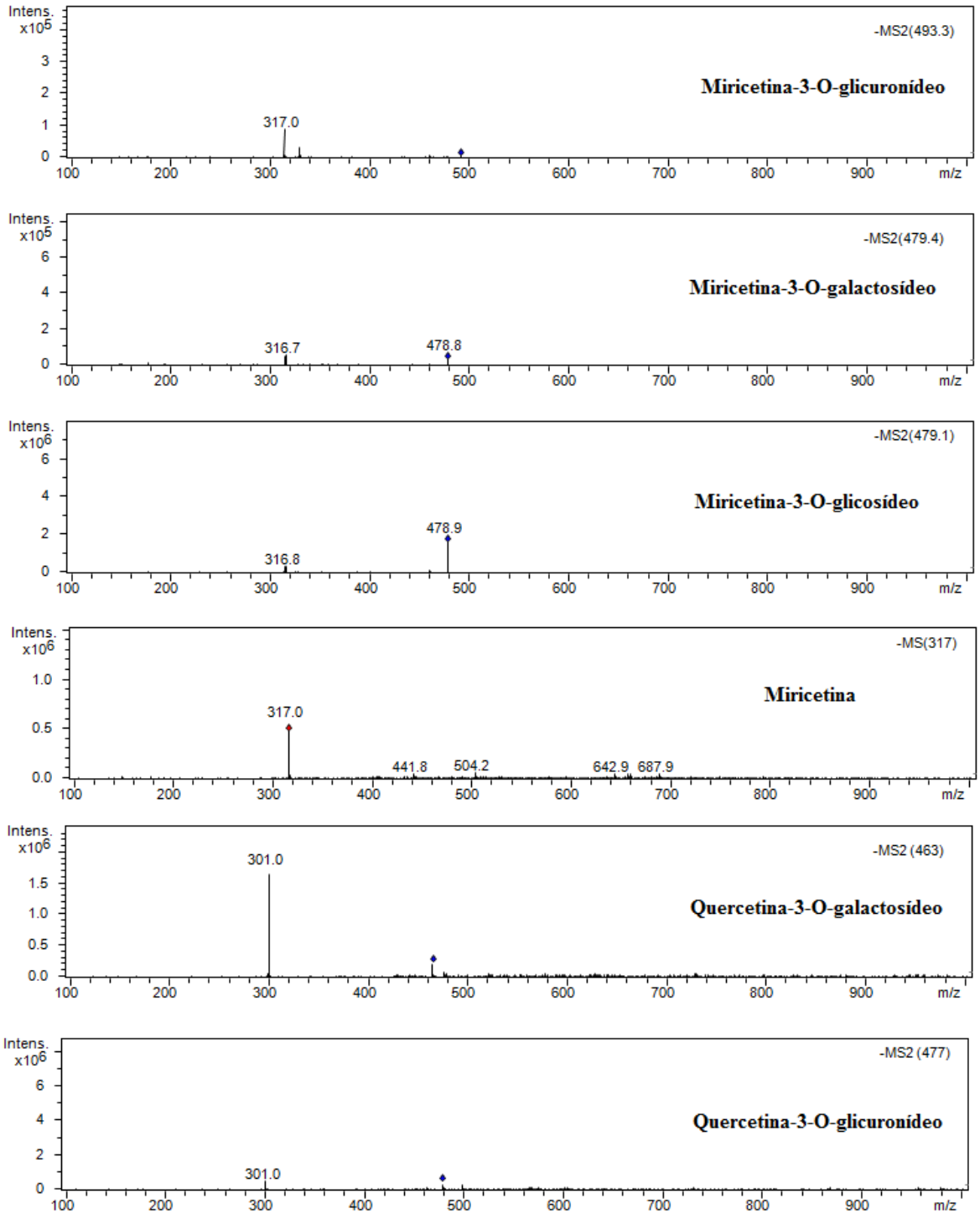

Intens.
x10^6
3
2
1
0
301.0
+MS2(505.4)
Peonidina-3-(6"acetil)-glicosídeo
200
400
600
800
1000
m/z

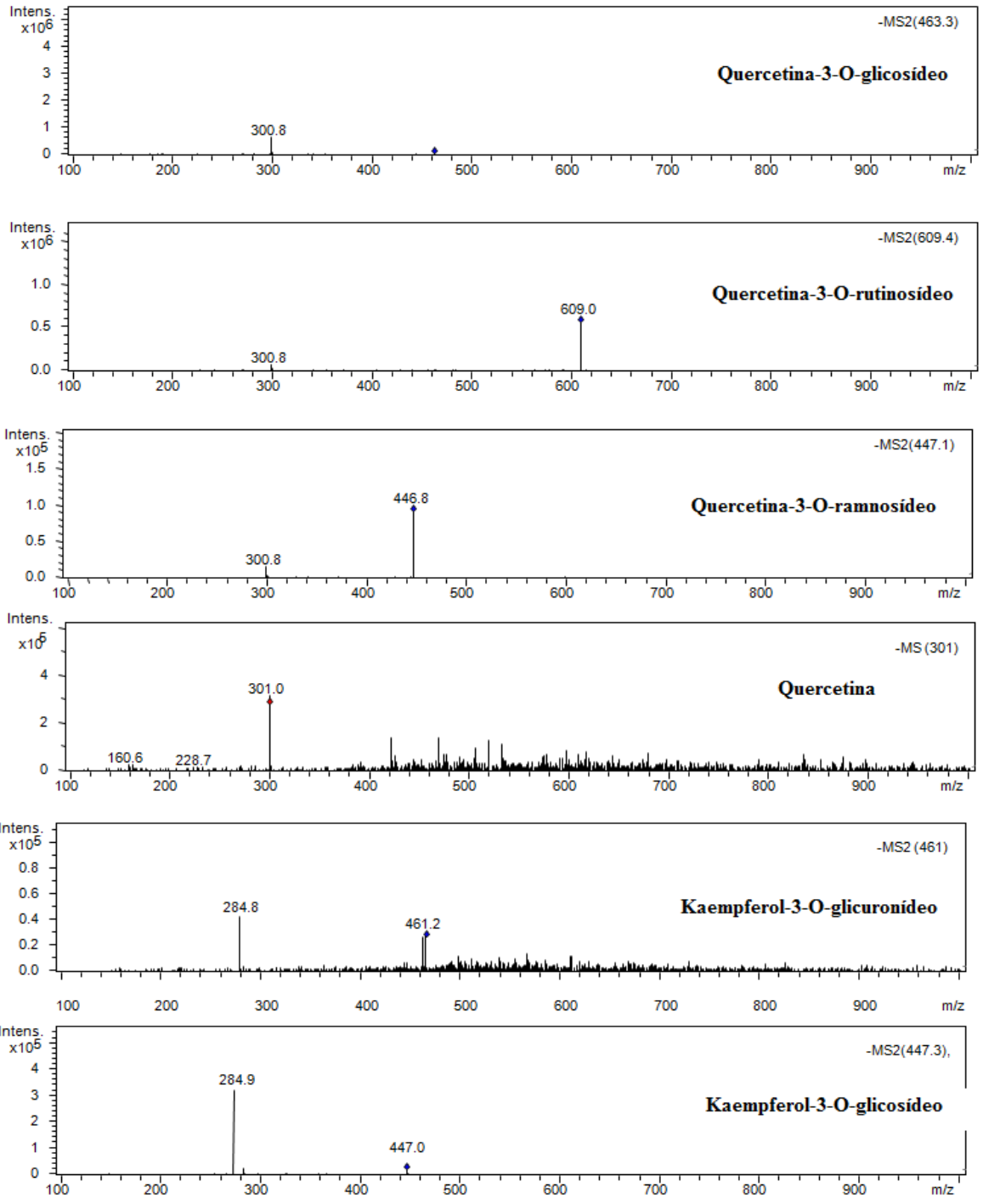

Intens.
x10^7
1.5
1.0
0.5
0.0
331.0
+MS2(535.4)
Malvidina-3-(6"acetil)-glicosídeo
200
400
600
800
1000
m/z

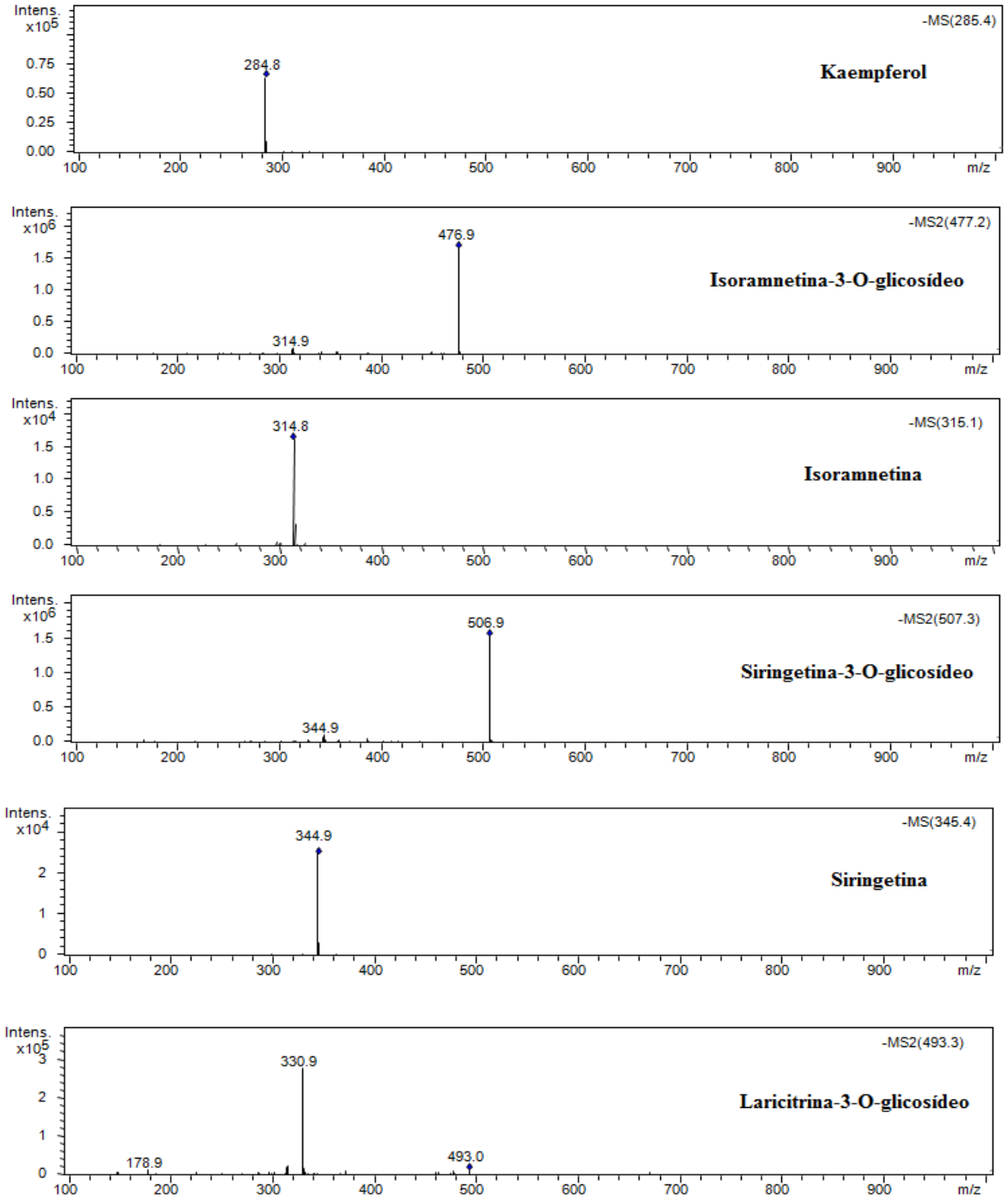

Intens.
x10^6
+MS2(561.4)
399.0
10-carboxi-piranomavidina-3-glicosídeo
0.5
0.0
200
400
600
800
1000
m/z
Intens.
x10^5
+MS2(517.3)
355.0
Piranomavidina-3-glicosídeo
1.5
1.0
0.5
0.0
Intens.
x10^5
+MS2(531.4)
369.0
10-carboxi-piranopeonidina-3-glicosídeo
Intens.
x10^5
+MS2(603.3)
399.0
10-carboxi-piranomavidina-3-(6''acetil)-glicosídeo
4
2
0
Intens.
x10^5
+MS2(559.4)
355.0
Piranomavidina-3-(6''acetil)-glicosídeo
397.0
Intens.
x10^5
+MS2(707.3)
399.0
10-carboxi-piranomavidina-3-(6''-p-cumaroil)-glicosídeo

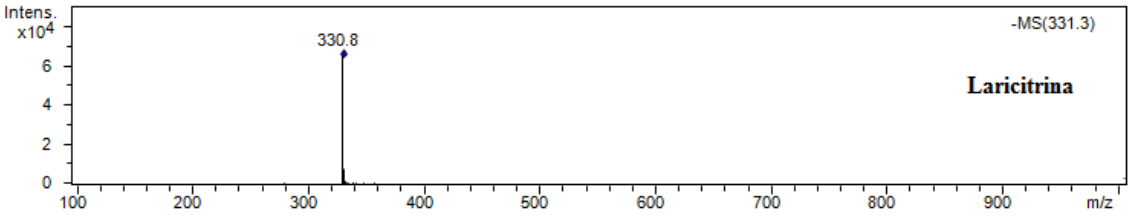

Intens.
x10^5
+MS2(625.3)
463.0
10-(3‴,4‴-hidroxi)-fenil-piranomalvidina-3-glicosídeo
m/z
Intens.
x10^6
+MS2(609.4)
447.0
10-(4‴-hidroxi)-fenil-piranomalvidina-3-glicosídeo
m/z
Intens.
x10^6
+MS2(651.4)
447.1
10-(4‴-hidroxi)-fenil-piranomalvidina-3-(6''acetil)-glicosídeo
m/z
Intens.
x10^5
+MS2(581.5)
419.0
10-(4‴-hidroxi)-fenil-piranodelfinidina-3-glicosídeo
m/z
Intens.
x10^5
+MS2(711.6)
403.1
10-(4‴-hidroxi)-fenil-piranocianidina-3-(6''-p-cumaroil)-glicosídeo
m/z
Intens.
x10^5
+MS2(741.6)
433.1
10-(4‴-hidroxi)-fenil-piranopetunidina-3-(6''-p-cumaroil)-glicosídeo
m/z

Apêndice 5. Espectros de massas (ESI-MS/MS) dos diferentes flavonols encontrados nas amostras de uvas e subprodutos do processo da vinificação.

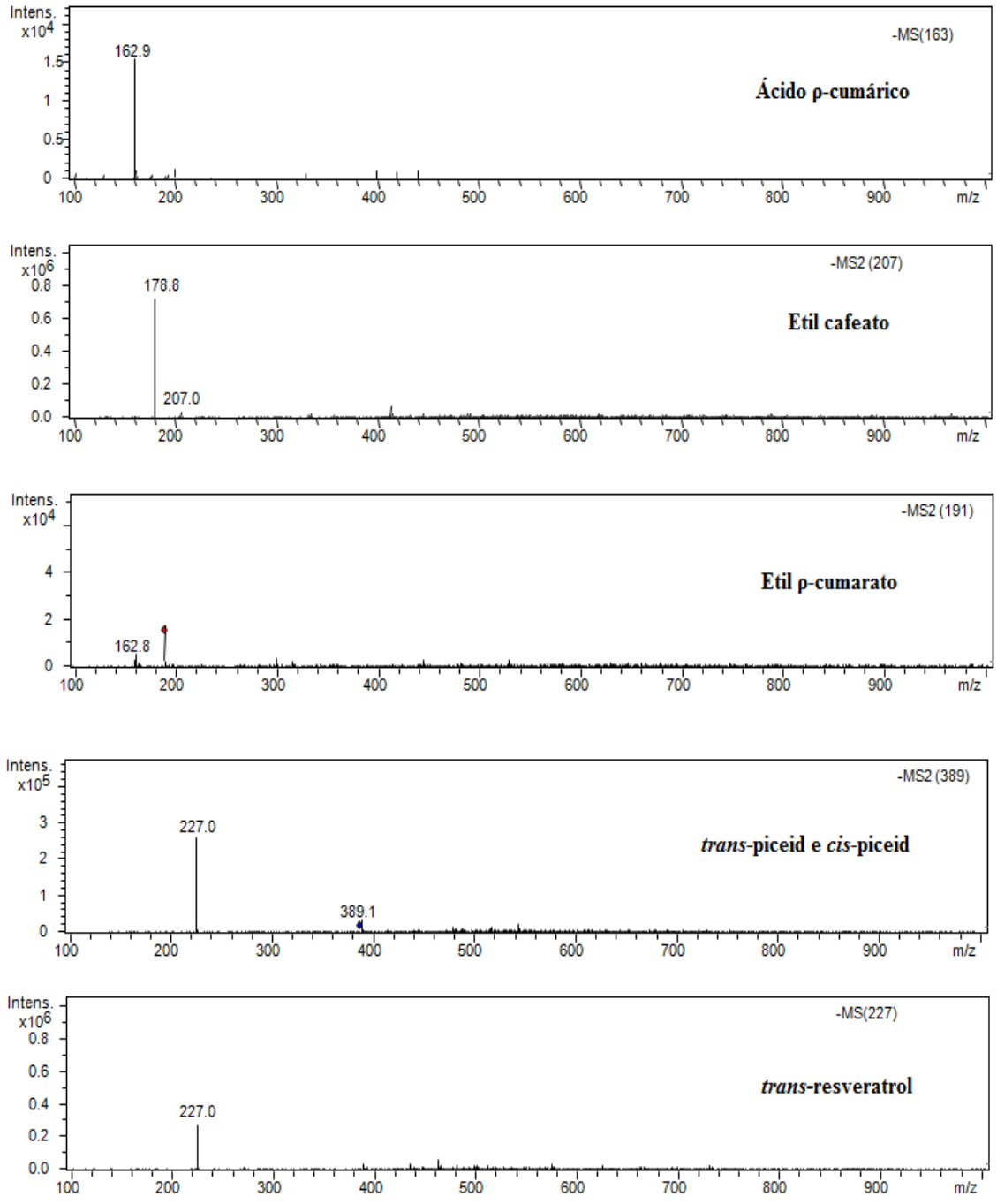


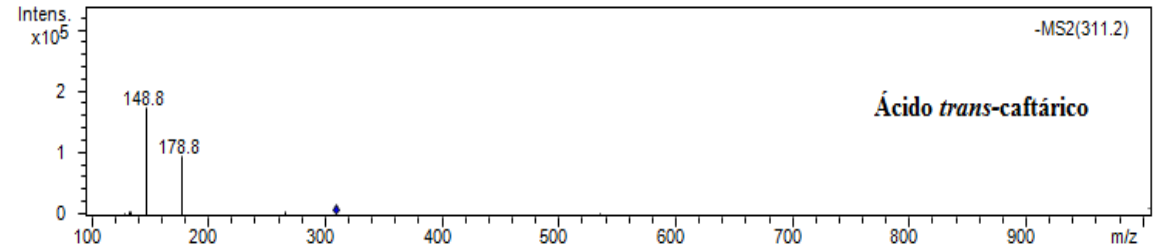

Intens.
x10^6
-MS2(463.3)
Quercetina-3-O-glicosídeo
300.8
m/z
Intens.
x10^6
-MS2(609.4)
Quercetina-3-O-rutinosídeo
609.0
300.8
m/z
Intens.
x10^5
-MS2(447.1)
Quercetina-3-O-ramnosídeo
446.8
300.8
m/z
Intens.
x10^5
-MS (301)
Quercetina
301.0
160.6
228.7
m/z
Intens.
x10^5
-MS2 (461)
Kaempferol-3-O-glicuronídeo
284.8
461.2
m/z
Intens.
x10^5
-MS2(447.3),
Kaempferol-3-O-glicosídeo
284.9
447.0
m/z

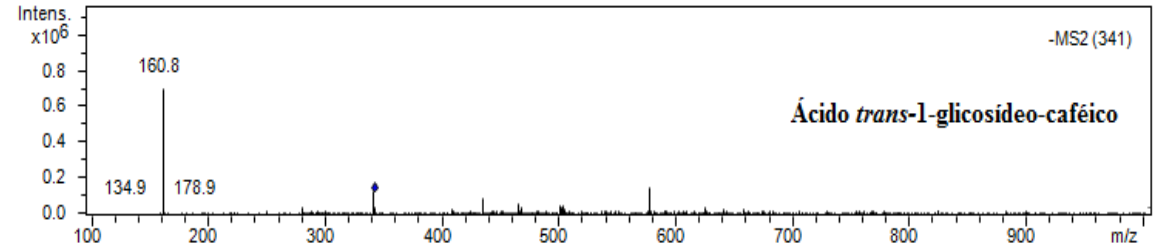

Intens.
x10^5
0.75
0.50
0.25
0.00
284.8
-MS(285.4)
Kaempferol
100 200 300 400 500 600 700 800 900 m/z
Intens.
x10^6
1.5
1.0
0.5
0.0
476.9
314.9
-MS2(477.2)
Isoramnetina-3-O-glicosídeo
100 200 300 400 500 600 700 800 900 m/z
Intens.
x10^4
1.5
1.0
0.5
0.0
314.8
-MS(315.1)
Isoramnetina
100 200 300 400 500 600 700 800 900 m/z
Intens.
x10^6
1.5
1.0
0.5
0.0
506.9
344.9
-MS2(507.3)
Siringetina-3-O-glicosídeo
100 200 300 400 500 600 700 800 900 m/z
Intens.
x10^4
2
1
0
344.9
-MS(345.4)
Siringetina
100 200 300 400 500 600 700 800 900 m/z
Intens.
x10^5
3
2
1
0
330.9
178.9
493.0
-MS2(493.3)
Laricitrina-3-O-glicosídeo
100 200 300 400 500 600 700 800 900 m/z

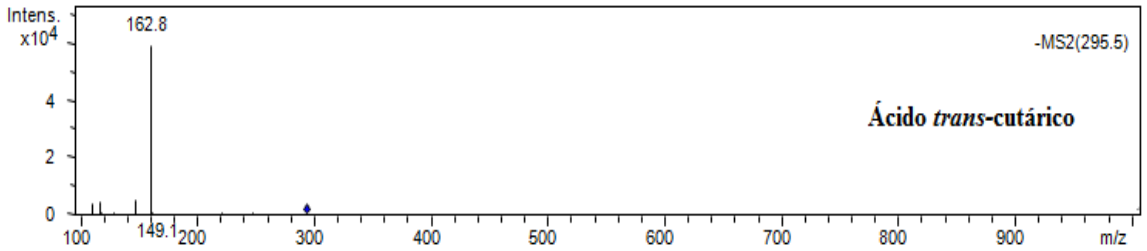

Intens.
x10^4
6
4
2
0
330.8
-MS(331.3)
Laricitrina
100
200
300
400
500
600
700
800
900
m/z

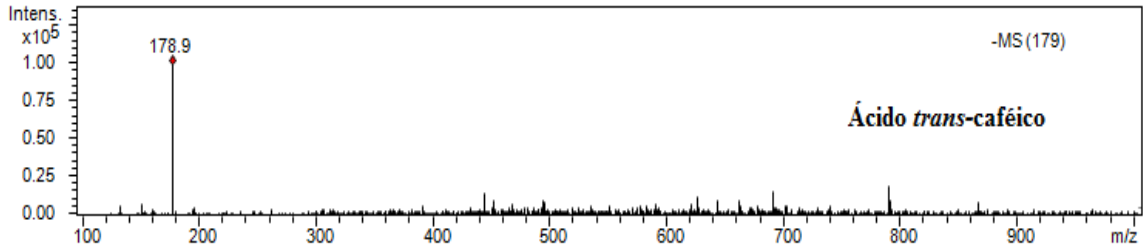
Apêndice 6. Espectros de massas (ESI-MS/MS) dos diferentes ácidos hidroxicinâmicos e estilbenos encontrados nas amostras de uvas e subprodutos do processo da vinificação.

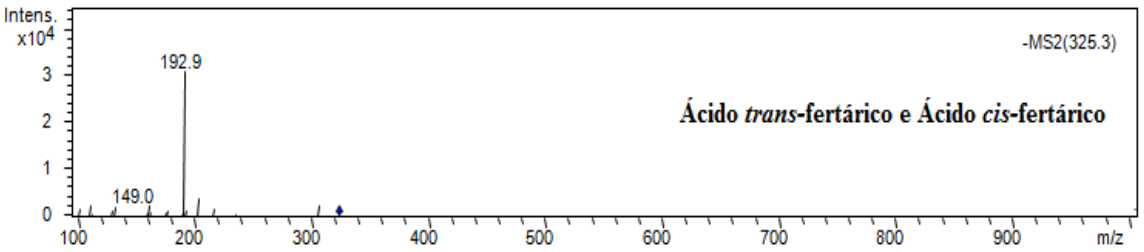



Intens.
x10^5
2
1
0
148.8
178.8
-MS2(311.2)
Ácido trans-caftárico
100
200
300
400
500
600
700
800
900
m/z

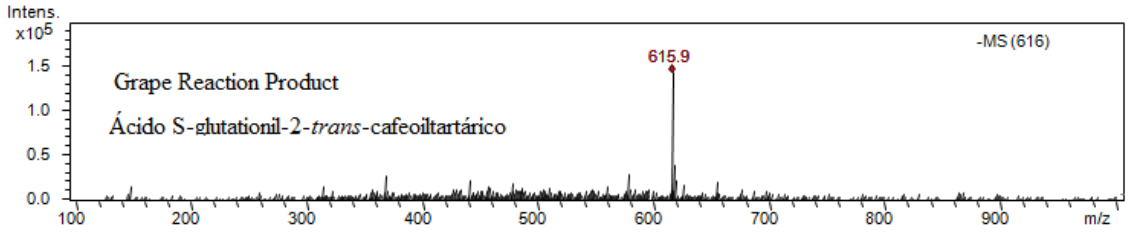

Intens.
x10^6
0.8
0.6
0.4
0.2
0.0
160.8
134.9
178.9
-MS2 (341)
Ácido trans-1-glicosídeo-caféico
100
200
300
400
500
600
700
800
900
m/z


Intens.
x10^4
4
2
0
162.8
149.1
-MS2(295.5)
Ácido trans-cutárico
100
200
300
400
500
600
700
800
900
m/z

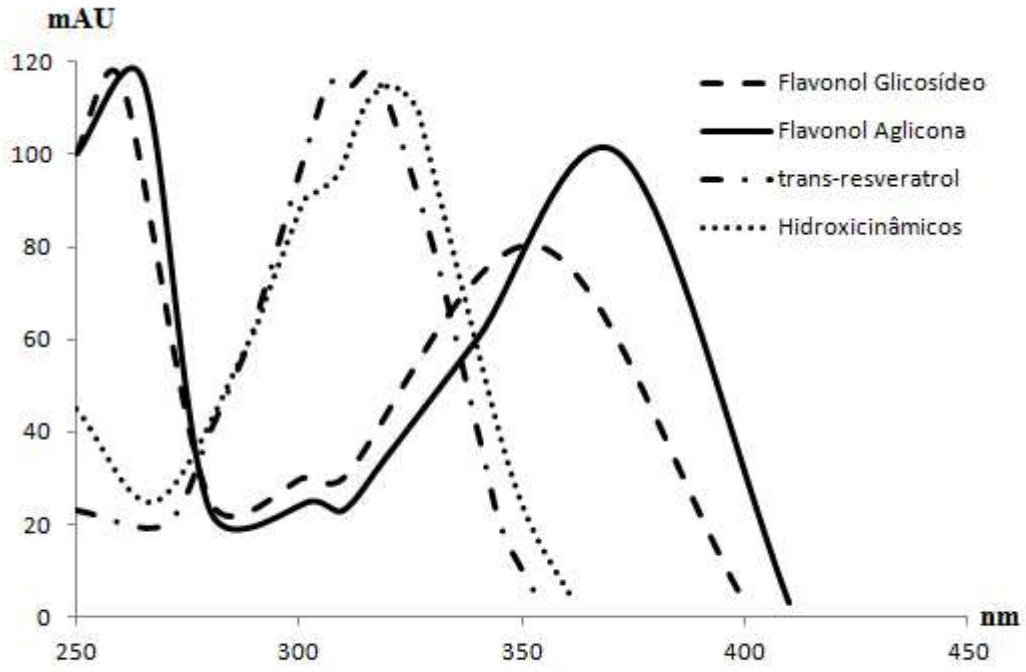

Intens.
x10^5
1.00
0.75
0.50
0.25
0.00
178.9
-MS (179)
Ácido trans-caféico
100
200
300
400
500
600
700
800
900
m/z


Intens.
x10^4
3
2
1
0
192.9
149.0
-MS2(325.3)
Ácido trans-fertárico e Ácido cis-fertárico
100
200
300
400
500
600
700
800
900
m/z

Apêndice 7. Espectros de massas (ESI-MS/MS) do GRP (Grape Reaction Product) encontrado nas uvas e subprodutos do processo da vinificação da cultivar BRS Lorena.



Apêndice 8. Espectros de UV-vis (nm) dos compostos fenólicos presentes nas uvas e subprodutos do processo da vinificação.



Apêndice 9. Comprimentos de onda (nm) dos compostos fenólicos encontrados nas uvas e subprodutos do processo da vinificação.

Composto fenólico	UV-vis (nm)
Miricetina-3-glicuronídeo	(257), 260, (301), 354
Miricetina -3-galactosídeo	(257), 262, (298), 354
Miricetina -3-glicosídeo	(255), 261, (298), 355
Miricetina	(253), 265, (303), 372
Quercetina-3- galactosídeo	255, (265), (302), 353
Quercetina -3- glicuronídeo	257, (264), (300), 354
Quercetina -3- glicosídeo	256, (265), (395), 354
Quercetina -3-rutinosídeo	255, (264), (301), 354
Quercetina -3-ramnosídeo	255, (266), (295), 348
Quercetina	255, (265), (301), 370
Kaempferol -3- glicuronídeo	265, (300), (325), 348
Kaempferol -3- glicosídeo	265, (300), (325), 348
Kaempferol	252, (264), (306), 366
Isoramnetina-3- glicosídeo	255, (265), (300), 354
Isoramnetina	255, (265), (305), 370
Siringetina-3- glicosídeo	255, (264), (301), 357
Siringetina	253, (265), (305), 371
Laricitrina -3- glicosídeo	256, (262), (301), 356
Laricitrina	253, (265), (304), 372
Ácido *trans*-caftárico	(300), 328
Ácido *trans* -1-glc-caffeic acid	(302), 329
Ácido *trans* -cutárico	(300), 312
Ácido *trans*-caféico	(298), 323
Ácido *trans* -fertárico	(300), 326
Ácido *cis*- fertárico	(300), 323
Ácido ρ-cumárico	(298), 315
Etil cafeato	(300), 322
Etil ρ-cumarato	(300), 310
trans -piceid	304-(318)
cis-piceid	284
trans -resveratrol	306, 315
Ácido S-glutationil-2-*trans*-cafeoiltartárico (GRP)	327

*número entre parênteses significa ombro no espectro de UV-visível.