UNIVERSIDADE DE SÃO PAULO
FACULDADE DE CIÊNCIAS FARMACÊUTICAS

Departamento de Alimentos e Nutrição Experimental
Programa de Pós-Graduação em Ciência dos Alimentos
Área de Bromatologia

Aplicação de metil jasmonato em uvas (*Vitis labrusca* L.) visando enriquecimento dos frutos quanto aos compostos fenólicos, flavonoides e estilbenos

Laís Moro

Tese para obtenção do grau de
DOUTOR

Orientador: Prof. Dr. Eduardo Purgatto

São Paulo
2019

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Laís Moro

Aplicação de metil jasmonato em uvas (*Vitis labrusca* L.) visando enriquecimento dos frutos quanto aos compostos fenólicos, flavonoides e estilbenos

Comissão julgadora para obtenção do grau de Doutor

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4º Examinador

São Paulo,_____ de 2019

Aos meus pais, Domingos e Mari, pelo incentivo,

Amor e carinho.

A Giovani, pelo amor, incentivo e paciência.

A minha família e amigos.

Dedico e Ofereço

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Resumo

As uvas (*Vitis. Sp*) são particularmente ricas em compostos fenólicos, como flavonóis, antocianinas e estilbenos. Estudos relacionados aos efeitos benéficos sobre a saúde exercidos por estes compostos, incentivam pesquisas que visem proporcionar o aumento em sua concentração nos frutos. Dentre o arcabouço de possibilidades para atingir tal resultado, alguns hormônios vegetais, e seus derivados como o metil-jasmonato (MeJa), tem mostrado resultados promissores em diversos frutos. Neste contexto, o presente trabalho teve como objetivo induzir o aumento no conteúdo de compostos fenólicos, flavonoides e estilbenos em uvas (*Vitis labrusca*. L.), através da aplicação de MeJa no período de pré-colheita. Foi realizada a otimização da aplicação do tratamento em uvas *Concord* e *Isabel Precoce* na região Sul durante as vindimas de 2015 e 2016. Os melhores resultados foram observados a partir da aplicação de MeJa em 2 períodos (*véraison* e 2 semanas pré-colheita). Houve aumento de *trans*-resveratrol especialmente em uvas *Isabel Precoce* em relação a frutos não tratados, e o perfil de compostos voláteis apresentou vias biosintéticas responsivas ao MeJa em ambas vindimas. A identificação de antocianinas demonstrou não haver modificação no perfil, sendo observadas diferenças apenas entre cultivares. Posteriormente, o tratamento foi avaliado em ambas cultivares nas regiões Sul (Bento Gonçalves, RS) e Sudeste (Caldas, MG) durante as vindimas de 2017 e 2019. Estas uvas foram processadas, e o suco foi avaliado quanto aos estilbenos durante o armazenamento (6 meses). Foi observado aumento do conteúdo de estilbenos nas uvas, e o suco elaborado com estas uvas, em comparação ao elaborado a partir de uvas não tratadas. Visando compreender o efeito de modo mais amplo, nas modificações proporcionadas pelo MeJa, em vias metabólicas que não sejam o alvo de nosso projeto, realizou-se um estudo metabolômico, nas uvas e suco de uvas cultivadas nas regiões Sul e Sudeste após aplicações do MeJa, durante a vindima de 2017. De modo geral, foram detectados como fortes marcadores estilbenos, antocianinas, ácidos fenólicos e flavonoides. O presente estudo demonstrou o potencial do MeJa na indução de compostos bioativos, com potencial na promoção da saúde, visto que marcadores presentes nas uvas se mantiveram após o processo térmico utilizado para a elaboração do suco.

Palavras-chave: uva, *Vitis labrusca*, metil jasmonato, estilbenos, metabolomica

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Abstract

Grapes (*Vitis* sp.) are considered a major source of phenolic compounds, such as flavonols, anthocyanins and stilbenes. Studies related to the beneficial effects exerted by these compounds on health, have encouraged researches that aimed to increase their concentration in fruits. Among the framework of possibilities to achieve these results, some plant growth regulators and its volatile ester methyl-jasmonate (MeJa), have shown promising results in many fruits. On this context, the present work aimed induce phenolic compounds, flavonoids and stilbenes on grapes (*Vitis labrusca*. L.), through pre harvest MeJa application. Treatment application optimization was performed on Concord and Isabel Precoce grapes on Brazilian south region on 2015 and 2016 harvest. Our best results were observed with MeJa application at 2 periods (*véraison* and 2 weeks before harvest). An increase of *trans*-resveratrol was observed specially for Isabel Precoce grapes in comparison with non-treated group, volatile compounds profile present biosynthetic pathways responsive to MeJa treatment on both harvests. Anthocyanin identification didn't display profile changes, being observed only differences between cultivars. Following, the treatment was evaluated on the same cultivars, on south (Bento Gonçalves, RS) and southeast (Caldas, MG) regions on 2017 and 2019 harvests. These grapes were processed and the juice evaluated regarding stilbenes content during storage (6 months), in comparison to non-treated grapes. In order to better understand the modifications promoted by MeJa treatment besides the target by our project, we performed a metabolomic study on grape and grape juice grown on south and southeast regions after MeJa treatment on 2017 harvest. In summary, we detect as strong markers stilbenes, anthocyanins, flavonoids and phenolic acids. The present study evidence the treatment with MeJa in inducing bioactive compounds, with potential on health promotion, since markers detected on grapes were detected even after thermal processing used for grape juice extraction.

Key words: grapes, *Vitis labrusca*, methyl jasmonate, stilbenes, metabolomics

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1. Introdução

A fruticultura de clima temperado, apesar de possuir uma área de produção inferior em relação às espécies de clima tropical e subtropical, destaca-se no cenário nacional, seja na produção dos frutos para consumo *in natura*, muitas vezes associado ao agroturismo, como para o aproveitamento pela indústria. Dentre as frutas mais produzidas destaca-se a uva, por representar 45% da produção total e 64% das exportações brasileiras das frutas de clima temperado (Fachinello et al., 2011). As uvas cultivadas no Brasil, são classificadas como europeias (*Vitis vinifera* L.), denominadas finas, e americanas ou híbridas (cruzamento entre europeias e americanas), denominadas comuns. Entre as cultivares americanas ou híbridas estão a *Vitis labrusca* L., *Vitis bourquina* L. e híbridos envolvendo várias espécies americanas e também *Vitis vinifera* L.

As cultivares americanas e seus híbridos, são muito utilizadas para a produção de vinhos comuns de mesa, sucos e também para o consumo *in natura.* Geralmente apresentam alta produtividade e são mais resistentes às doenças fúngicas, adaptando-se bem às condições ambientais brasileiras. Estas cultivares representam aproximadamente 80% das uvas processadas, sendo as cultivares Ives, Isabel, Concord e Niágara, responsáveis por 50% da produção total de uvas no Brasil (Giovannini, E. 2005; Lona, A. 2009; Toaldo et al., 2015).

Destacam-se as regiões vitícolas sul e sudeste brasileiro pelo potencial enológico e diversidade climática (Granato et al., 2016; Toaldo, et al. 2015; Regina et al., 2011). Na região Sul, o estado do Rio Grande do Sul (RS) é responsável pela produção de 90% das uvas brasileiras, possui quatro indicações de procedência (IP), que demarcam a origem das uvas e da produção, sendo: Altos Montes, Monte Belo, Pinto Bandeira e Farroupilha. Além disso, possui a única Denominação de Origem no Brasil, composta pelos municípios de Bento Gonçalves (61,07%), Garibaldi (33,49%) e Monte Belo do Sul (5,44%), no Vale dos Vinhedos (Tonietto et al., 2013). Conforme a classificação de Köppen, a região de Bento Gonçalves (RS) é classificada como *Cfa*, (subtropical) com uma temperatura média, no mês mais gelado abaixo de 18°C e a temperatura média do mês mais quente inferior a 22°C. A pluviosidade no mês mais seco, é superior a 40mm (Alvares et al., 2013). O estado de Minas Gerais (MG), apresenta características únicas, que possibilitam a intervenção no ciclo da videira, desviando a colheita para um período mais seco, bem definido, cujas características

favorecem o amadurecimento das uvas (Tonietto et al., 2006; Regina et al., 2011). De acordo com a classificação de Köppen o clima da região de Caldas (MG) é do tipo *Cwb*, temperado quente (mesotérmico), sendo a temperatura média do mês mais frio entre 18°C e - 3°C, enquanto a temperatura média do mês mais quente é inferior a 22°C. Durante a época mais seca, o inverno, pelo menos um mês apresenta precipitação com média inferior a 60mm (Tonietto et al., 2006).

As uvas (*Vitis. Sp*) são consideradas uma das maiores fontes de compostos fenólicos, pois contém um grande número de classes de metabolitos secundários, possuindo uma composição de polifenóis muito rica tanto qualitativa como quantitativamente. Já foi bem demonstrada a associação de benefícios a saúde com o alto teor de compostos fenólicos, os quais incluem, o resveratrol, os flavonoides quercitina, catequinas, procianidinas, antocianinas entre outros. A grande diversidade entre as cultivares resulta em uvas com diferentes características, tanto de sabor quanto de coloração, o que certamente está associado ao conteúdo e ao perfil dos polifenóis (Toaldo, et al., 2015; Xia, et al; 2010; Abe, et al., 2007).

O resveratrol (3, 5, 4'-trihidroxiestilbeno) é uma fitoalexina, cuja síntese em plantas está associada a resposta aos estresses bióticos e abióticos. Sua biossíntese tem como precursor a fenilalanina (Figura 1), substrato para a fenilalanina amonialiase (PAL) (EC 4.3.1.5) formar ácido cinâmico, que por sua vez, através da catálise da cinamato-4-hidroxilase (C4H) (EC 1.14.13.11), origina o ácido *p*-cumárico. Após a catálise da coumaroil-CoA ligase (4CL) (EC 6.2.1.12), forma-se a *p*-coumaroil-CoA que, junto a 3 moléculas de manolil-CoA, formam o *trans*-resveratrol, esta reação é catalisada pela estilbeno sintase (STS) (EC 2.3.1.95) (Stuart et al., 2013).

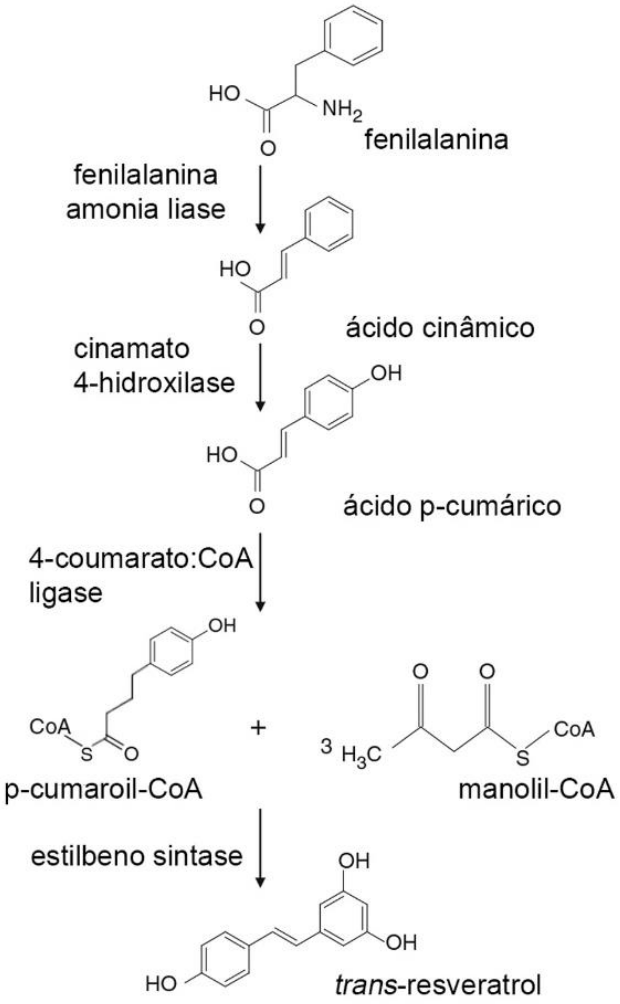

Figura 1. Via biosintética do resveratrol. Adaptado de Stuart, et al., (2013).

Visando facilitar o armazenamento, translocação e a proteção contra a degradação oxidativa, a glicosilação do *trans*-resveratrol forma o *piceid*. Além deste, o *trans*-resveratrol pode ser convertido a compostos como pterostilbeno e viniferina, através de metoxilação e oligomerização oxidativa, respectivamente (Figura 2).

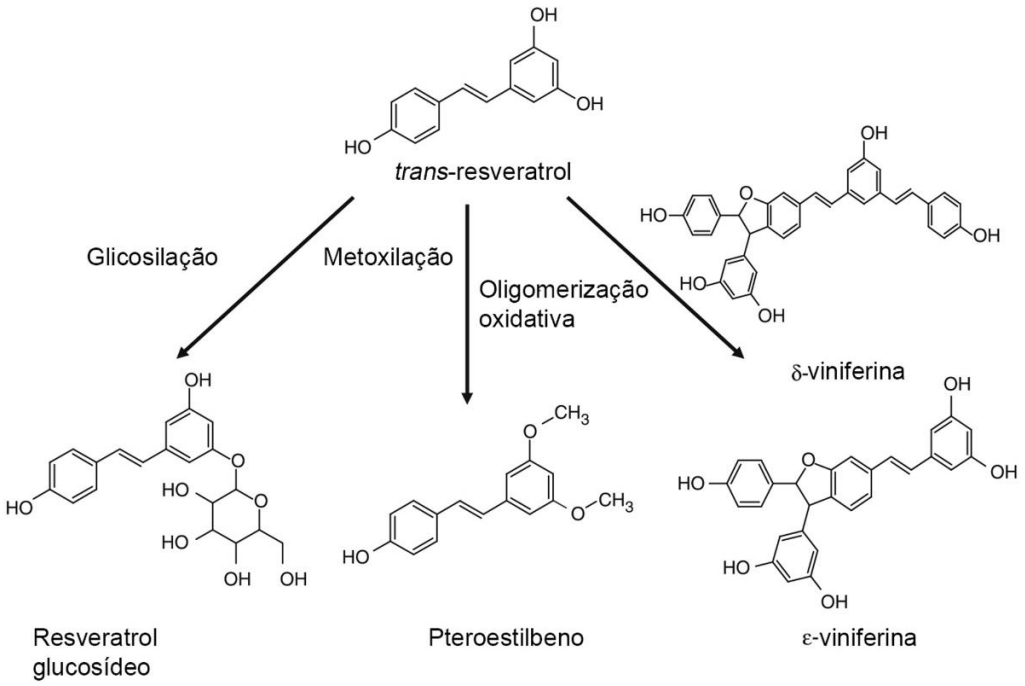

Figura 2. Derivados do *trans*-resveratrol. Adaptado de Stuart, et al., (2013).

Desde sua descoberta em 1940, o resveratrol tem recebido bastante atenção da comunidade científica. Os estudos relacionados aos efeitos na saúde da ingestão de vinho ou suco de uva tornaram-se mais frequentes, e colocaram em destaque o que foi denominado de “paradoxo francês”. Este sugere que os indivíduos franceses têm uma incidência relativamente baixa de doenças coronárias (cardio-circulatórias), apesar de suas dietas ricas em gorduras saturadas, devido ao consumo regular de vinho, que contém compostos antioxidantes, tais como antocianinas, taninos e especialmente resveratrol, que possui a capacidade de reduzir os níveis de lipoproteínas de baixa densidade (LDL) séricas (Wang et al., 2015; Sautter et al., 2005; Renaud et al., 1992). Também há registros de estudos que atribuem ao resveratrol atividades anti-inflamatória, antiobesidade, antidiabetes e neuroprotetivas (Stuart & Robb, 2013; Xia et al., 2010). As mais recentes e promissoras pesquisas indicam que o resveratrol é capaz de mimetizar os efeitos de uma dieta de baixas calorias, cujos efeitos incluem o aumento da longevidade em animais de laboratório, através da ativação de diferentes formas de proteínas da família das sirtuínas (Guarente, L. 2017). Muitos estudos estão sendo realizados no sentido de identificar se o mesmo pode ocorrer em seres humanos. Além disso, alguns derivados de resveratrol, apresentam atividades biológicas similares as encontradas neste composto, podendo

apresentar maior biodisponibilidade *in vivo* que o próprio resveratrol (Stuart et al., 2017).

Em função dos resultados obtidos dos efeitos benéficos do resveratrol e seus derivados sobre a saúde, diversas propostas vêm sendo apontadas para aumentar os níveis de estilbenos na uva e, por conseguinte, em seus subprodutos como o vinho e o suco. Os vinhos brasileiros apresentam-se como uma boa fonte de estilbenos (Vitrac et al., 2005), mas ainda há possibilidades para aumentar as concentrações destes compostos na bebida. Estudos demonstraram que a suplementação de resveratrol em vinho tinto até 200 mg/L, não altera a palatabilidade do produto e os níveis do estilbeno permanecem estáveis (Gaudette et al., 2011). Vinhos e sucos enriquecidos com resveratrol tornam-se meios efetivos de aumentar o consumo de estilbenos na dieta. Contudo, esta é uma alternativa que pode encarecer o preço final do produto tornando-o, assim, pouco atraente para os consumidores.

Estratégias que independam da obtenção de culturas geneticamente modificadas ou mesmo da seleção de clones com variação genética natural, podem ser empregadas em campo ou na pós-colheita, com o intuito de aumentar a concentração de compostos do metabolismo secundário. Neste aspecto, diversos tratamentos com reguladores de crescimento vegetal têm sido propostos para aumentar o teor de compostos bioativos em frutos e hortaliças e, assim, aumentar o valor nutricional dos produtos hortícolas e sua atração para um público cada vez mais informado e exigente no que tange sua saúde.

Dentre os hormônios vegetais que vem sendo estudados para esta finalidade, estão os jasmonatos. Esta classe hormonal desempenha um importante papel como regulador intracelular de diversos processos de desenvolvimento e respostas de defesa (Vezzuli et al., 2007).

O ácido jasmônico (AJ) é sintetizado nas plantas a partir do ácido linolênico que é liberado principalmente das membranas dos plastídeos e convertido ao produto final na via dos octodecanóides (Taiz & Zeiger, 2004; Kerbauy, 2004). Especificamente, a oxigenação do ácido linolênico, pela enzima lipoxigenase (LOX; EC 1.13.11.12), que gera o ácido 13-hidroxi-linolênico, o qual é convertido pela aleno-óxido sintase (AOS; EC: 4.2.1.92), a enzima passo-limitante da biossíntese do AJ (Soto, et al., 2012).

O ácido jasmônico é catabolizado formando vários conjugados no citosol, dentre os quais, o éster volátil metil-jasmonato (MJ), que é produto da ação de uma

carboxi-metil transferase (EC: 2.1.1.141), sendo a reação reversa catalisada pela metil-jasmonato esterase.

O metil-jasmonato ocorre naturalmente nas plantas, está presente na maioria das frutas e tem um histórico seguro de exposição alimentar, sendo considerado um agente flavorizante não tóxico pela FAO/WHO. Além disso, é classificado pela *Food Drug and Administration* (FDA) como substância *"Generally Recognized as Safe* (GRAS)*".* Este hormônio vegetal é indutor de resistência sistêmica adquirida (SAR-indutor), desencadeando respostas de defesa, ajudando a proteger a planta contra-ataques de insetos, fungos e bactérias (Pfeife, et al., 2013). Este mecanismo de aumento da resistência pode proporcionar efeitos direto e indireto, como a expressão gênica e síntese de fenólicos, fitoalexinas como quitinases e β, 1,3, glucanases, diversos compostos antioxidantes enzimáticos e não enzimáticos, inibidores de proteases, alcaloides, e aumentando compostos aromáticos que são desagradáveis para patógenos ou possuem atividade bactericida ou fungicida (Asghari, M., 2019).

Diversos estudos indicam que AJ aumenta a biossíntese de compostos fenólicos em diversas plantas (Figura 3) através da ativação da via do shikimato e ativando diversos sinais para a expressão gênica e atividade de enzimas como a fenilalanina amônia liase (PAL; EC 4.3.1.24); chalcona sintase (CHS; EC 2.3.1.74) e estilbeno sintase (STS; EC: 2.3.1.95), proporcionando um aumento significativo na síntese de estilbenos e flavonoides como antocianinas e taninos condensados (Fig 3).

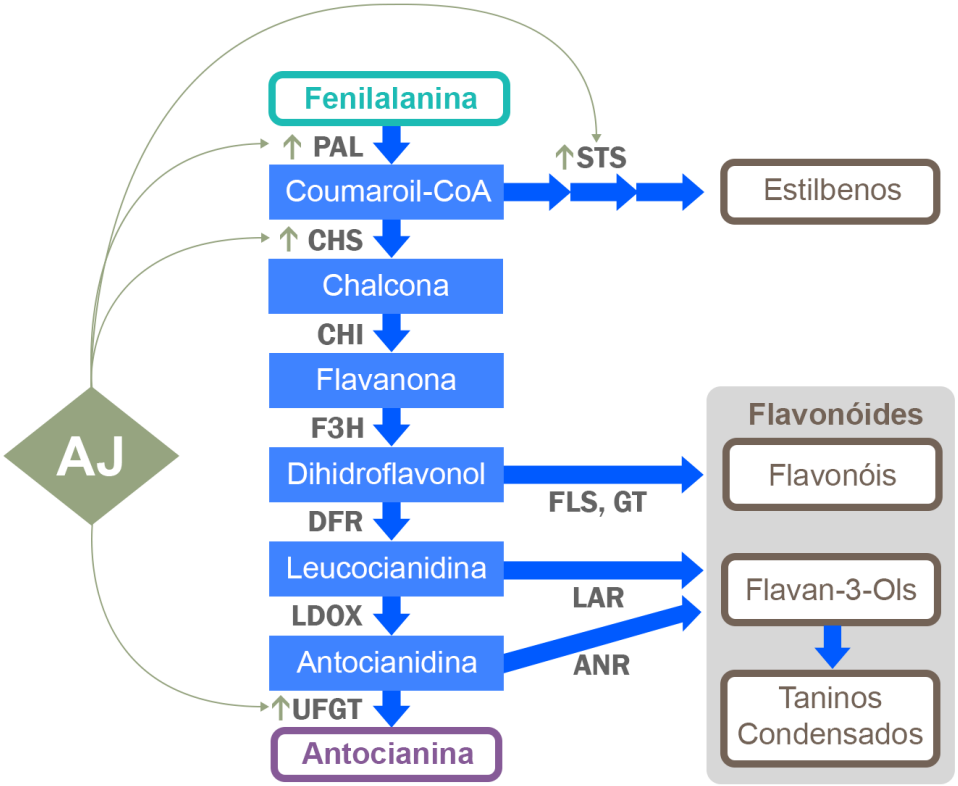

Figura 3. Mecanismo de aumento da biossíntese de fenólicos através de jasmonatos. Adaptado de Asghari, M (2019). CHI - chalcona isomerase; F3H – flavanona 3-hidroxilase; DFR – dihidroflavonol redutase; LDOX: leuconianidina dioxygenase; UFGT - UDP glicose: flavonóide-3-O-glicosiltransferases. GT – glicosiltransferases; FLS – flavonol sintase; LAR – leucoantocianidina redutase; e ANR – antocianidina returase;

Aplicações repetidas de metil-jasmonato em vinhas *V. vinifera* em desenvolvimento, aumentaram substancialmente os níveis de *trans*-resveratrol e viniferina nas bagas (Larronde et al. 2003; Vezzulli et al. 2007). Em cultura de células de videiras, o metil-jasmonato estimulou a síntese de estilbenos (Portu et al., 2016; Voung et al., 2014). Vezzulli e colaboradores (2007) observaram aumento na síntese de *trans*-resveratrol após a aplicação de 10 mM MJ (dissolvido em 100% etanol) em *V. vinifera* cv. Barbera, durante o amadurecimento através de três aspersões nos cachos em intervalos de 48 horas na mudança da cor dos frutos, sendo coletadas as amostras no 8º dia após os tratamentos.

Contudo, devido a sua complexidade, poucos estudos com aplicação de MJ em uvas são realizados a campo, sendo em sua maioria, realizados *in vitro* (cultura celular), ou em casas de vegetação em ambientes controlados. O destaque do estudo aqui proposto foi avaliar a viabilidade de sua utilização em um ambiente com a

complexidade de variáveis como variações meteorológicas, interação com outras plantas e outros fatores. Além disso, os estudos desenvolvidos até o momento foram realizados com cultivares de *Vitis vinifera* L., visando o enriquecimento de uvas e a elaboração de vinhos. No entanto, no Brasil aproximadamente 80% das uvas processadas são *Vitis labrusca* L. originando vinhos de mesa, geleias, e especialmente suco.

Destaca-se o suco de uva brasileiro, por ser elaborado com estas uvas e apresentar as características de aroma e sabor apreciados pelos consumidores nacionais e de vários outros países. Observa-se nos últimos anos, um aumento na elaboração de suco de uva, decorrente da crescente demanda (Gráfico 1).

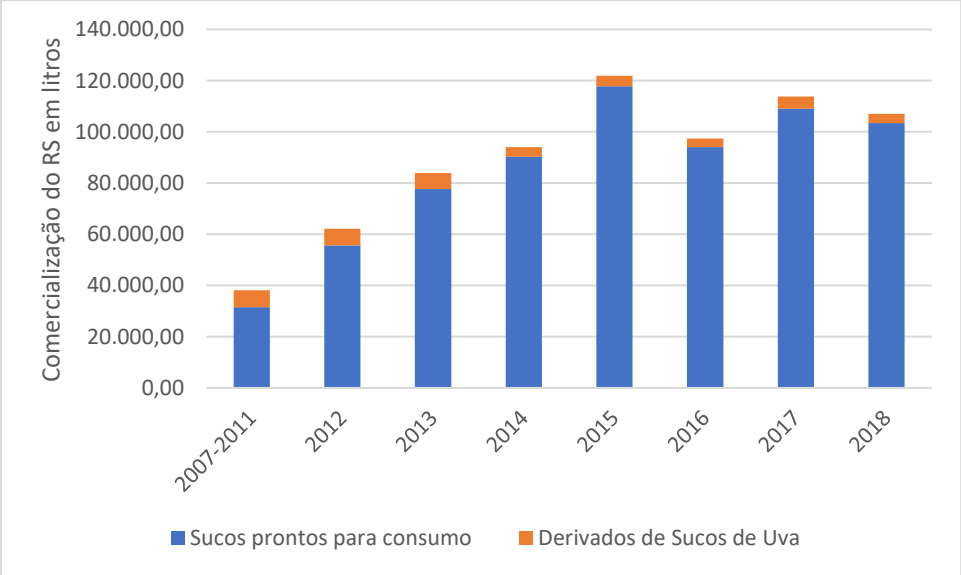

Gráfico 1. Evolução da comercialização de suco de uva no Rio Grande do Sul, dados: IBRAVIN (2019)

Do total do suco elaborado na vindima de 2018, 92,6% apresenta-se na forma natural/ integral, seguido por reprocessado/reconstituído (5,4%), polpa de uva (1,51) e mosto de uva (0,12) (IBRAVIN, 2019).

Este acréscimo na elaboração de suco de uva, recebeu um incentivo nos últimos anos, decorrente do aumento de consumo. Neste último ano (2017/2018) o consumo de suco de uva aumentou 23,28% (IBRAVIN, 2019). Além deste, houve a inclusão do suco de uva nas refeições das escolas públicas, através da Lei Nº 13.247, de 08 de setembro de 2009 do estado do Rio Grande do Sul e Lei Promulgada Nº 14.995, de 16 de dezembro de 2009 do estado de Santa Catarina. Disponibilizando um produto mais saudável para crianças e estimulando o setor vitícola, especialmente

pequenos produtores e agronegócios familiares (Dachery et al., 2016). Em todo país, um grande número de pequenos empreendimentos familiares (cerca de 50.000 produtores), elaboram o suco pelo sistema de panela extratora por arraste de vapor (Figura 4) (Guerra et al., 2016).

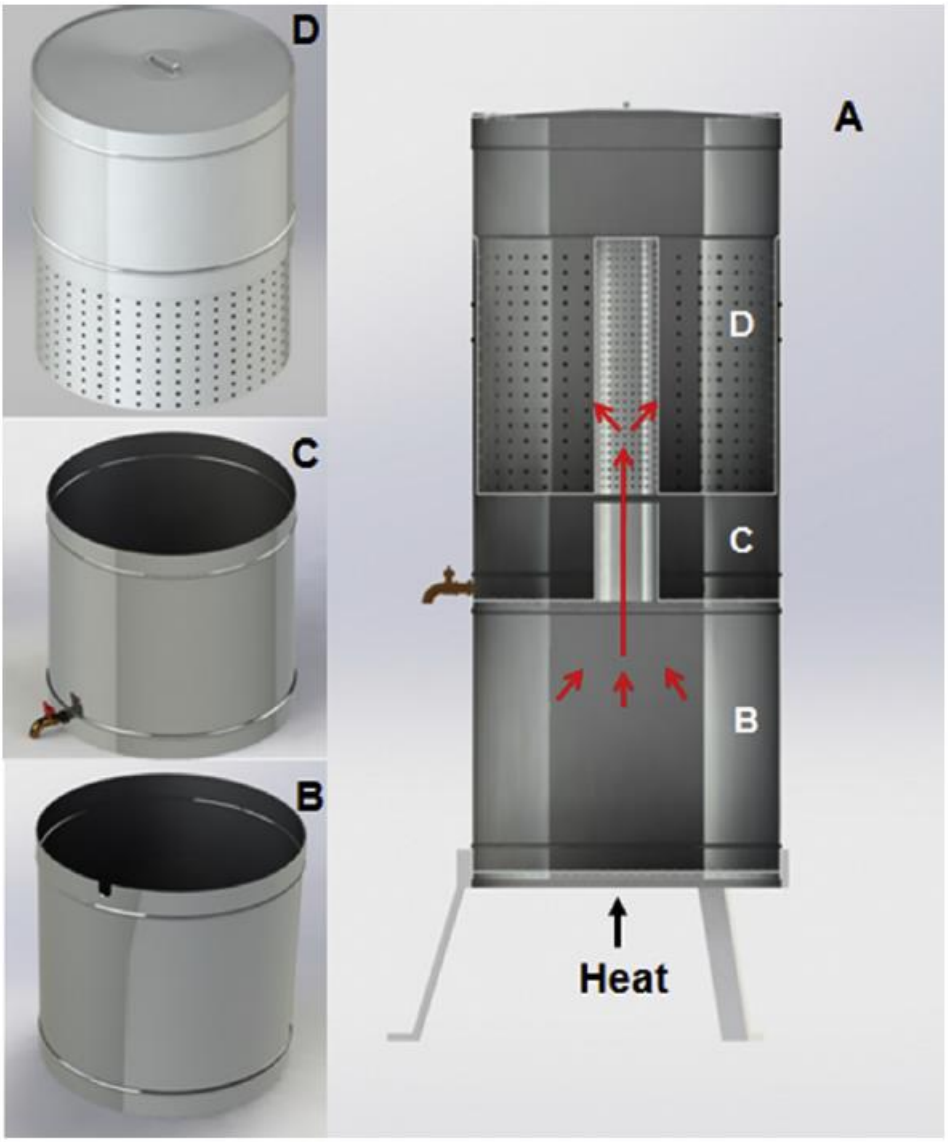

Figura 4. Sistema de Extração de arraste de Vapor. Adaptado de Dachery et al., (2017). A - Visualização interna da panela extratora. B - Reservatório de água; C - coletor do suco; D – armazenamento das uvas.

Este sistema consiste de uma fonte de calor (caldeira, fornalha, vaso de aquecimento ou queimador a gás ou a óleo Diesel), que aquece um recipiente (panela) contendo água potável (Figura 4 - B). Na parte superior está acoplada uma segunda panela (Figura 4 - D), com pequenos orifícios em sua parte inferior, a qual contém a

uva desgranada e intacta. O vapor d'água formado pela fervura, sobe e passa através das bagas de uva, amolecendo-as. Desse modo, o suco das bagas amolecidas é liberado e recolhido (Figura 4 - C). O suco assim obtido pode ser imediatamente engarrafado, ainda quente, ou ser resfriado para a decantação das borras para mais tarde sofrer pasteurização (o suco é colocado de volta na panela e aquecido) e envase (Guerra et al., 2016; Dachery et al., 2017).

Em estudos da ingestão de suco de uva elaborado com *V. labrusca* L., foi observado que 1 hora após o consumo de suco foi suficiente para determinar um aumento do status antioxidante. Os rápidos efeitos verificados no plasma, neste estudo, foram associados a capacidade dos compostos presentes no suco, como compostos fenólicos, de modular mecanismos oxidativos enzimáticos e não enzimáticos, sem afetar o status glicêmico (Toaldo et al., 2015). Também foi observado um efeito protetivo do consumo de suco de uva, contra peroxidação lipídica, através da limpeza de radicais peroxil na membrana fosfolipídica das células (Toaldo et al., 2016).

Em um cenário no qual os consumidores estão priorizando dietas mais saudáveis e aumentando seu conhecimento sobre procedência, qualidade e segurança de alimentos, uvas enriquecidas em seus compostos bioativos, assim como seus produtos derivados, como o suco de uva, se destacam perante o mercado consumidor. O suco de uva apresenta-se como uma boa fonte natural de polifenóis com efeitos bioativos para a população, particularmente para crianças, jovens e adultos que não consomem bebidas alcoólicas.

2. Objetivo

Através de aplicação do hormônio vegetal metil-jasmonato no período de pré-colheita em uvas (*Vitis labrusca* L.), aumentar nas bagas os níveis de estilbenos, dentre os quais o resveratrol, assim como outros compostos fenólicos, levando a obtenção de produto com maior potencial bioativo, quando comparado aos frutos não tratados.

3. Descrição das Sessões

O presente trabalho foi subdividido em três sessões, a primeira sessão engloba a otimização do período de aplicação dos tratamentos, a segunda contempla a análise metabolômica de uvas e suco de uva, enquanto a sessão 3 avalia o conteúdo de estilbenos nas uvas e suco de uvas *Vitis labrusca* L.

Sessão 1

Com o objetivo de otimizar o período de aplicação do tratamento com metil jasmonato (MeJa), em uvas *Concord* e *Isabel Precoce* (*Vitis labrusca* L.), foram realizados testes durante a vindima de 2015. Os melhores resultados foram observados a partir da aplicação de MeJa em 2 períodos (*véraison* - período de mudança de cor dos frutos e 2 semanas prévias a colheita). Este protocolo foi utilizado para as aplicações em ambas cultivares em 2016, onde foram avaliados o conteúdo de açúcares solúveis, ácidos orgânicos, antocianinas, compostos voláteis e estilbenos. Foi possível aumentar em 60% o conteúdo de *trans*-resveratrol em uvas *Isabel Precoce* (colhidas em 2016) no grupo tratado com MeJa. O perfil de compostos voláteis destas uvas, demonstrou a abrangência do tratamento, indicando vias biosintéticas de compostos voláteis responsivas ao MeJa em ambas vindimas. A identificação de antocianinas demonstrou não haver modificação no perfil entre grupos, sendo observadas diferenças apenas entre cultivares.

O trabalho desta sessão, relacionado a influência do tratamento com MeJa na biossíntese de compostos voláteis, foi publicado na revista *Acta Horticulturae* (doi: 10.17660/ActaHortic.2019.1248.60). Enquanto a otimização dos tratamentos para o aumento dos estilbenos, foi submetido a revista Oeno one.

Sessão 2

Visando compreender o efeito de modo mais amplo, nas modificações proporcionadas pelo MeJa, em vias metabólicas que não sejam o alvo de nosso projeto. Realizou-se um estudo metabolômico, através de análise *fingerprinting*, que possibilitou a caracterização de um amplo número de metabólitos, em uma abordagem qualitativa e quantitativa. Para isso, foram avaliadas as uvas e suco de uvas cultivadas nas regiões Sul (Bento Gonçalves, RS) e Sudeste (Caldas, MG) após aplicações do MeJa, durante a vindima de 2017. Para as uvas, MeJa influenciou diversos flavonoides, especialmente antocianinas, estilbenos e ácidos fenólicos

(hidroxicinamicos) em ambas regiões, sendo a cultivar Concord mais responsiva que Isabel Precoce. De modo geral, foram detectados como fortes marcadores os estilbenos (resveratrol; *cis* e *trans* - piceid); antocianinas (derivados de malvidina); ácidos fenólicos (ácidos coumarico e cafeico). Para o suco elaborado a partir das uvas tratadas com o MeJa, foram observados como marcadores os estilbenos (resveratrol); antocianinas (derivados de cianidina e petunidina); ácidos fenólicos (cafeico); o flavonol rutina e os principais flavan-3-ol catequina e epicatechina. Este estudo demonstrou o potencial do MeJa na indução de compostos bioativos, com potencial na promoção da saúde, visto que diversos marcadores detectados nas uvas se mantiveram após o processo térmico utilizado para a elaboração do suco.

O trabalho desta sessão, relativo à análise metabolômica das uvas, foi submetido a *Metabolomics* ISSN: 1573-3882 (Print) 1573-3890 (Online). Enquanto a análise metabolômica dos sucos de uvas será submetida para publicação na revista *Food Research International* (ISSN: 0963-9969).

Sessão 3

Baseado nos resultados obtidos na seção I, na qual são apresentados os resultados sobre o melhor período para a aplicação de MeJa em *Vitis labrusca* L., avaliou-se a efetividade do tratamento perante a influência de diferentes fatores edafoclimáticos. Para isso, foram realizadas as aplicações do MeJa em uvas cultivadas nas regiões Sul (Bento Gonçalves, RS) e Sudeste (Caldas, MG) durante a vindima de 2017 e 2019. Além disso, estas uvas foram processadas, e o suco elaborado, foi avaliado quanto ao teor de estilbenos durante o período de armazenamento de 6 meses. Os resultados do tratamento foram expressivos para as sementes das uvas cultivadas na região Sudeste. Para os sucos, as maiores concentrações de *trans*-resveratrol foram observadas nas avaliações iniciais, a partir de uvas tratadas com MeJa, em ambas vindimas. Melhor manutenção de estilbenos durante o armazenamento, foi observada em sucos elaborados a partir de uvas tratadas com MeJa, cultivadas na região Sudeste, especialmente para *Isabel Precoce* na vindima 2017.

SESSÃO 1

Methyl jasmonate application to increase volatile compounds of *Vitis labrusca* L. grape berries cultivated under subtropical conditions

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Abstract

There has been increased demand for eco-friendly means to improve the bioactive content of fruits. Various studies have focused specifically on methyl jasmonate (MJ), a plant growth regulator. Field applications of MJ appear to induce secondary metabolism in *Vitis vinifera* L. grapes; however, few studies have focused on the species *Vitis labrusca* L., even though its cultivars represent approximately 80% of processed grapes in Brazil. Therefore, this study aimed to evaluate the best period for MJ application on *V. labrusca* L. grapes in the field and the impact on free volatile compounds. The project was conducted in Rio Grande do Sul state in south Brazil over two consecutive years. Free volatile compounds were extracted through solid-phase microextraction, identified using gas chromatography-mass spectrometry (GC-MS), and the results were analyzed by multivariate statistics. Optimal results were found when MJ application occurred during two periods, véraison and pre-harvest. For both harvests, an increase in esters and aldehyde levels without interference in organic acid content was found. Increasing volatile compounds in *V. labrusca* grapes could be an interesting area for future study, since a decrease in total content occurs during processing, particularly for juice and jellies.

Keywords: pre-harvest, methyl jasmonate, VOCs, *V. labrusca,* Concord, Isabel

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1. Introduction

In Brazil, American cultivars of *Vitis labrusca* L. represent more than 80% of processed grapes, with ‘Isabella’ and ‘Concord’ being among the most commonly cultivated types, at around 50% of total grape production (Toaldo et al., 2015). Fruit from these cultivars have a significant amount of volatile esters, responsible for the

“fruity” character and “foxy” smell. These characteristics often cause grape byproducts to be rejected among consumers, particularly European wine drinkers, although these products are generally accepted in the United States and among a specific sector of Brazilian wine and grape juice consumers (Biasoto et al., 2014; Ribéreau-Gayon et al., 2006).

‘Isabella’ grapes are known for their strawberry-like aroma, due mainly to furaneol, while ‘Concord’ grapes have a characteristic “foxy” aroma due to the presence of methyl anthranilate, amino acetophenone, and furanone (Ghaste et al., 2015; Keller, 2010). Many factors affect the biosynthesis of these compounds and, because of their importance for flavor, increased demand exists to improve these secondary metabolites in fruits, preferably in an eco-friendly manner. Among the promising options is methyl jasmonate (MJ), a volatile ester derivative of jasmonic acid known to act as a signaling molecule upon biotic stress and to be involved in plant defense mechanisms (Koo and Howe, 2009). MJ has been applied to control some microbial pathogens, for instance the fungus *Erysiphe necator* (Belhadj et al., 2006). Furthermore, a chemically synthesized MJ, 2,3-dihydroxypropyl jasmonate, has been shown to be an efficient elicitor to enhance resveratrol accumulation in cell suspension cultures of *Vitis vinifera* L. × *V. labrusca* ‘Kyoho’ (Shen et al., 2012). Additionally, MJ tends not to leave a residue in treated foods, as it has high vapor pressure and tends to diffuse into the atmosphere (Flores et al., 2015; Reyes-Díaz et al., 2016). Thus, MJ appears to be a viable alternative to improve volatile compound content. Portu et al. (2017) found that MJ foliar application in the warmest part of Spain’s Rioja wine region increased the levels of anthocyanins and stilbenes in ‘Tempranillo’ grapes. However, to our knowledge, no information exists on volatile compound content after MJ application to *V. labrusca* grapevines cultivated under subtropical conditions.

The literature largely agrees that the chemical composition of grapes in terms of volatile compounds depends on many factors, including soil composition, sunlight exposure, pathogen attacks, and agronomical procedures (Granato et al., 2016). Brazil’s southern region processes approximately 90% of the grapes cultivated in the country. According to the Köppen classification, the southern region’s climate is type Cfa, a subtropical climate. The average temperature is below 18°C in the coldest month and above 22°C in the warmest month (Mello, 2017; Alvares et al., 2013). These climate characteristics may influence not only the biosynthesis of volatile compounds, but also the vine response to MJ treatment. Therefore, this study aims to evaluate the

impact of MJ treatment on grapes of ‘Isabel Precoce’ (a spontaneous somatic mutation of ‘Isabel’) and ‘Concord’ grown under subtropical conditions in terms of free volatile compounds over two consecutive years.

2. Material and Methods

2.1. Plant Material and Field Treatments

The field experiment was conducted on commercial vineyards of Isabel Precoce (spontaneous somatic mutation of the cultivar Isabel - *Vitis labrusca* L.) and Concord grape cultivars (*Vitis labrusca* L.*)*, with five-year-old and 20-year-old*,* respectively, located in Bento Gonçalves, Rio Grande do Sul state, Brazil (29° 10′ 26″ S e 51° 31′ 7″ W, altitude: 671 m). Vines were grafted onto *1103-Paulsen* rootstock trained on a tendone, overhead trellises system with a between-row and within-row spacing of 2.8 × 1.5 m. Winter pruning was performed, leaving two to three buds per spur. The vineyard was managed according to standard viticultural practices for the cultivar and region as described by Camargo U. A. (2004).

Climatic conditions were monitored through a meteorological station and the data was collected from Instituto Nacional de Meteorologia (INMET - http://www.inmet.gov.br/portal/).

The MJ solution was prepared according to Vezzulli et al. (2007) at a concentration of 10 mM; it was applied manually by spraying directly into the clusters at *véraison* three times (approximately 50 mL per cluster on days 1, 3, and 6 after *véraison*) and/or once approximately two weeks before harvest. Control fruits (CN) were sprayed with water (approximately 50 mL per cluster). The treatments were applied in completely randomized blocks with an experimental design consisting of three replicates (sampling number, n=3) of six vines per treatment, with 20 clusters per vine. The grapes were harvested during the summer seasons of 2015 and 2016 when they reached commercial maturity for harvest; a random set of 40 berries per vine was collected and stored at -80 °C until analysis.

During 2015, tests were performed to evaluate treatment efficacy. Isabel Precoce grapes received the treatment at *véraison* (with three applications) plus a single application two weeks before harvest (identified as group M). In addition to the control group (identified as group CT), another block was treated with MJ (single application) only two weeks before harvest (identified as group F). Concord grapes were treated twice: prior to *véraison* (with three applications) plus a single application two weeks before harvest.

In terms of the results, we observed a better response with the MJ application in two periods: at *véraison* with three applications (on days 1, 3, and 6) plus a single application approximately two weeks before harvest. These treatments were used on both cultivars during the 2016 harvest.

2.2. Soluble Sugar Content and Organic Acids

Organic acid content was determined after the separation of sugars through an anion exchange resin (Bio-Rex 5, Bio-Rad Laboratories, Hercules, CA, EUA), as described by McCord et al. (1984). Tartaric and malic acids were quantified by high-performance liquid chromatography in a HP1100 system (Hewlett-Packard Company, Palo Alto, CA, EUA) coupled with a diode-array detector. The HP1100 system was equipped with a SupelcoGel C-610H 30 cm x 7.8 mm column (Supelco Sigma-Aldrich, Bellefonte, PA, EUA); the runs were isocratic, with 0.1% H3PO4 as the mobile phase, as described by Mota et al. (2010).

The soluble sugar content was extracted and evaluated as described by Gomez, Lajolo, Cordenunsi (2002).

2.3. Free Volatile Compound Content

Sample preparation occurred with 3 g of grape tissue originating from 10 random berries, which were added to a 20 mL solid-phase microextraction (SPME) glass vial (Supelco) containing 7 g of saturated NaCl solution. The vial was tightly capped with a Teflon/silicone septum (Supelco) and stored at -20°C until analysis. The analysis of free volatile compounds was performed according to Sun et al. (2011), with some modifications. SPME was executed using a 100 µm fiber coated with divinylbenzene/carboxen/polydimethylsiloxane (Supelco, Bellefonte, PA), which was thermally conditioned for one hour at 270°C before use. For the sampling of volatiles, the vials were incubated at 40°C for 10 minutes. The SPME fiber was then exposed to the sample for 40 minutes at 40°C under agitation with a magnetic stir bar.

Compound identification was performed via GC-MS using a HP6890 chromatograph coupled with a HP5973 mass selective detector (Agilent Technologies, Palo Alto, CA) fitted with a Sulpelcowax 10 (30mts x 0.25 mm D.I. x 0.25 µm) column. The SPME fiber was inserted into the inlet at 250°C in splitless mode. The purge was activated at 2 minutes, and the carrier gas in constant flow mode was helium (1 mL/min.). The oven temperature was held at 35°C for 3 minutes, programmed at 6°C/min. to 250°C, and held for 5 minutes isothermally. Mass spectra were acquired

over *m*/*z* 33–250. Chemstation software version G1701EA E.02.00.493.33 was used for data acquisition. To determine retention indices, a C7 C30 n-alkane standard was run under the same conditions, and linear retention indices were calculated using standard approaches. The FD value was geometrically averaged from the data of two replicates using the equation FD=2(a+b)/2. The mass spectra of volatile compounds were compared to those in the NIST version 2011 library and confirmed with the spectral data from MassBank of North America (http://mona.fiehnlab.ucdavis.edu/).

2.4. Statistical Analysis

Soluble sugars and organic acids were expressed as mean ± standard deviation (n=3). Statistical analysis was performed using Graph Pad Prism software version 5.01 (GraphPad Software, CA, USA). Differences among treatments were analyzed using analysis of variance and the Student–Newman–Keuls test, assuming a significance level of 0.05.

Heatmap and principal component analysis (PCA) were used as statistic multivariate techniques using MetaboAnalyst 3.0 (http://www.metaboanalyst.ca) (Xia et al., 2015).

3. Results and Discussion

Grapes were harvested in 2015 and 2016 when they reached a total soluble solids content of 16 and 14 °Brix, respectively, for ‘Isabel Precoce’. ‘Concord’ grapes were harvested with a total soluble solids content of 15 °Brix for both harvests.

Grape berries harvested in 2015 began to grow in 2014, with a total annual rainfall of 2.043 mm, an average temperature of 18.2°C, and a relative humidity of 78% (Figure 1). These conditions included excessive rainfall, especially during budding, and the above-average temperature was conducive to fungi infections (Alves and Tonietto, 2015). In addition, the harvest was advanced approximately 15 days to prevent major losses.

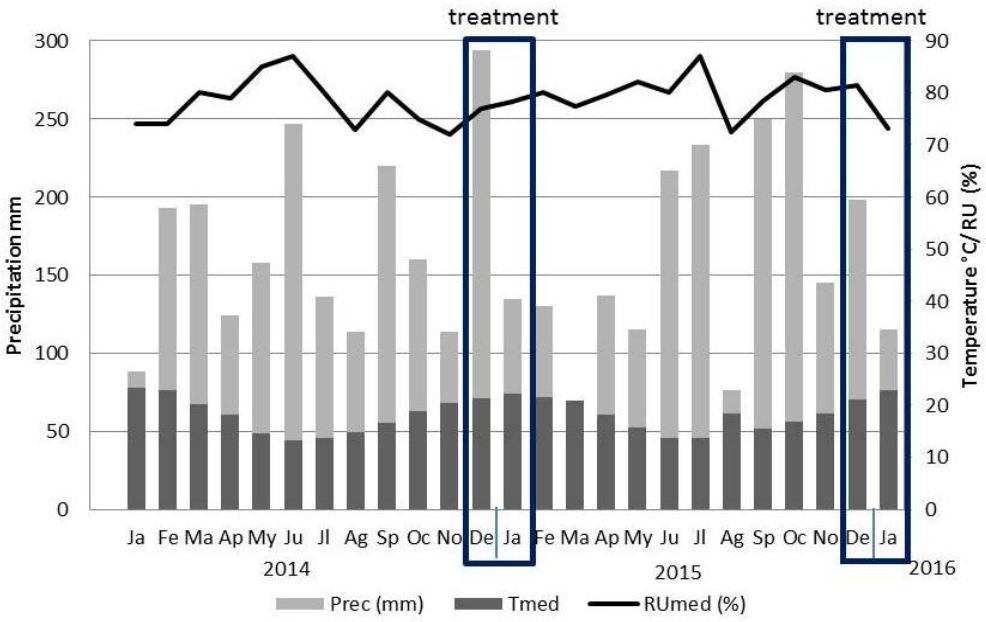


Figure 1: Mean precipitation (mm), mean temperature (°C), mean relative humidity (RU %) of Bento Gonçalves, (Rio Grande do Sul state) Brazil, from 2014 to 2016. Ja-January, Fe-February, Ma-March, Ap-April, My-May, Ju-June, Jl-July, Ag-August, Sp-September, Oc-October, No-November, De-December.

Grapes from the 2016 harvest were grown in 2015 (Figure 1), with an annual rainfall of 1.971 mm, an average annual temperature of 18.0°C, and a relative humidity of 80.1%. Despite these conditions, grapes from the 2016 harvest experienced low productivity, particularly on early harvested grapes, as Isabel Precoce. Budding was advanced due to high temperatures during August, with considerable damage caused by the severe mid-September frost that reduced vineyard productivity. Adding to these conditions, October and November brought significant rain, unfavorable for the flowering period. Hydric stress hindered vegetative growth and was conducive to fungi infections (Alves et al., 2016).

Despite these climate particularities, we opted to evaluate both harvests to observe treatment responses under the unusual weather conditions of 2016.

3.1. Soluble Sugar and Organic Acid Content

Sugar content creates fruit's sweet taste, decreases the perception of astringency and bitterness. Besides, sugars have been reported to regulate gene expression, including for those involved in the synthesis of secondary compounds (Belhadj et al., 2008; Davis et al., 2012; Keller, M., 2010). Due to its importance, the soluble sugar content of both harvests was evaluated. For Concord and Isabel Precoce

grapes (Table 1), the MJ treatment at *véraison* and/or near harvest significantly affect the glucose, fructose, and sucrose content only on 2015 harvest.

Table 1. Soluble sugar and organic acid content for Concord and Isabel Precoce grapes (n=3) harvested in 2015 and 2016 in Bento Gonçalves, Brazil

	Soluble sugars (g/100g grape f.w)			*Organic acids (g/100g grape f.w.)*	
	Glucose	**Fructose**	**Sucrose**	**Malic acid**	**Tartaric acid**
Concord					
C15	3.06 ± 0.12	4.06 ± 0.27	0.03 ± 0.01	0.04 ± 0.01	0.07 ± 0.01
M15	3.06 ± 0.01	3.84 ± 0.03	0.02 ± 0.01	0.05 ± 0.01	0.08 ± 0.01
Isabel Precoce					
C15	3.80 ± 0.24	4.90 ± 0.39	0.06 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
M15	3.74 ± 0.27	2.84 ± 0.05 a	0.02 ± 0.01 a	0.07 ± 0.01	0.05 ± 0.01
MF15	2,18 ± 0.12 c	5.11 ± 0.03 b	0.09 ± 0.01 a	0.06 ± 0.01	0.05 ± 0.01
Concord					
C16	3.48 ± 0.44	5.36 ± 0.68	0.08 ± 0.02	0.07 ± 0.01	0.04 ± 0.01
M16	3.50 ± 0.11	5.27 ± 0.33	0.09 ± 0.02	0.08 ± 0.00	0.05 ± 0.00
Isabel Precoce					
C16	3.61 ± 0.39	4.52 ± 0.43	0.12 ± 0.05	0.08 ± 0.01	0.06 ± 0.01
M16	3.70 ± 0.06	4.51 ± 0.02	0.15 ± 0.02	0.07 ± 0.01	0.06 ± 0.01

C: Control group; M: Methyl jasmonate treated in two periods; MF: Methyl jasmonate treated in one period. For each compound, different letters in the same column indicate significant difference with control ($p \leq 0.05$)

Grapes sweetness interacts with acidity, phenolic and volatile compounds to create flavor perception. In grapes, the predominant acids are tartaric and malic acids, which together may account for over 90% of the total acidity (Ford, C. M., 2012; Keller, M., 2010). Organic acid content was not influenced by MJ treatment (Table 1); the harvest neither period nor cultivar type influenced organic acid content for either harvest.

According to Keller, M. (2010), the majority of changes to grape composition are caused by climate variability, annual weather differences, and vineyard location. As the experiment was replicated at the same location, MJ treatment did not appear to significantly affect organic acid in vines grown on subtropical conditions.

Regarding soluble sugar contents, we were able to observe difference significate to soluble sugar content only for Isabel Precoce grapes MJ treated on 2015

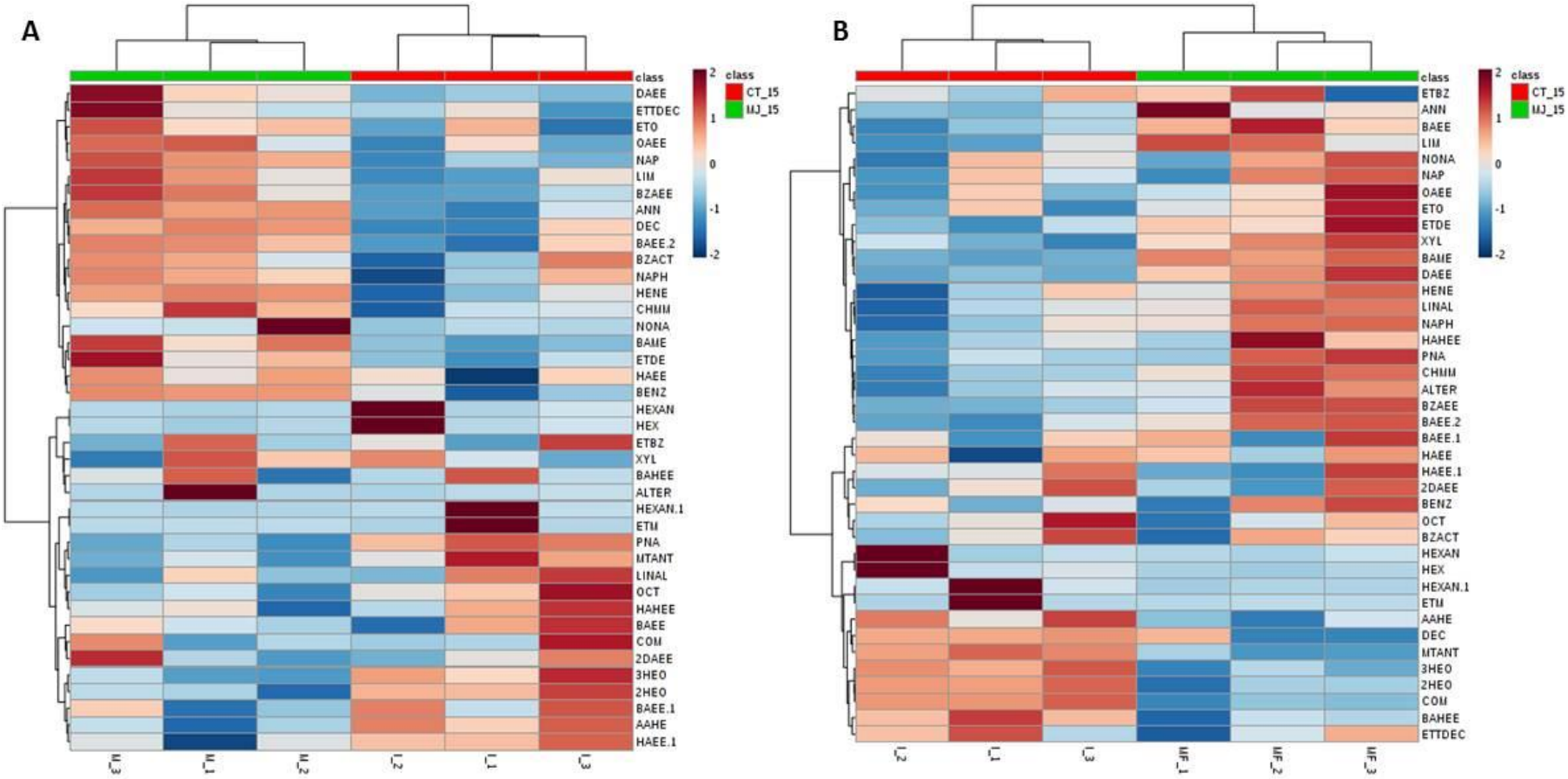
harvest. MJ treated fruits at one period (MF15) present higher levels of fructose and sucrose while M15 present lower levels of sucrose. However, these significative changes do not occur on 2016 harvest. We believe that lower levels observed on M15 might be related to the answer of the treatment on other compounds, since glycosylation is a functional form to storage and translocate molecules as volatile compound, anthocyanins and stilbenes (Flamini et al., 2013). However, to confirm this hypothesis further studies are needed regarding glycosylated volatile compounds, since season (environmental conditions - Figure 1) might affect soluble sugar content as volatile composition, being a dominant factor (Garde-Cerdan et al., 2018).

3.2. Free Volatile Compounds

We identified 59 free volatile compounds in both cultivars and harvests, through NIST library and spectral data was confirmed through MassBank of North America. Among these compounds we detect: esters (twenty-seven compounds), aldehydes (seven compounds), alcohols (thirteen compounds), ketone (four compounds), aromatic hydrocarbons (five compounds), and terpene (two compounds) (Table 2).

Besides the define clusters (Figure 2) of CT (red) and MJ (green), we can also visualize on the left, three defined clusters of free volatile compounds for Isabel Precoce 2015 harvest (Figures 2A and 2B). The first cluster presents compounds as octanoid acid ethyl ester (OAEE) that were stimulated by MJ application; the second one does not demonstrate a clear pattern of variation as *p*-xylen (XYL), and the third has compounds that diminish with MJ treatment as 2-hexenal (HEX). Isabel Precoce grapes that received MJ treatment at both periods (*véraison* and pre-harvest) (Figure 2A) demonstrate higher intensity (red) and homogeneity for compounds enhanced by MJ treatment than fruits that receive the treatment at only one period (Figure 2B). Based on this result, we choose to perform the application on 2016 with 4 treatments divide between *véraison* (3 applications) and pre-harvest (single application) for both cultivars.

The control and MJ-treated groups demonstrated different profiles of free volatile compounds. Differences highlighted by the hierarchical clustering were also reviewed in the scores plots (Supplementary material FS1), in which the comparative analysis made differences more evident between the control and MJ groups from each cultivar and harvest.



Figures 2. Free volatile compound heatmaps of 'Isabel Precoce' grapes harvested in 2015 after MJ treatment in two periods (A) or one period (B). Samples I_(1-3) belong to control group (CT), samples M_(1-3) belong to group treated with methyl jasmonate (MJ) in two periods, and samples MF_(1-3) belong to group treated once with MJ (F).

Table 2. Free volatile compounds identified on Isabella and Concord grapes (n=3) harvested in 2015 and 2016 in Bento Gonçalves, Brazil.

Compound	Code	Aromatic note[1]	R.I.[2]	R.I.[3]	CAS Number
Ester					
Ethyl trans-2-octenoate	ETO	Fruity, pineapple,	1557	1530	2351-90-8
Butanoic acid, ethyl ester	BAEE	Floral, apple	1015	1015	105-54-4
Butanoic acid, 2-methyl-, ethyl ester	BAME	Apple	1028	1028	7452-79-1
Hexanoic acid, ethyl ester	HAEE	Apple, fruity	1199	1193	123-66-0
Acetic acid, hexyl ester	AAHE	Fruity, herbal	1276	1234	142-92-7
Heptanoic acid, methyl ester	EAME	Floral, fruity			
2-Hexen-1-ol, acetate, (E)-	HOA	Fruity	1315	1294	2497-18-9
2-Hexenoic acid, ethyl ester	HAEE	Pineapple, apple	1336	1308	1552-67-6
Octanoic acid, ethyl ester	OAEE	Wine, fruity, floral	1438	1401	106-32-1
Butanoic acid, 3-hydroxy-, ethyl ester	BAHEE	-	1490	1485	5405-41-4
Nonanoic acid, ethyl ester	NONAEE	Oily note, fruity	1509	1509	123-29-5
2-Octenoic acid, ethyl ester	EOCT	Fruity green	1557	1529	7367-82-0
Propanedioic acid, diethyl ester	PADE	Light	1582	1549	105-53-3
Tetrahydrofuran-2-acetic acid ethyl ester	THFAE	N.D.	1586	1587	2434 02 8
2-Furancarboxylic acid, ethyl ester	FCAEE	N.D.			
Decanoic acid, ethyl ester	DAEE	Grape	1639	1614	110-38-3
Ethyl trans-4-decenoate	ETTDEC	Sweet, orange,	1680	1642	76649-16-6
Benzoic acid, ethyl ester	BZAEE	chamomile, floral, Herbal	1650	1647	93-89-0
Hexanoic acid, 3-hydroxy-, ethyl ester	HAHEE	Fresh	1650	1653	2305-25-1
Butanedioic acid, diethyl ester	BADE	Pleasant	1662	1650	123-25-1
Ethyl trans-2-decenoate	ETDE	Oily note, pear	1750	1743	7367-88-6
Pentanedioic acid, diethyl ester	PEADE	Grape, fresh	1768	1758	818-38-2
Benzeneacetic acid, ethyl ester	BAEE	Fruity, sweet	1754	1765	101-97-3
Benzoic acid, 2-hydroxy-, ethyl ester	BEAHEE	Mint, floral	1806	1804	23726-93-4
Ethyl-trans-2, cis-4-Decadienoate	ETDEC	Pear			
Methyl anthranilate	MTANT	Honey, floral	2236	2229	134-20-3
2-Butenoic acid, ethyl ester,	BAEE	Citric, caramel	1161	1119	623-70-1
Diethyl carbonate	DlC	N.D.			
Aldehyde					
Hexanal	HEXAN	Grass	1053	1052	66-25-1
Decanal	DEC	Orange	1485	1475	112-31-2
Benzaldehyde	BENZ	Almond, burnt	1516	1504	100-52-7
Benzeneacetaldehyde	BZACT	Honey, sweet	1516	1504	100-52-7
Octanal	OCT	Lemon,	1290	1253	124-13-0
2-hexenal, (e)-	HEX	Herbaceous, leaf	1216	1181	6728-26-3
Nonanal	NON	Citric,	1390	1355	124-19-6
Alcohol					
Cyclopentanol, 2-methyl-, trans-	COM	N.D.	1344	1341	25144-04-1
1-Octen-3-ol	OCT	Sweet, herbal	1449	1412	3391-86-4
1-Hexanol, 2-ethyl-	HENE	Rose, herbaceous	1479	1456	104-76-7
1-hexanol	HEXAN	Herbaceous	1316	1312	111-27-3
Linalool	LINAL	Floral, , citric	1535	1516	78-70-6
1-nonanol	NONA	Herbaceous	1666	1631	143-08-8
Alpha.-terpineol	ALTER	Oily note, mint,			

Phenylethyl alcohol	PNA	Honey, floral	1894	1890	60-12-8
3-Hexen-1-ol,	3HEO	Herbaceous		1279	928-95-0
2-Hexen-1-ol,	2HEO	N.D.	1315	1294	2497-18-9
2,6-Octadien-1-ol, 3,7-dimethyl-, (E)-	GER	Rose	1754	1765	101-97-3
(S)-3-Ethyl-4-methylpentanol	ETM	N.D.		1527	1000144-07-1
Terpinen-4-ol	CHMM	N.D.	1593	1576	20126-76-5
Ketone					
2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-, (E)-	BTOTC	Apple, honey	1803	1806 1606	23726-93-4
2(1H)-Naphthalenone, 3,4,5,6,7,8-hexahydro-	NAPH	N.D.		1703	13837-12-2
3(2H)-Furanone, 4-methoxy-2,5-dimethyl-	FURMD	Caramel, sweet	1576	1566	4077-47-8
5-Hepten-2-one, 6-methyl-	HOM	Citric	1312	1300	110-93-0
Aromatic hydrocarbon		N.D.			
Naphthalene	NAP	Soil	1748	1728	91-20-3
Styrene	ST	Balsamic, sweet			
Ethylbenzene	ETBZ	Floral	1119	1081	100-41-4
Benzene, 1,3-dimethyl-	BZ DM	Floral	1133	1102	108-38-3
P-Xylene	XYL	Floral	1133	1093	106-42-3
Terpene					
D-limonene	LIM	Citric, mint	1190	1158	5989-27-5
Alpha.-phellandrene	APH	Mint	1449	1412	3391-86-4

[1] Described in Burdock, G. A., 2010.
[2] Kovatz index from NIST version 2011 library
[3] Kovatz index calculated
N.D.: Not Described

The 2016 Isabel Precoce harvest (Figure 3) demonstrates two defined clusters, CT (red) and MJ (green). Hierarchical clustering of the compounds can be visualized on the left, with, two defined clusters of free volatile compounds. The first one is encompasses compounds such as octanoid acid ethyl ester (OAEE) that presents higher concentration on MJ-treated grapes and the second one present compounds as benzaldehyde (BENZ) with opposite behavior, i.e., with lower concentrations on MJ-treated grapes.

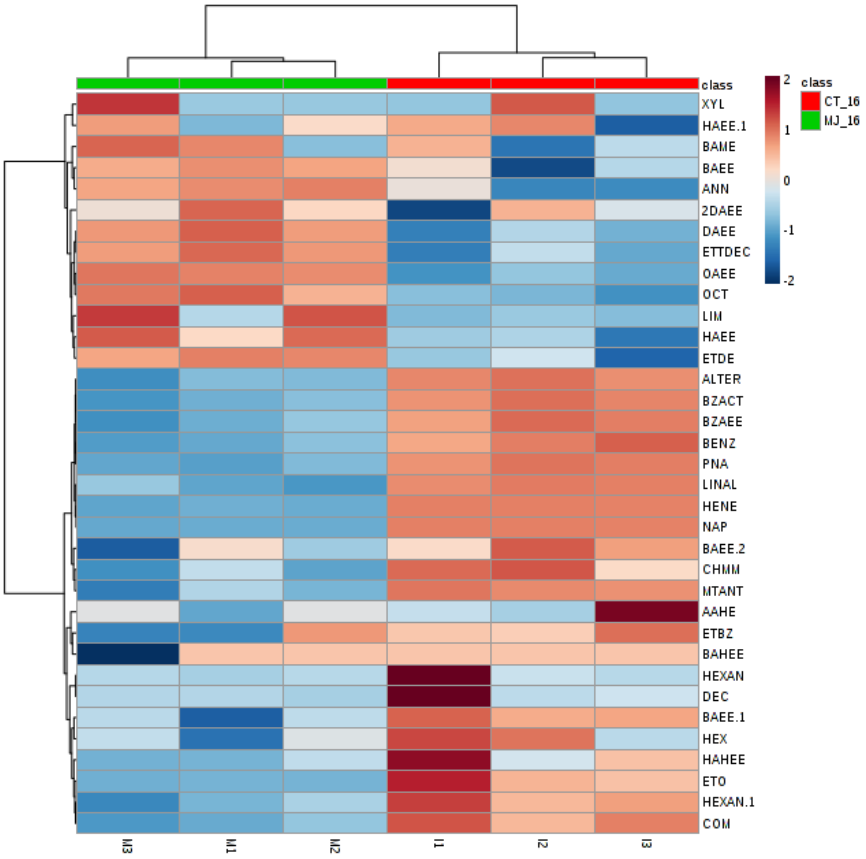


Figure 3. Heatmap of free volatile compounds of Isabel Precoce grapes harvested in 2016 (CT: Samples I_(1-3) belong to control group (CT), and samples M_(1-3) belong to group treated with methyl jasmonate (MJ) in two periods.

Besides the vintage effect, we observed that some compounds display the same behavior with MJ treatment. Through variable importance in projection scores, calculated by partial least square discriminate analysis, we identified esters whose content increased with MJ treatment. Among these esters, five compounds responded positively to MJ in 2015: dodecanoid acid ethyl ester (2DAEE); decanoid acid ethyl ester (DAEE); octanoid acid ethyl ester (OAEE); hexanoic acid ethyl ester (HAEE); and ethyl trans-2-cis-4-decadienoate (ETDEC). These compounds are fatty acid derivatives. The two esters that also responded positively to MJ treatment for both harvests (octanoic acid, ethyl ester - OAEE and decanoic acid, ethyl ester - DAEE) have an aroma described as having fruity, grape, and brandy notes (Burdock, G. A., 2009). Viana et al. (2018) also found these compounds on *V. labrusca* L. grapes. In agreement with Garde-Cerdan et al., (2018), we also observed lower levels of 3-hexen-1-ol (3HEO) on MJ treated grapes, the decrease on this compounds promote by MJ it was also reported by Gutiérrez-Gamboa, et al (2019) on Tempranillo (*Vitis vinifera* L.).

Our study was conducted on V. labrusca grapes, but it seems that this response to MJ treatment is conserved in the genus Vitis; however, further studies are needed to confirm this hypothesis. Since 3HEO at high levels can provide herbaceous flavors to the wines, our result are promising for grapes MJ-treated grapes that will be used for wine production (Gutiérrez-Gamboa, et al 2019).

Regarding other C6 aldehydes, previous studies found that MJ application can induce lipid peroxidation and lipoxygenase (LOX; EC 1.13.11.12) activity and thus the volatiles derived from this pathway (Gómez-Plaza et al., 2012). We believe this might also occur also on *Vitis labrusca* grapes (Ju et al., 2016; Moreno et al., 2010).

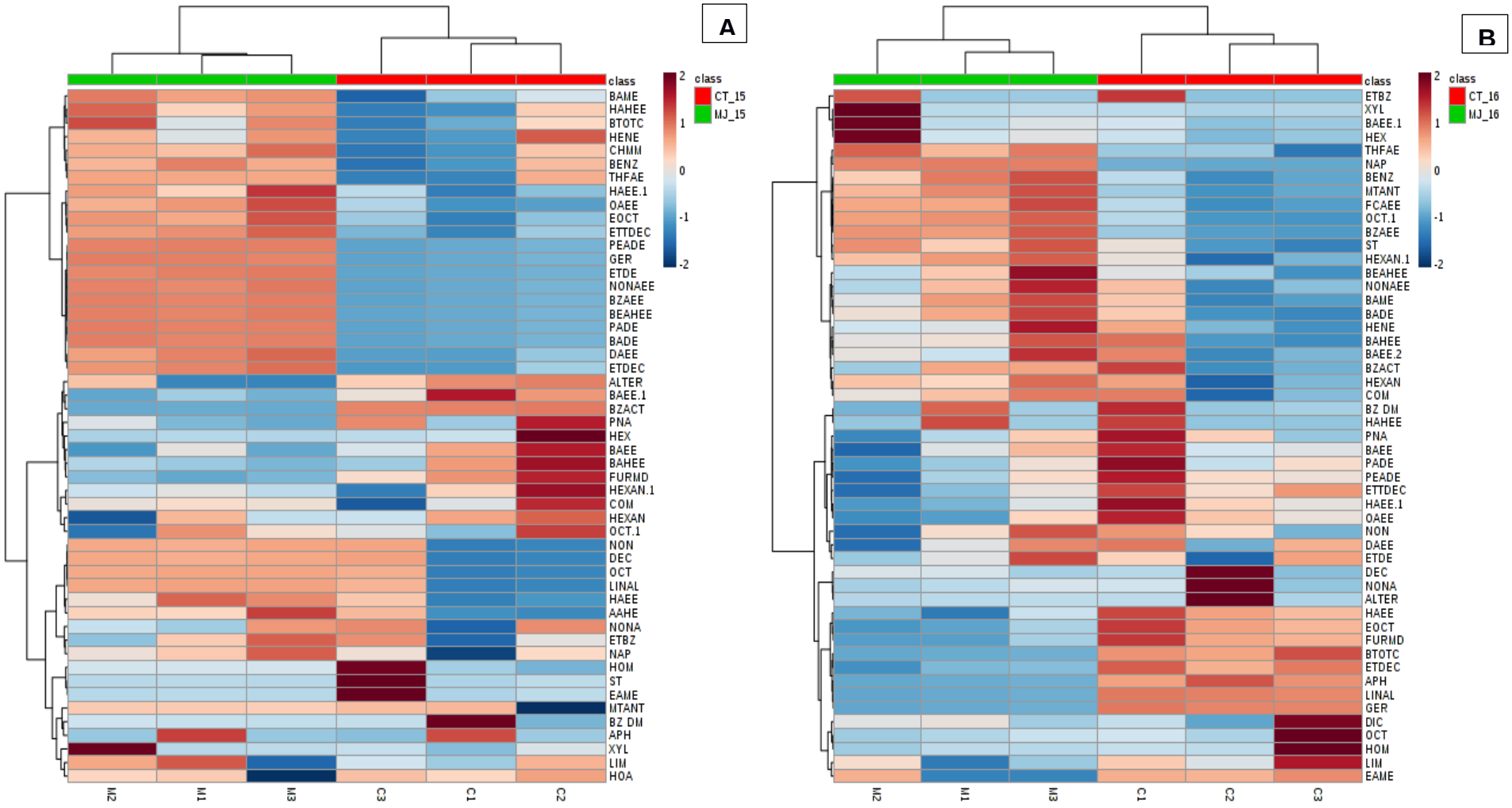
For ‘Concord’ grapes, analyzing the heatmap of free volatile compounds for the 2015 harvest (Figure 4A) revealed that besides CT and MJ clusters, three defined clusters of compounds appeared on the left. The first cluster was stimulated by MJ application, e.g., decanoic acid ethyl ester (DAEE), while the second cluster contains compounds presenting a higher concentration in the CT group, e.g., hexanal (HEXEN). The free volatile compound profile from the 2016 ‘Concord’ harvest (Figure 4B) shows a clustering behavior similar to the previous year, with lower compounds responsive to MJ treatment than for 2015 harvest grapes.

Analyzing MJ treatment for both harvests over the same period of application – at véraison and 2 weeks before harvest (Figures 2B and 4B) – we observed a smaller number of free volatile compounds for ‘Isabel Precoce’ grapes compared to ‘Concord’ grapes. As both cultivars were grown in the same region and had the same protocol for free volatile compound extraction, it appears the number of free volatile compounds responding to MJ treatment relates to the cultivars. We identified 54 and 40 free volatile compounds for ‘Concord’ and ‘Isabel Precoce’ grapes, respectively.

Through variable importance in projection scores, we identified 13 compounds stimulated by MJ treatment for the 2015 harvest of ‘Concord’ grapes. However, only six compounds were stimulated by MJ treatment during the 2016 harvest. The compounds included esters, alcohol, and aldehyde, derived from fatty acids and amino acids, particularly monoterpenes and phenolic acid derivatives. However, from all these compounds, only tetrahydrofuran-2-acetic acid ethyl ester (THFAE) and benzoic acid ethyl ester (BZAEE) were responsive to MJ treatment in ‘Concord’ grapes in both years. These esters are derived from phenolic acids and monoterpenes, respectively.

Amino acid-derived volatiles, including methoxypyrazines and benzenoids, are derived from the phenylalanine formed from a shikimate acid pathway. This pathway

mediates the flow of carbon from the metabolism of carbohydrates. Environmental stresses can activate these pathways and further promote the accumulation of secondary metabolites (Liu et al., 2013). As MJ is involved in plant defense responses against insects, pathogens, and several types of abiotic stresses (Zhao et al., 2016), these increases in phenolic acid derivatives appear to be a direct response to MJ treatment.



Figures 4. Heatmaps of volatile compounds of 'Concord' grapes harvested in 2015 (A) and 2016 (B). Samples C_(1-3) belong to control group (CT), samples M_(1-3) belong to group treated with methyl jasmonate (MJ) in two periods.

Among the compounds whose content diminishes with MJ treatment of ‘Concord’ grapes in both harvests, we highlight phenylethyl alcohol (PNA), with an aromatic note described as floral and pleasant (Burdock, 2009). PNA is a characteristic compound detected in V. labrusca grapes cultivated in southern Brazil (Viana et al., 2018). This lower content of benzenoids found in MJ-treated grapes was also described by Gutiérrez-Gamboa et al. (2019).

Naphthalene, linalool, furanones, and particularly methyl anthranilate – described as characteristic volatiles of ‘Concord’ grapes – are considered responsible for the distinctive “foxy” flavor of the grapes. This aroma, described as the “naphthalene note” or “like furniture polish”, causes significant consumer rejection, particularly for wine. However, the fruity flavor of wines produced with V. labrusca grapes is widely demanded by an important sector of Brazilian wine and grape juice consumers (Biasoto et al., 2014; Flamini and Traldi, 2010; Ribéreau-Gayon et al., 2006; Sun et al., 2011).

Methyl anthranilate did not respond to MJ treatment for the ‘Concord’ grapes during the 2015 harvest, but it was increased in the grapes of the 2016 harvest. This response appears to be related to the abnormal climatic conditions of 2015, alongside which production was reduced and vines were stressed after flowering.

4. Conclusion

This study provides evidence that MJ application is an eco-friendly means to enhance volatile compounds in V. labrusca grapes without interfering with organic acid content. Therefore, the effect of pre-harvest MJ application is associated with the phenological state at the time of application. Optimal results are achieved when application occurs during two periods, véraison (with three applications) and approximately 2 weeks before harvest. The differences in soluble sugar content and tartaric and malic acids among harvests are more evident than for the MJ treatment itself. Volatile compounds responded positively to MJ treatment for both cultivars and harvests. In ‘Isabel Precoce’ grapes, MJ treatment particularly enhanced esters, fatty acids, and terpenoid derivatives, while, in ‘Concord’ grapes, MJ enhanced esters, alcohol, and aldehyde compounds derived from fatty acids and amino acids, besides monoterpenes and phenolic acid derivatives.

Further studies are being conducted in other Brazilian regions to better understand the response of V. labrusca grapes to MJ treatment in different edaphoclimatic conditions.

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Methyl Jasmonate pre-harvest applications improving stilbenes in *Vitis labrusca* L. grapes grown in subtropical climate

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Abstract

Context and purpose of the study

Grapes (*Vitis* sp.) are considered a major source of phenolic compounds such as flavonols, anthocyanins and stilbenes. Studies related to the beneficial effects of these compounds on health have encouraged researches aiming to increase their concentration in fruits. On this behalf, several plant growth regulators such as jasmonic acid and its volatile ester, methyl-jasmonate (MeJa), have demonstrated promising results in many fruits. However, Brazilian subtropical climate might interfere on treatment response. The present study aims to evaluate the period of MeJa application in the pre-harvest period in Concord and Isabel Precoce grapes (*Vitis labrusca* L.).

Material and methods

Grapes cultivated in Brazil's southern region (Bento Gonçalves, RS, altitude: 671 meters) receive a MeJa solution into different periods during ripening. Grapes were harvested, analyzed and the results compared to non-treated fruit (control group). Edaphoclimatic conditions and cultural practices were monitored (climate data, soil, fertilization, pruning, etc.). Anthocyanins and stilbenes were quantified by analytical reversed-phase liquid chromatography (Agilent Technologies, model 1260 Infinity) equipped with a DAD.

Results

The results obtained after a 2-years study has provided evidence that MeJa application is an eco-friendly means to enhance compounds such anthocyanin and stilbenes in *Vitis labrusca* L. grapes, grown even in subtropical climate. The effectiveness of MeJa application seems to be related to phenological state in the period of application. Best results were obtained with two applications: during *véraison* and approximately two weeks before harvest. Our results suggest that MeJa treatment enhances *trans*-resveratrol and piceid contents when grapes are treated in these conditions. Further studies are being conducted in other Brazilian regions with the objective of better understanding the behavior *of Vitis labrusca* L. grapes towards MeJa treatment in different edaphoclimatic conditions.

Keywords: *Vitis labrusca* L., stilbenes, anthocyanins, pre-harvest, elicitors, methyl jasmonate

1. Introduction

Grapes and wine are important dietary sources of anthocyanins, flavanols, and stilbenes, particularly resveratrol-(3,5,4'–trihydroxy-stilbene) an important phytoalexin, produced by plants as a defense compound with antifungal properties (Toaldo et al., 2015; Wang et al., 2010). In humans, it has been demonstrated that resveratrol has effects on cardiovascular diseases, diabetes mellitus, obesity, inflammation and several types of cancer (Dvorakova, et al., 2017; Tsai et al., 2017; Toaldo et al., 2015; Stuart et al., 2013).

There is an increased demand for an eco-friendly means to improve secondary metabolites in fruits. In this context, methyl-jasmonate (MeJa), a naturally occurring plant growth regulator, has been tested since it does not leave residues in treated fruits, once has a high vapor pressure and tends to readily diffuse into the atmosphere (Flores et al., 2015). Some studies tested the effect of MeJa treatment in *Vitis vinifera* L. grapes (Portu, et al., 2016; Voung et al., 2014, Vezzuli, et al., 2007). However, the effect of its application on *Vitis labrusca* L. grapevine cultivated under subtropical conditions remains unknown. In Brazil, 90% of total grape processing occurs at Rio Grande do Sul state. American varieties and its hybrids correspond to 89% of processed grapes, being mostly *Vitis labrusca* L. red grapes (IBRAVIN, 2017; Mello, L. M. R, 2017).

It is known that grapes chemical composition depends on many factors including soil, the degree of berry ripeness, sunlight exposure, pathogenesis, and agronomical management (Granato et al., 2016). According to Köppen, Brazilian Southern region is classified as type Cfa, i.e., a subtropical climate that covers 6.5% of the Brazilian territory, characterized by elevated humidity and hot summer (Alvares et al., 2013). It seems that humidity might influence stilbenes biosynthesis during ripening period triggering the pathway, as the micro-climate around the clusters (Bavaresco et al., 2007). Since grape and wines are main source of stilbenes for Brazilian population, elicitors that might improve its contents are desirable. Therefore, the aim of this study is to evaluate the effect of different periods of MeJa applications into grapevines with the purpose of enhancing stilbenes and anthocyanin on grapes cultivated under subtropical conditions. In this study, two *Vitis labrusca* L. cultivars were studied: *Isabel Precoce*, a spontaneous somatic mutation of cultivar Isabel, and *Concord* during the 2015 and 2016 harvests.

2. Material and Methods

2.1. Plant Material and Field Treatments

The field experiment was conducted in 5-year-old and 20-year-old commercial vineyards of Isabel Precoce (spontaneous somatic mutation of cultivar Isabel) and Concord grape cultivars (*Vitis labrusca* L.*)* respectively, located in Bento Gonçalves, Rio Grande do Sul state, Brazil (29° 10′ 15″ S e 51° 31′ 08″ W, altitude 671 m). Vines were grafted on *1103-Paulsen* rootstock trained on tendone, overhead trellises system with a between-row and within-row spacing of 2.8 × 1.5 m respectively. Winter pruning was performed leaving 2–3 buds per spur. The vineyard was managed according to the standard viticultural practices for the cultivar and region. (Figure 1 A).

Weather conditions were recorded by a meteorological station approximately 5 km from the vineyard, and the data collected from Instituto Nacional de Meteorologia (INMET).

Fig 1. Methyl jasmonate application on grapes cultivated at Bento Gonçalves, Brazil.

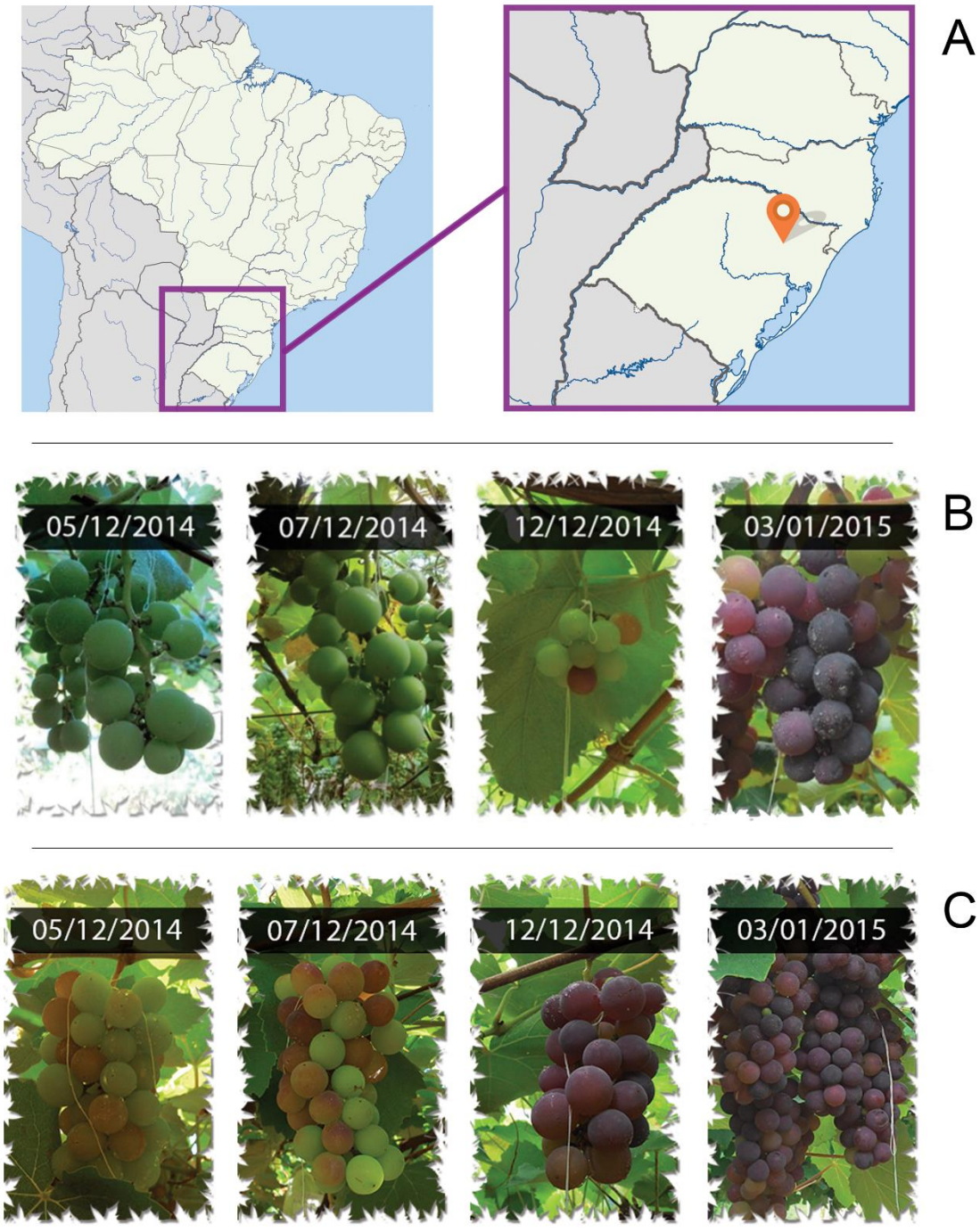

Figure 1. Methyl jasmonate application on grapes cultivated at Bento Gonçalves, Brazil on 2015 harvest (A). Applications on Concord grapes before *véraison* and pre-harvest (B), applications on Isabel Precoce grapes at véraison and pre-harvest (C).

The methyl jasmonate (MeJa) solution was prepared according to Vezzulli et al., (2007) at a concentration of 10 mM and was applied manually by spraying it into the clusters at *véraison*, three times (50 ml per application on days 1, 3, and 6) and/or a single application approximately two weeks before harvest. The control (CTRL)

group was sprayed with water (50 ml per application) at the same time and following the same procedures as the MeJa-treatments. The treatments were applied in completely randomized blocks with an experimental design consisting of three replicates (sampling number, n=3) of six vines per treatment, with 20 clusters per vine. The grapes were harvested during the summer seasons of 2015 and 2016 when reached the commercial point of harvest.

In the first year of our study (2015), some tests were performed to evaluate the treatment efficacy. Besides control group (identify as CNTR) another block was treated with MeJa (single application) only 2 weeks before harvest (identified as MeF) (Figure 1 B). Concord grapes were treated in two periods: previous to *véraison* (with tree applications) plus a single application 2 weeks before harvest. Isabel Precoce grapes receive the treatment at *véraison* (with tree applications) and 2 weeks before harvest (identified as MeJa) (Figure 1 C).

Based on the results, we observed a better response with MeJa treatment, regarding stilbenes and anthocyanin content, in two periods: at *véraison* with tree applications (on day 1, 3, and 6) plus a single application approximately two weeks before the harvests. This method were used on both cultivars during the 2016 harvest.

2.2. Stilbene Extraction and Quantification

Stilbenes were extracted from grapes peels as described by Liu et al (2013). *Trans*-resveratrol, piceid and viniferin were quantified by analytical reversed-phase liquid chromatography (Agilent Technologies, model 1260 Infinity) equipped with a DAD. The column used was Prodigy 5 ODS3 reversed-phase silica (250 mm x 4.6 mm, i.d., 5 µm, Phenomenex Ltd., Torrance, CA), using a gradient program described by Liu et al (2013). Stilbenes were identified according to the retention times and the UV–Vis data obtained from authentic standards of *trans*-resveratrol, piceid and viniferin (Extrasynthese, Genay, France). The results were expressed as mg/100g grape peel fresh weight (f.w).

2.3. Anthocyanin Extraction and Quantification

Anthocyanins were extracted as described by Ribeiro et al (2015) and quantified by analytical reversed-phase liquid chromatography (Agilent Technologies, model 1260 Infinity) coupled with UV-Vis detection. The column used was Prodigy 5 ODS3 reversed-phase silica (250 mm x 4.6 mm, i.d., 5 µm, Phenomenex Ltd., Torrance, CA),

and the elution solvents were A, (water/formic acid/acetonitrile 95:1:3 v/v/v) and water/formic acid/acetonitrile (48:1:51, v/v/v; eluent B) using a gradient program as described by Ribeiro et al (2015). The calibration was performed by injecting authentic aglycone standards of malvidin (Extrasynthese, Genay, France). The results were expressed as mg malvidin aglycone/100 g of grape fresh weight (f.w).

2.4. LC-ESI-MS/MS

Anthocyanin identification was carried out on a liquid chromatograph, model Prominence (Shimadzu, Japan) coupled to an íon trap mass spectrometer, model Esquire HCT (BrukerDaltonics, German) equipped with an electrospray ionization (ESI) source. Separation conditions were the same as described for LC-DAD analysis. The positive mode was recorded for anthocyanins in the range m/z 100-1000 at full scan mode. For MS/MS experiments, the molecular ion isolated in the íon trap was followed by ESI at 3500V. The identification of individual anthocyanins was performed by correlating the absorbance and mass spectra obtained, with those previously reported in the literature and in studies of their fragmentation patterns. To confirm the identity of each anthocyanin, the retention time was compared with commercial standards.

2.5. Statistical Analysis

Anthocyanins and stilbenes were expressed by means ± standard deviation (SD, *n*=3). Statistical analysis was performed using Graph Pad Prism software version 5.01 (GraphPad Software, CA, USA). Differences among treatments were analyzed by ANOVA and the Student–Newman–Keuls test, assuming a significance level of 0.05.

3. Results and Discussion

Grapes were harvested in 2015 and 2016, when reached the total soluble solids (TSS) content 16 and 14°Brix, respectively, for Isabel Precoce cultivar. In Concord grapes, the TSS were 15°Brix in both harvests.

Grapes harvested in 2015 were grown in 2014, under a total annual rainfall of 2.043 mm, an average temperature of 18.2 °C and a relative humidity of 78% (Table 1). These conditions present an excessive rainfall, especially during budding (Table 1) and the above-average temperature was conducive to fungi infections (Alves et al.,

2015). In addition, the harvest was advanced approximately 15 days to prevent major losses in early harvested cultivars.

Table 1: Climatic conditions during berry development.

Berry Stage Development	Month/Year	T (ºC)	P (mm)	RU (%
flower	Oct/14	18.9	160	75
berry	Nov/14	20.5	114	72
véraison	Dec/14	21.4	294	77
harvest	Jan/15	22.3	135	78.4
flower	Oct/15	16.8	280	83.1
berry	Nov/15	18.5	145	80.5
véraison	Dec/15	21.2	198	81.4
harvest	Jan/16	22.8	115	73.2

Table 1: Climatic conditions during berry development in Bento Goncalves, Rio Grande do Sul state, Brazil. T: Temperature mean (C); P: Precipitation (mm); RU: Relative Humidity (%); Oct: October; Nov: November; Dec: December; Jan: January.

Grapes from the 2016 harvest, were grown in 2015 under an annual rainfall of 1.971 mm, an average annual temperature of 18.0 °C and a relative humidity of 80.1% (Table 1). Despite the values presented, grapes from the 2016 harvest have low productivity in all regions, particularly those harvested early (Alves et al., 2016).

3.1. Anthocyanin and Stilbene Quantifications

The increase in anthocyanin content by MeJa applications was previously reported in *Vitis vinifera* L. varieties, through pre-harvest treatment, and post-harvest treatments on strawberries and raspberries (Flores, et al., 2015; Moreno et al., 2010; Moro et al., 2017). However, no data have been found concerning the effects of pre-harvest MeJa applications on *Vitis labrusca* L. grapes growth under subtropical conditions.

Our result reveal that, for Concord grapes, the anthocyanin content in the 2015 harvest, was not enhanced (Table 2) by the treatment application before *véraison* plus a single application two weeks before harvest (Figure 1-B). We believe that the fruit was not at a development stage receptive for the treatment because, in the 2016 harvest, when the treatment was applied at *véraison* and two weeks before harvest, MeJa treatment enhanced approximately 10% anthocyanin content at a significant level (Table 2).

The application of exogenous MeJa stimulates *MYB* transcriptional factors, and enzymes involved in the phenyl propanoid biosynthetic pathway, such as phenylalanine ammonialyase (*PAL*) (EC 4.3.1.24) and chalcone isomerase (*CHI*; EC 5.5.1.6) (Moro et al., 2017; Flores, et al., 2015). However, the response of pre-harvest applications of MeJa seems to be related to the crop, doses and phenological stage of development (Días et al., 2016).

Regarding Isabel Precoce grapes (Table 2), higher anthocyanin contents in the 2015 harvest were found in the treatments performed at two times: *véraison* and before harvest (Figure 1-C). As detected on MeF, (2015 harvest, Table 2), a single treatment before harvest was not enough to stimulate anthocyanin biosynthesis. However, in the 2016 harvest, with the treatment application at two moments, we do not see the same result as with 2015; the content found in the CNTR group was higher than in the MeJa group.

Table 2: Chemical analysis of Concord and Isabel Precoce grapes.

		Anthocyanin (g/100 g f.w)	Stilbenes (mg/100 g peel f.w)		
		malvidin aglycone	*trans*-resveratrol	piceid	viniferin
Harvest 2015					
Concord	CNTR	39.12 ± 1.84	0.003±0.001	<LOQ	17.38 ± 2.56
	MeJa	39.37 ±3.66	0.002±0.001	<LOQ	13.46 ± 2.68
Isabel P.					
	CNTR	57.92 ± 2.57	0.009±0.004	<LOQ	13.31 ± 1.97
	MeJa	135.39± 2.70 ^{c}	0.012±0.002 ^{c}	<LOQ	9.98 ± 0.46
	MeF	67.43± 1.51	0.006±0.001	<LOQ	11.43 ± 6.80
Harvest 2016					
Concord	CNTR	34.55 ± 3.10	0.010±0.001	6.52 ± 0.95	19.86 ± 2.19
	MeJa	39.33 ± 3.67 ^{A}	0.143±0.198 ^{C}	23.75± 2.93	17.90 ±2.92
Isabel P.					
	CNTR	113.06±2.45	0.05±0.001	4.41 ± 1.30	9.42 ± 0.55
	MeJa	31.73 ± 5.33	0.09±0.001	11.38 ±0.61	8.03 ± 1.85

Table 2: Chemical analysis of Concord and Isabel Precoce grapes (n=3) cultivated at Bento Gonçalves, Brazil. CNTR – Control group and MeJa - methyl Jasmonate treated in two periods; MeF - methyl Jasmonate treated in one period; (a,b,c) Different lower case letters denote significant differences between CN and MJ grapes harvested in 2015 according to ANOVA (Student–Newman–Keuls test, α=0.05). (A–C) Different capital letters indicate significant differences between grapes harvested in 2016 according to ANOVA (Student–Newman–Keuls test, α = 0.05). < L.O.Q - above limit of quantification.

The main result expected after MeJa application was the increase in stilbene concentration in grapes. Studies have demonstrated resveratrol effects on diabetes

mellitus, obesity, inflammation and some antineoplasic (Dvorakova, et al., 2017; Tsai et al., 2017; Stuart et al., 2013). It is also suggested that resveratrol directly activates sirtuins that are conserved mediators of longevity, and reassemble the effects of calorie restriction diets (Guarente, L. 2017).

On Concord grapes (Table 2), the treatments applied before the *véraison* and a single application two weeks before harvest did not enhance the biosynthesis of *trans*-resveratrol. These results agree with the findings for anthocyanin contents in the same samples. A series of R2R3-*Myb* transcriptional factors (TF) seem to be involved in the control of different branches of the phenyl propanoid pathway influencing the anthocyanin content in grapes (He et al., 2010), and could be investigated since previous studies in raspberries indicate that members from R2R3-Myb TF family associated to anthocyanin synthesis are MeJa-responsive (Moro et al., 2017). Likewise, the *trans*-resveratrol content was not affected by a single MeJa application (one period application) in Isabel Precoce grapes harvested in 2015.

The application of MeJa treatment at two periods (*véraison* and two weeks before harvest) promotes a higher concentration of *trans*-resveratrol content in Isabel Precoce grapes. With this result, a two-period application was adopted as protocol for 2016 treatments at both cultivars. As expected, the treatment enhanced *trans*-resveratrol content on the peels of both cultivars. It appears that both periods are sensitive to hormonal stimulus, promoting a higher increase in *trans*-resveratrol content. The *véraison,* characterized by the softening and coloring of the berry, is a key stage associated with changes in several metabolic pathways. Some studies revealed that *Myb* 14 has an important role in both the transcription of stilbene synthase (*STS;* EC: 2.3.1.95) mRNA and biosynthesis of resveratrol. Also, it is known as a regulator of enzymes involved in the first steps of flavonoid biosynthesis, such as phenylalanine ammonia lyase (*PAL*; EC 4.3.1.24) and chalcone isomerase (*CHI*; EC 5.5.1.6) (Moro et al., 2017; Flores, et al., 2015; Wang et al., 2015; Chong et al., 2009). However, the Myc family of transcription factors in red *V*. *vinifera* L. grapes seems to be only expressed after *véraison* and regulates anthocyanin biosynthesis during ripening by strict control of the expression of specific anthocyanin biosynthesis genes, particularly anthocyanidin 3-O-glucosyltransferase (*UFGT*; EC: 2.4.1.115) (He et al., 2010).

The piceid content was below the limit of quantification for both cultivars in 2015 harvest. However, the piceid contents in MeJa-treated grapes from 2016 harvest were approximately four times higher for Concord grapes and three times higher for Isabel Precoce grapes, then their respective control groups. Piceid is a glycoside derivative from *trans*-resveratrol, generally, is the most abundant form found in grapes. Some studies suggest that piceid presents higher bioavailability *in vivo* than resveratrol (Stuart et al., 2013). As *trans*-resveratrol, piceid accumulation in grapes is stress related, and when stilbene synthase is over expressed, a predominant accumulation of *trans*-piceid was detected (Fabris et al., 2008; Stuart et al., 2013).

For both harvests, viniferin content (Table 2) in control group grapes were higher than the MeJa group, despite no other statistical difference being found. As viniferin is a *trans*-resveratrol derivative due to the oxidation of the basic stilbene by 4-hydroxystilbene peroxidases, its accumulation can be induced in response to biotic and abiotic stresses (Flamini et al., 2013; Chong et al., 2009). We hypothesize that as resveratrol is a common subtract for piceid and viniferin for 2016 harvest elevated levels of piceid were detected in the MeJa group, these lower levels of viniferin may be caused by substrate competition.

3.2. LC-ESI-MS/MS

HPLC–MS/MS chromatograms of *Concord* and *Isabel Precoce* anthocyanin extracts reveal 14 peaks identified as anthocyanins. Although Concord and Isabel Precoce grapes presented different anthocyanins profile (Figure 2), CNTR and MeJA groups from both harvests presented similar profiles of anthocyanins, within the cultivar, indicating that MeJa treatment does not affect the anthocyanin profile, only their quantity.

Fig 2. LC-ESI-MS/MS chromatogram of the anthocyanin profiles

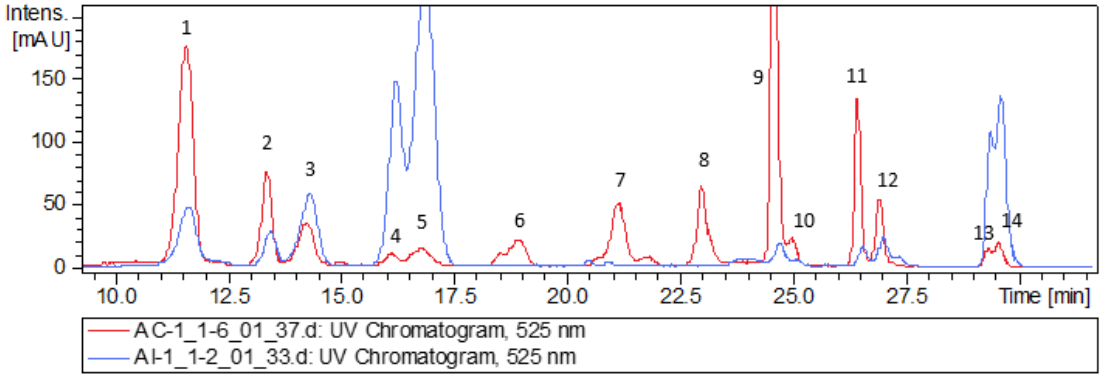

Figure 2. LC-ESI-MS/MS chromatogram of the anthocyanin profiles of Concord (red) and Isabel Precoce (blue) grapes cultivated at Bento Gonçalves, Brazil.

The anthocyanins identified had the same elution order as those in the work of Ribeiro et al (2015). The most intense signals detected regarding Concord grapes were delphinidin 3-glucoside (peak 1), delphinidin-3-coumaroyl glucoside (peak 9), cyanidin 3-p-coumaroyl glucoside (peak 11). For Isabel Precoce grapes the results were as follows: peonidin 3-glucoside (peak 4); malvidin 3-glucoside (peak 5); peonidina-3-coumaroyl glucoside (peak 13) and malvidin-3-coumaroyl glucoside (peak 14) (Figure 2).

Anthocyanin identification was performed by mass spectral analysis by HPLC–MS/MS considering the generated fragments and data from the literature. Table 3 present the 14 peaks identified for Isabel Precoce and Concord grapes (respectively), retention time, and the fragmentation obtained by collision-induced dissociation in the MS^2 and MS^3.

Table 3: LC-MS/MS data of anthoctanins extracted from Isabel Precoce and Concord grapes

Peak	Compound	Rt (min)	[M]+*m/z*	MS/MS	IP	CD
1	Delphinidin 3-glucoside	11.6	465	303/257	x	
2	Cyanidin 3-glucoside	13.4	449	287	x	
3	Petunidin 3-glucoside	14.3	479	317/302	x	
4	Peonidin 3-glucoside	16.1	463	301/286	x	
5	Malvidin 3-glucoside	16.8	493	331/316/299	x	
6	Delphinidin 3-acetyl glucoside	19.0	507	303/272.99/228.93/149		x
7a	Cyanidin-3-*p*-coumaroyl glucoside-5-glucoside	20.7	757	595/287/203		x
7b	Delphinidin-3-*p*-coumaroyl glucoside 5-glucoside	21.2	773	611/465/303/256/176		x
7 c	Petunidin 3-acetyl glucoside	21.7	521	317		x
8	Cyanidin-3-*p*-coumaroyl glucoside-5-glucoside	23.0	757	595/287/231/165		x
9	Delphinidin-3-coumaroyl hexoside	24.6	611	303/257	x	
9a	Malvidin - 3 -*O*- acetyl glucoside	24	535	331/179	x	
10	no id	25.1			x	
11	Cyanidin 3-*p*-coumaroyl glucoside	26.5	595	287/212	x	
12	Petunidin-3-*p*-coumaroyl glucoside	27	625	317/301/274	x	
13	Peonidin-3-*p*-coumaroyl glucoside	29.3	609	301	x	
14	Malvidin-3-*p*-coumaroyl glucoside	29.6	639	331/179	x	

Table 3: HPLC–MS/MS data of anthocyanins extracted from Isabel Precoce (IP) and Concord (CD) grapes cultivated at Bento Gonçalves (RS), Brazil.

4. Conclusion

In summary, this study provided evidence that MeJa application induces the accumulation of anthocyanin and stilbenes in *Vitis labrusca* L. grapes even in subtropical climate. The effect of the pre-harvest application of the plant growth regulator can be associated with the phenological state at the period of application. The optimal results appear when conducted at two periods, during *véraison* (with three applications) and approximately two weeks before harvest. Our results suggest that MeJa treatment enhances *trans*-resveratrol and piceid contents when grapes are treated in these conditions.

Further studies are being conducted in other Brazilian regions with the objective of better understanding the behavior of *Vitis labrusca* L. grapes towards MeJa treatment in different edaphoclimatic conditions.

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SESSÃO 2

LC-MS untargeted approach shown that methyl jasmonate application on *Vitis labrusca* L. grapes increases phenolics at subtropical Brazilian regions

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Abstract

Introduction *Vitis labrusca* L. grapes are largely cultivated in Brazil, but the tropical climate affect negatively the phenols content, especially anthocyanin. Therefore, researches are focusing on increase grape phenols content; with methyl jasmonate application (MeJa) considered a good alternative.

Objectives Aim was to investigate with an untargeted approach the metabolic changes caused by the MeJa pre-harvest application on two *Vitis labrusca* L. cultivars grape, both grown in two Brazilian regions.

Methods Isabel Precoce and Concord grapes cultivated under subtropical climate, on south and southeast of Brazil, received MeJa pre-harvest treatment. Grape metabolome was extracted and analyzed with a MS based metabolomics protocol by UPLC-HRMS-QTOF.

Results Unsupervised data analysis reveal clear separation between the two regions and the two cultivars, while supervised data analysis revealed biomarkers between MeJa and control group. Between the biomarkers, stilbenes, hydrocinnamates, flavonols and anthocyanins were identified.

Conclusion These results suggest that MeJa can be used as elicitor to secondary metabolism in grapes grown even under subtropical climate, affecting phenolic biosynthesis.

Keywords: methyl jasmonate**,** phenols, *Vitis labrusca*, LC-MS, stilbenoids, pigments.

1. Introduction

Brazil grape production is based on *Vitis labrusca* L. and its hybrids that cover approximately 90% of the vineyards (IBRAVIN, 2018) and is differentiated by region since the viticulture in the south is directed to juice and wine production, while at northeast to table grapes (Fachinello et al. 2011). According to the Köppen classification (Alvares et al. 2013), the south region (RS in Fig. 1A) is considered subtropical, characterized by warm temperate, elevated humidity and hot summer; while the southeast region (MG in Fig. 1A) is mesotermic, also warm temperate, but with dry winter and warm summer. Viticulture and grape production on subtropical (warm and rainy) regions is challenging, not only due to fungal infections, but also because grape phenolic composition is strongly affected by biotic and abiotic factors. For example, temperature and sunlight plays an important role affecting anthocyanic profile and quantity, while humidity may affect the biosynthesis of stilbenes (Flamini, Mattivi, et al. 2013). Extreme high temperatures may decrease anthocyanins levels through degradation and affect their profile by increasing the content of acetylated and coumarylated anthocyanins (Pillet et al. 2016). Elevated humidity positively affect stilbenes biosynthesis since they protect the plant against exogenous attack, as fungi infection (Flamini, Mattivi, et al. 2013).

Since polyphenols are strongly positively correlated with fruits/food quality and nutritional value, viticulturists and scientists study various methods to favor their biosynthesis. Among the alternatives are several plants grow regulators. Abscisic acid increased the expression of enzymes from anthocyanin pathway and transcriptional factors in grapes cultivated under subtropical climate (Koyama et al. 2018). Methyl jasmonate (MeJa) was used on grapes (Gómez-Plaza et al. 2012; Portu et al. 2018) and others non-climacteric fruits as berries (Moro et al. 2017; Saavedra et al. 2016) and eggplant (Fan et al. 2016). MeJa participate in many plants abiotic stress response, by playing a key role in several plant cell communication and signaling processes (Per et al. 2018) and is already classified as a Generally Recognized As Safe (GRAS) substance by the U.S. Food and Drug Administration, (Pfeifer et al. 2013) therefore presents a good alternative to increase secondary metabolites on grapes.

Since MeJa gave promising results on *Vitis vinifera* L. cultivars in Europe (Javier Portu, Pilar Santamaría, Isabel López-Alfaro, Rosa López 2015; Portu, Santamaría, et al. 2015; Vezzulli et al. 2007); the aim of this study was to investigate if MeJa treatment

on *Vitis labrusca* L. grapes cultivated under the subtropical climate of Brazil could also have a positive response into the polyphenolic profile and quantity. In order to gain a wider view about the changes on the grape metabolic fingerprint, an untargeted LC-MS workflow was followed.

2. Material and Methods

2.1. Experimental design

MeJa application was conducted in 2016, in *V. labrusca* L. grapes grafted onto *1103-Paulsen*, into two different regions in Brazil. In the south, the experiments were done in Bento Gonçalves (29° 10′ 26″ S e 51° 31′ 7″ W, 671 meters altitude) in a 5-year-old commercial vineyard of Isabel Precoce grapes (Fig. 1A) and a 20 year-old vineyard of Concord (Fig. 1A). Both vines were trained on tendone system with a between row and within-row spacing of 2.8 × 1.5 m, respectively. In southeast, the experiment was conducted in Caldas (21° 55’ 23’’ S e 46° 23’ 15’’ W, 1.131 meters altitude), in a 7-year-old vineyard of Isabel Precoce and Concord (Fig. 1A) trained on vertical shoot positioning system with a between-row and within-row spacing of 2.5 × 1.5 m.

Weather conditions were recorded and collected by the Instituto Nacional de Meteorologia (http://www.inmet.gov.br/portal/).

As shown in Fig. 1A for each region/cultivar two blocks were used, control versus treatment. For the south region, each block comprised 10 vines, while for southeast 18 vines. Each plant represented a biological replicate. The MeJa solution was prepared according to (Vezzulli et al. 2007), at a concentration of 10 mM of MeJa dissolved in water. Approximately 50 mL of treated solution were applied into the cluster by spraying at *véraison* with tree applications (at days 1, 3, and 6) plus a single application approximately 2 weeks before harvest (approximately 15,5° Brix), while water was used for control (CtRl) group (Fig. 1B). The treatments were applied at least 2 hours before rain.

Grape clusters from 6 to 8 vines from each condition/variety/region were harvested at their technical maturation; and later 20 berries per vine were sampled, frozen and lyophilized (STOKES, USA). The lyophilized samples were transported to Italy, grounded using an analytical mill (IKA, Germany), and stored at -80 until analysis (Fig 1F).

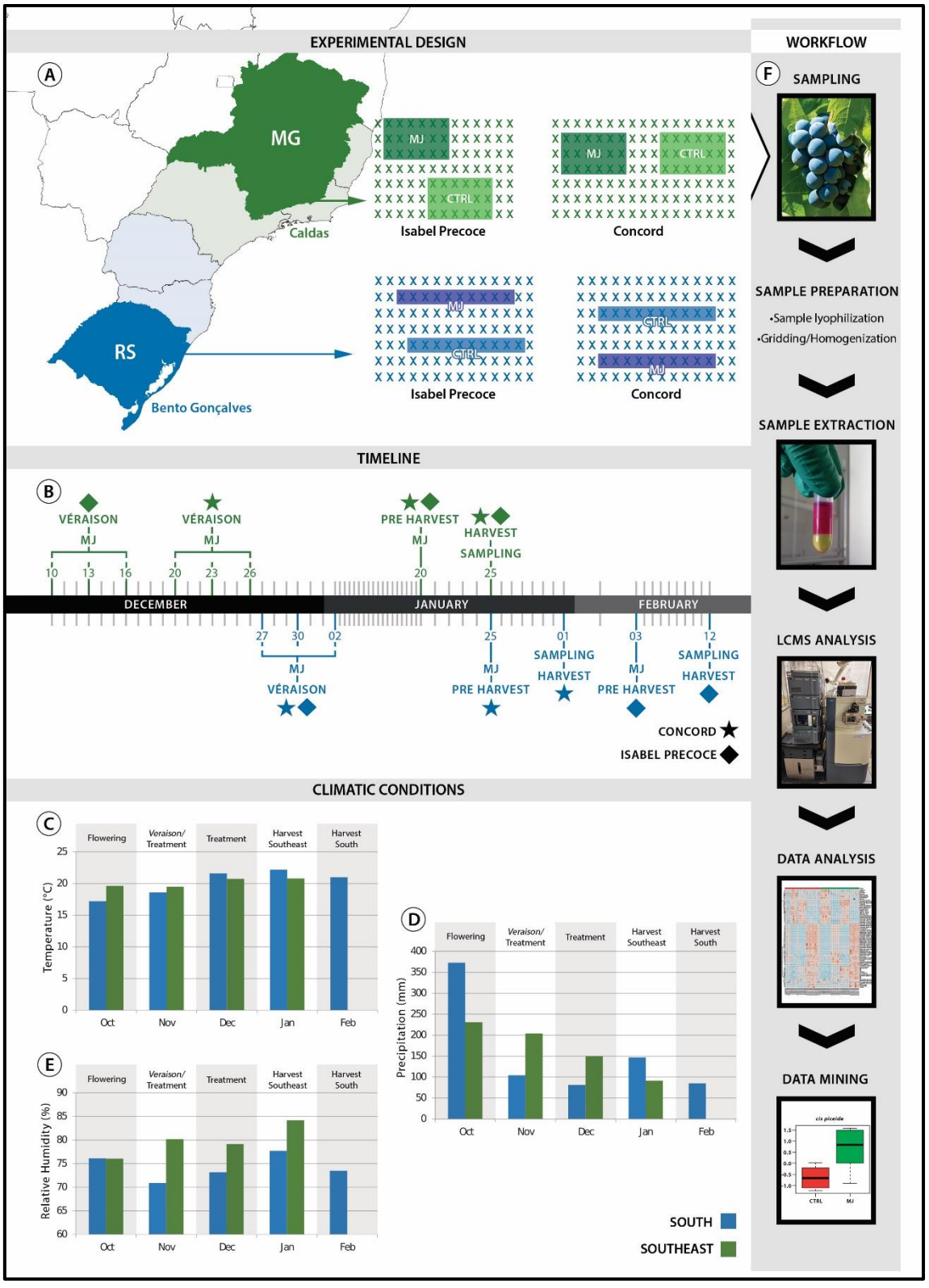

Figure 1. Experimental design of the study (A); Timeline of MeJa application (B); Climatic condition described as mean temperature (C), mean precipitation (D) and mean relative humidity (E) in southern (blue) and southeastern (green) regions of Brazil; Workflow (F).

2.2. Sample preparation

Extraction and analysis were made according to a randomized order (http://www.random.org/sequences/). The extraction was performed as described by Degu et al. (2015) with slight modifications. Briefly, 100 mg of frozen tissue powder was transferred to 2 mL Eppendorf tube, and metabolites were extracted in 1 mL methanol/chloroform/water extraction solution (2.5/1/1 v/v/v). The mixture was then vortexed for 1 min and centrifuged for 5 min at 10,000 RPM (Sigma, Germany) at 4°C, and the supernatant was decanted into the new tubes. The supernatant was mixed with 400 µl of chloroform and 400 µl of MiliQ water and then centrifuged at 5 min at 10,000 RPM at 4°C. The upper water/methanol phase was filtered 0.22 µm (Millipore) and transferred to MS vials for LC-MS analysis. A quality control (QC) sample was prepared as pooled mixture of all samples.

2.3. UPLC–QTOF Analysis

A Waters Acquity UPLC coupled with an electrospray ionization (ESI) interface to a Synapt HDMS QTOF MS (Waters, Manchester, UK) operating in W-mode and controlled by MassLynx 4.1 software was used. The LC-MS conditions were previously described in Arapitsas et al. (2014).

2.4. Data analysis

For quality control during the runs and data analysis, we used PCA (Principal Component Analysis) plots generated by Progenesis QI (Version 2.0.0.0.0, nonlinear Dynamics), checking the distribution/clustering of the QC injections (Arapitsas, et al. 2016). Progenesis QI parameters used for alignment were done on default mode by Progenesis QI with peak picking performed at maximum level; and the first minute and the last six minutes of the run excluded of data processing. Tentative biomarkers were considered the “compounds” that according the Progenesis QI statistical analysis had max fold range > 1.5 and Anova (*p* value) < 0.01. Progenesis QI consider as “compound” a group of the isotopic and adducts features belonging to the same metabolite.

Annotation was performed manually by comparing retention times and mass spectra accuracy with a mass tolerance of 5 ppm, based on the group previous experience with the specific instrumentation mass resolution (Shahaf et al. 2013), and in accordance the 4 levels described by Sumner et al. (2007). MS/MS data were also registered for further supporting the annotation of selected tentative biomarker.

The biomarkers and relative annotated metabolites peaks were also integrated semi manually using the TargetLynx tools of Waters MassLynx 4.1 software (Milford, MA). Further statistical analysis were performed on these integrated peaks by using MetaboAnalyst online platform version 4.0 (http://www.metaboanalyst.ca/, Chong et al. 2018), without normalization and data transformation, using Pareto scaling.

Raw LC–MS data and other details will be made publicly available for download with the accession number MTBLS784 from the MetaboLights public repository http://www.ebi.ac.uk/metabolights/ (Haug et al. 2013).

3. Results and discussion

For both regions, flowering happened in the end of October. Besides the high levels of precipitation (Fig 1D), pollination and fruiting were not affected. During the fruit development, the main difference between regions was the pluviometric content, which was higher on southeast than south region (Fig 1D), while the mean temperatures were the typical for each region (Fig 1C).

According to our previous experience, before the detailed investigation of a metabolomic dataset, is critical to be sure about the quality of the data. The sample set contained a good biological variability in term of number of plants, cultivars and regions (Fig. 1A). The question was one and clear: Which are the metabolic differences between control and treatment samples? Sample preparation and instrumental measurements were based into validated protocols ensuring good metabolic coverage and system robustness (Arapitsas et al. 2018; Arapitsas, Corte, et al. 2016). The tide cluster of the QC samples in the middle of all samples at the PCA plots of Fig. 2, was a first demonstration of the data set quality.

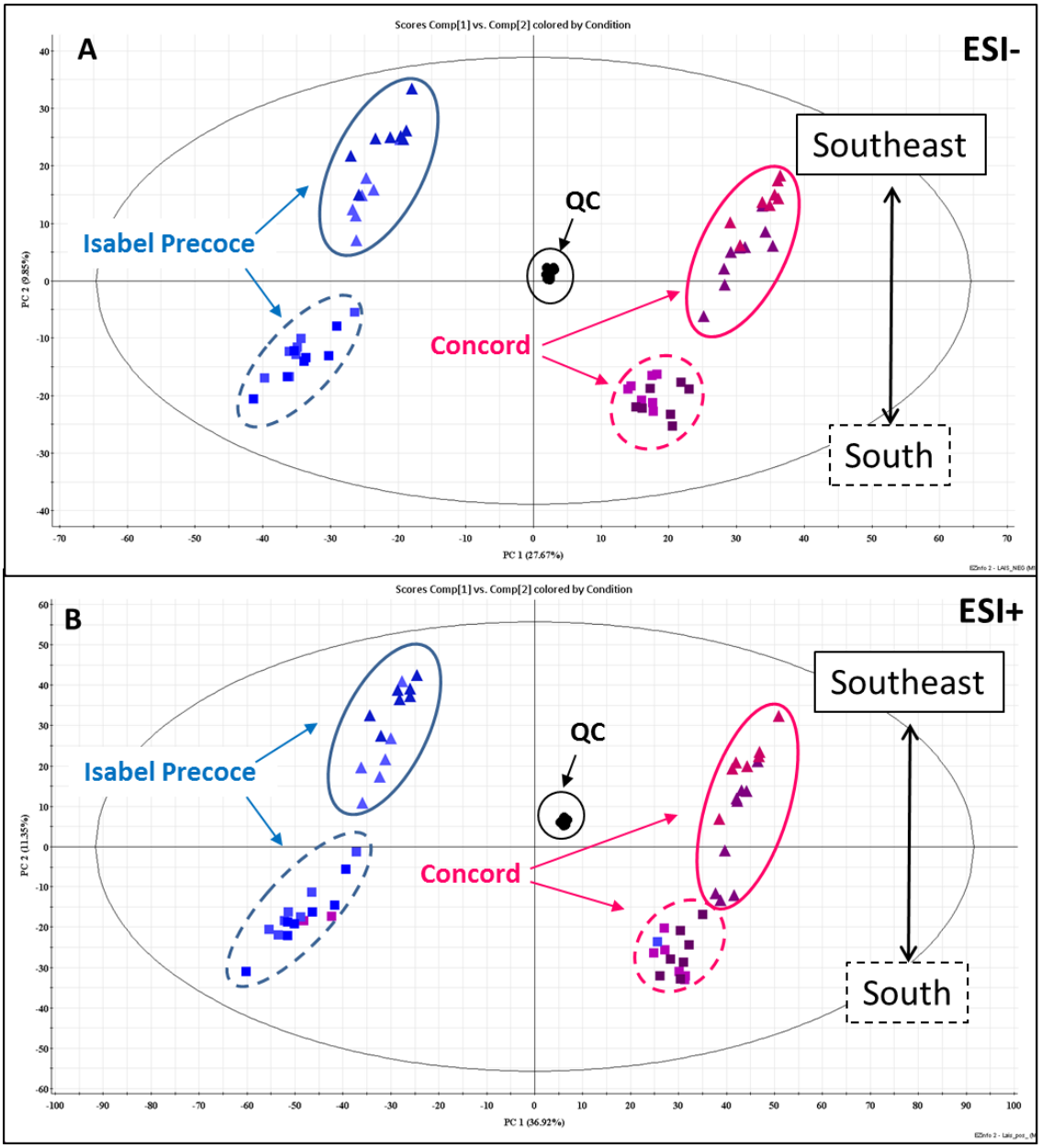

Figure 2. PCA plots of the ESI- mode dataset (A) and ESI+ mode (B). QC injections create a tide cluster, while in both cases PC1 separate the cultivars and PC2 the regions.

At first sight, both PCA plots (Fig. 2) based on the 1.880 detected “compounds” registered at ESI- mode (Fig. 2A) or the 3.628 “compounds” of ESI+ mode (Fig 2B), shown 4 clusters (plus the QC cluster), thus clear separation between the two cultivars and the two regions. Therefore, we observed that the cultivar and region were by far the main factors affecting the unsupervised sample distribution, pointing out the suitability of metabolomics to support cultivar and regional identification.

This highlighted region differentiation, could be explained as result of biotic, abiotic factors and growing conditions. Temperature and radiance can directly effect a series of events as the length of growing season, phenological stages, berry growth, synthesis and accumulation of compounds in the berries (Palliotti et al. 2014). However, the separation observed in Fig. 2 should not be only result of the environmental factors, but also depended on the conductive system used on grapevines at each region too. The grapes cultivated on the south were conducted on tendon system, being more shaded in comparison with those cultivated by vertical shoot positioning system at southeast region. These differences regarding conductive system effect directly the anthocyanin content (Palliotti et al. 2014). Analyzing dataset regarding the different regions, we were able to detect higher content of mono and 3,5-*O*-diglucoside anthocyanins on grapes cultivated on southeast region (Fig. 3). These grapes also presented higher amount of anthocyanins in comparison with south (Fig. 3).

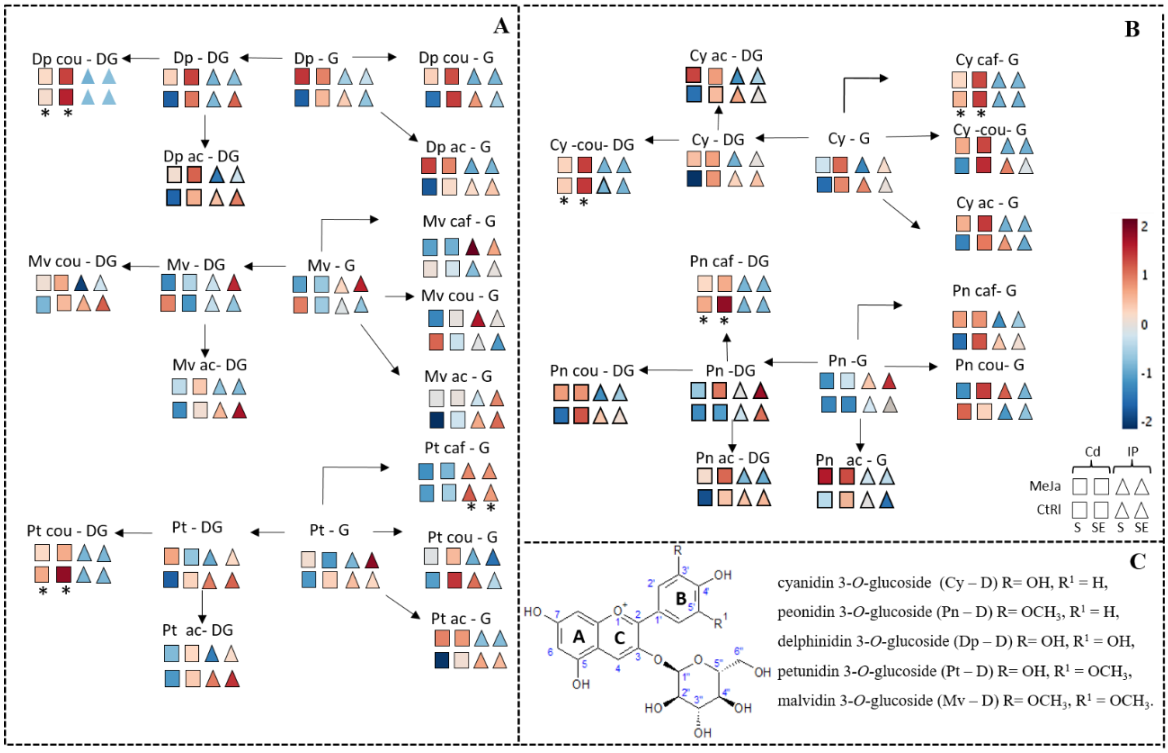

Figure 3. Annotated anthocyanins divided into 3'-hydroxy (A) and 3'5'-dihydroxy (B), their chemical structure (C), along with their behavior in our experiment. MeJA: treatment; CtRl: control group; Cd: Concord; IP: Isabel Precoce; S: southern region; and SE: Southeast. *p value < 0.05. The coloring of the square and triangle is in agreement with the heatmaps of Supplementary Figure SF1. Cy: cyanidin; Pn: peonidin; Dp: delphinidin; Mv: malvidin; Pt: petunidin, G: glucoside; DG: diglucoside; ac: acetyl; cou: *p*-coumaroyl; caf: caffeoyl.

On the other hand, the detection of the minor and specific effects of MeJa application was a challenging task, and specific supervised analytical strategies were followed for the data mining. Data were divided in four groups, where each group contained only the samples from one cultivar and one region, thus the four groups separated by the unsupervised PCA analysis of Fig. 2. From each group, only the compounds with max fold range > 1.5 and Anova (*p* value) < 0.01 (MeJa treatment versus control) were selected. Finally, as tentative biomarkers we consider the compounds, presenting the above characteristics in at least two groups. Such biomarker filtration gave 44 tentative markers for the ESI- mode and 17 for ESI+ mode analysis (Supplementary Table ST1). The majority of the biomarkers belonged to the phenolic family (anthocyanins, stilbens, hydroxycinamates, etc.) and we will present them in the following chapters.

3.1. Anthocyanins

Anthocyanins are natural colorants present in the red grapes skins and promote the organoleptic characteristics of grape products (Flamini, De Rosso, et al. 2013). Due to their importance and because some of the MeJa application biomarkers (Supplementary Table ST1) were identified as anthocyanins, we studied them in depth. We annotated 35 anthocyanins, such cyanidin, peonidin, delphinidin, malvidin and petunidin in the caffeoyl, acetyl and *p*-coumaroyl mono and diglucoside derivatives (Supplementary Figure FS1).

Fig. 3 shows the basic anthocyanin structure and their biosynthesis in grapes, providing information about their behavior in correlation to the region, cultivar and the treatment in the present experiment. It is known that *V. labrusca* L. grapes contain 3,5-*O*-diglucoside anthocyanins and that the anthocyanin acylation increases their stability (Flamini, Mattivi, et al. 2013). Moreover is well documented the positive correlation between sunlight exposure and flavonols/anthocyanins content (Flamini, Mattivi, et al. 2013; Gerós and Delrot 2016). For example, studies shows that in shaded grapes this decrease on anthocyanin content is related to the down-regulation of many genes coding enzymes both at beginning and in the end of the biosynthetic pathway (Gerós and Delrot 2016; Portu et al. 2018; Portu, Santamaria, et al. 2015).

Here, we observed that despite the subtropical climate conditions (Fig. 1), MeJa seems to exert a big impact on anthocyanin content, presenting as a good elicitor to improve the lower content that might be found on grapes cultivated under such climate.

Three derivatives of malvidin were identified as biomarkers for MeJa treatment (Supplementary Table ST1). Our results partially agreed with previous studies, were MeJa treatment has been reported to increase anthocyanin biosynthesis on *V vinifera* L. grapes (Portu et al. 2018; Portu, Santamaria, et al. 2015), because we observed different response between variety and region (Fig. 3). Generally, we observed higher content of acetylated anthocyanins on MeJa treated grapes (Fig. 3), with exception of Isabel Precoce grapes cultivated on southeast region. This profile might be a treatment response to higher levels of anthocyanins synthesized, since acylation of glucose increases the stability of anthocyanins (Flamini et al., 2013).

3.2. Non-anthocyanic flavonoids in MJ treated grapes

Non-anthocyanic flavonoids were a second big group influenced by the MeJa pre-harvest application, as we can notice in Figure 4 and Supplementary material ST1 and SF2.

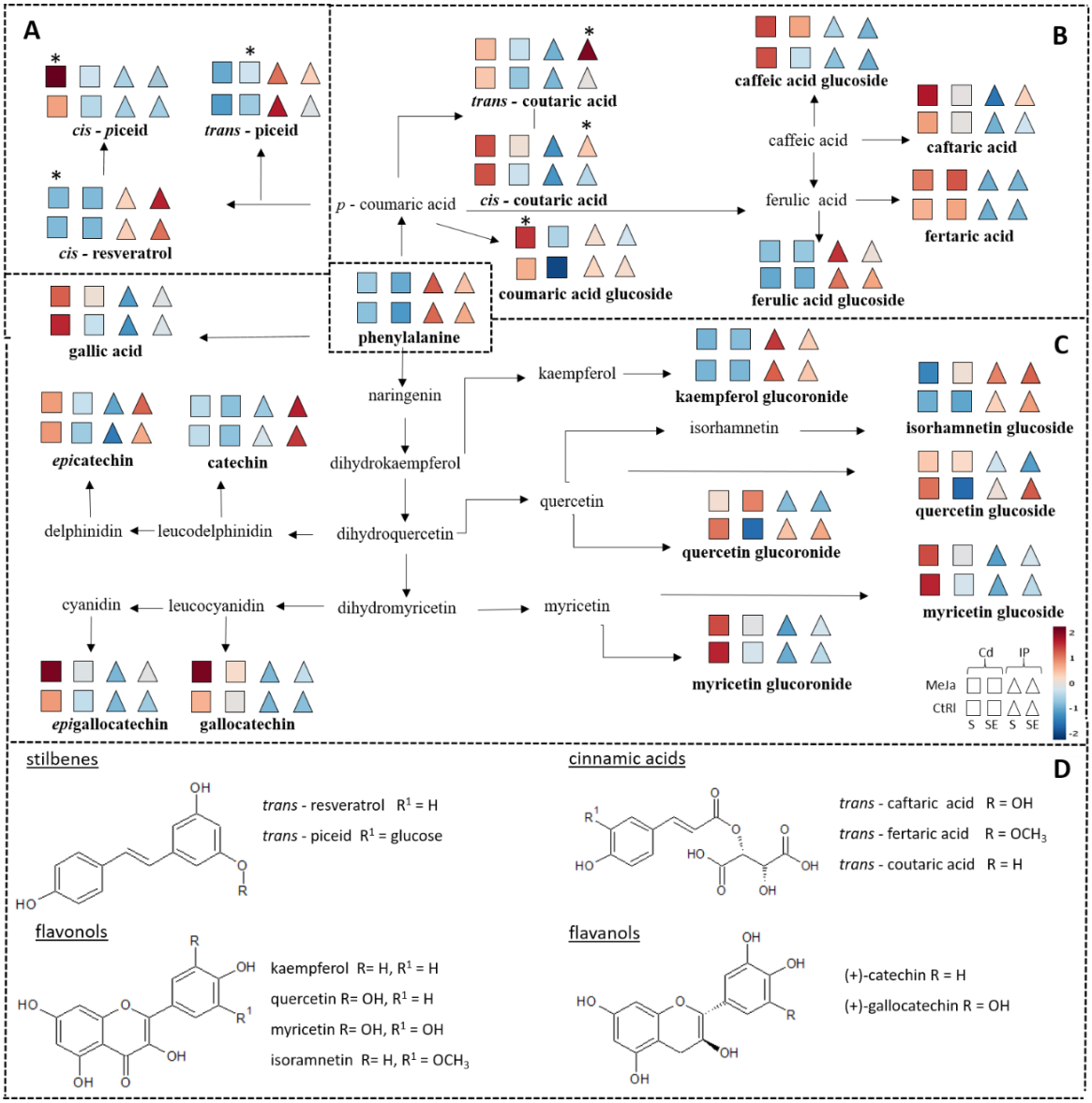

Figure 4. General metabolic pathway of non-anthocyanic phenolic compounds, object of the study, divided into Stilbens (A), hydroxycinnamates (B) and Flavonoids (C); their chemical structure (D), and their behavior in our experiment. MeJA: treatment; CtRl: control group; Cd: Concord; IP: Isabel Precoce; S: southern region; and SE: Southeast. *p value < 0.05. The coloring of the square and triangle is in agreement with the heatmaps of Supplementary Figure SF2.

Like anthocyanins also the biosynthesis of other flavonoids is modulated by light, protecting the berry from harmful UVB radiation (Gerós and Delrot 2016; Palliotti et al. 2014; Keller, M. 2010). Grapes from southeast region were cultivated by vertical shoot positioning system, having higher exposure to the sunlight, in comparison with the grapes cultivated on the south. As previous reported on literature it was expected an increase on quercetin glucoside (S. F. PRICE P. J. BREEN M. VALLADAO 1995) In our study, we did not observe a difference on quercetin glucoside content neither affected by region or by treatment; however, we observed an increase of isorhamnetin

glucoside on grapes treated with MeJa. Moreover, the B-ring three-substitute *epi*gallocatechin and gallocatechin where higher on Concord grapes of both regions. Kaempferol glucuronide contend also differentiate the two varieties, since higher levels were observed on Isabel Precoce from both regions, in comparison with Concord grapes.

3.3. Hydroxycinnamic acids

Some hydroxycinnamic derivaties (caffeic acid, caftaric acid and coumaric acid glucoside) were detected as strong MeJa treatment biomarkers (Fig. 4B, Supplementary Table ST1 and SF2). These metabolites are considered important in grapes both because of their high quantity and their impact to the sensorial character. However, similar researches in *V. vinifera* L. grapes (Portu et al. 2016, 2018) showed that foliar MeJa application did not affect hydroxycinnamates composition. The Concord and Isabel Precoce grapes in our study may have responded differently because although belonging to *Vitaceae* family, they compose a different specie. Nevertheless, we should not ignore that also viticulture practices and biotic factors play a role, so further experiments are necessary to confirm this behavior.

Our data show that grapes respond differently to MeJa treatment according to the region. For Isabel Precoce grown at southeast, it was observed an increase on caftaric acid, while for those grown at south region there were higher levels on ferulic acid (Fig 4B). Generally, Isabel Precoce had lower levels of caffeic acid glucoside and fertaric acid in respect to Concord.

3.4. Stilbenes

The main expected result following MeJa application was an increase in stilbenes content regardless grapevine growing condition (Garde-cerdán et al. 2016; Javier Portu, Pilar Santamaría, Isabel López-Alfaro, Rosa López 2015; Portu et al. 2018). In our study, we detected *cis*-resveratrol, *cis*- piceid and *trans*-piceid as biomarkers of MeJa treatment (Fig 4A and Supplementary table ST1). Piceid enhanced on all grapes studied, meanwhile, an increase on resveratrol content was observed only for Isabel Precoce samples. These results are in accordance with previous studies that suggest that MeJa enhance stilbenes biosynthesis (Portu et al. 2018; Ruiz-garcía et al. 2013) though up regulation of stilbene synthase.

3.5. Miscellaneous

Isabel Precoce grapes grown on south present lower content of gallic acid in comparison with the other three groups. Since gallic acid formation occurs as an alternative to the phenylpropanoid route by dehydrogenation of shikimic acid (Flamini, Mattivi, et al. 2013). It could be possible that a deviation of the path occur on these grapes. *V. labrusca* L. grapes are known to present higher content of hydrolysable tannin than *V. vinifera* (Narduzzi et al. 2015), however, glucogalloyl derivative it was not identified among our biomarkers.

The amino acid phenylalanine was positively affected by the treatment for Isabel Precoce grapes from both regions. Garde-cerdán et al. (2016) described similar result after foliar application of MeJa during pre-harvest on Tempranillo grapes. The same study also reported an increase on tryptophan content, but Gutiérrez-gamboa et al. (2018) did not register such behavior in a similar study with Tempranillo grapes. In our study, we did not detect significant changes on tryptophan content (Supplementary Figure SF3), but the product of the reaction between tryptophan and acetaldehyde (1,2,3,4-tetrahydro-β-carbonile-3-carboxilic acid) was among the biomarkers for Concord and Isabel Precoce grapes from south and southeast region respectively (Supplementary Table ST1). This compound which is a product of Pictet-Spengler condensation (Cox and Cook 1995) was previous described in wines and grapes (Herraiz et al. 1999). South Concord and southeast Isabel Precoce grapes presented opposite behavior with higher content detected at MeJa treated fruits grown at southeast (Supplementary Figure SF3). We believe that acetaldehyde concentrations could affect the formation of these markers, since acetaldehyde formation on plants is induced by stress conditions such as high light and temperature (Jardine et al. 2009), that are conditions found on the regions of our study (Fig 1). In addition, stress conditions might activate the octadecanoid pathway, increasing C6 aldehydes activity. This path may also be responsible for jasmonic acid biosynthesis as already shown on raspberries (Moro et al., 2017) where MeJa induced autocatalytic production, and consequently higher activity of this pathway. However, further studies are necessary to confirm this hypothesis.

4. Conclusion

This work pointed out the potential of MeJa pre-harvest application to increase the concentration of several phenolic compounds in grapes grown in warm and hot climate

and improve their quality. The unsupervised data analysis of the dataset, obtained through a LC-MS untargeted approach protocol, demonstrated the capability of metabolomics to distinguish cultivars and origin. On the other hand, the supervised data analysis and a robust workflow helped to understand the metabolic changes occurred in grapes due to MeJa treatment. Despite the biological variability caused by the environmental conditions and viticulture practices reported on our study, two period applications of MeJa were able to influence some of the major grape flavonoids (anthocyanins), stilbenes and phenolic acids (hydroxycinnamates) on both studied regions. In general, it seems that Concord grapes were more responsive to MeJa treatment, while Isabel Precoce grapes from south vineyards were the least responsive ones.

According to ours results, MeJa treatment could help to contribute in increasing grape health benefits and consequently their products.

Author contributions: LM and PA designed, conducted the experiments and analyzed data. LM and PA wrote the manuscript. All authors read and approved the manuscript.

Compliance with ethical standards: This article does not contain any studies with human and/or animal participants performed by any of the authors.

Conflict of interest: The authors declare that they have no conflict of interests.

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Supplementary Material

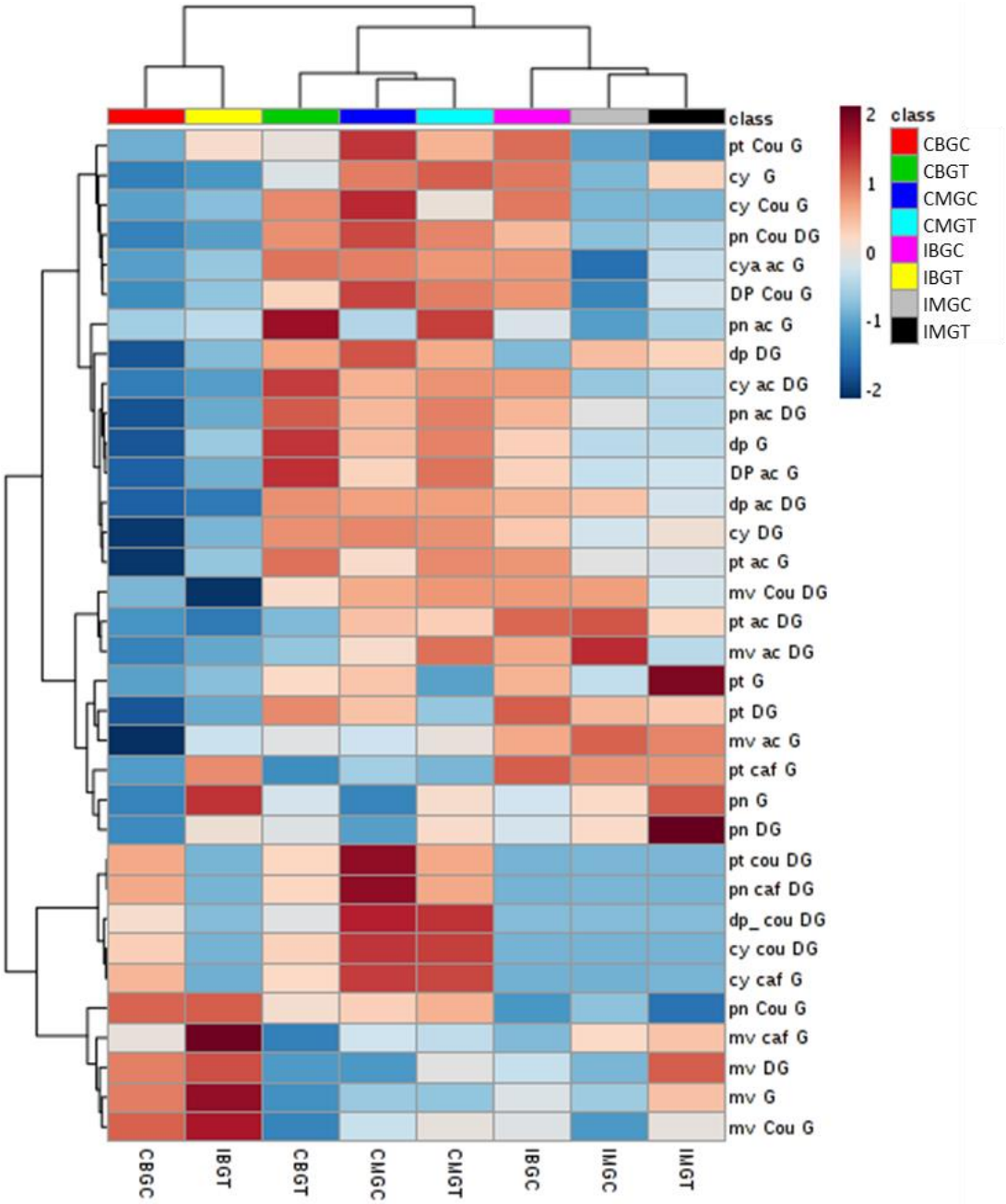

Figure SF1: Heatmap of anthocyanins annotated on our study; C- Concord, I- Isabel Precoce; cultivated on South (BG) and Southeast region (MG) of Brazil; C- control group T - MeJa treatment. Cy: cyanidin; Pn: peonidin; Dp: delphinidin; Mv: malvidin; Pt: petunidin, G-glucoside; DG- Diglucoside; Ac G - Acetyl Glucoside; Ac DG -Acetyl Diglucoside; Cou G – Coumaroyl Glucoside; Cou DG – Coumaroyl Diglucoside; Caf G - Caffeoyl Glucoside; Caf Dg - Caffeoyl Diglucoside.

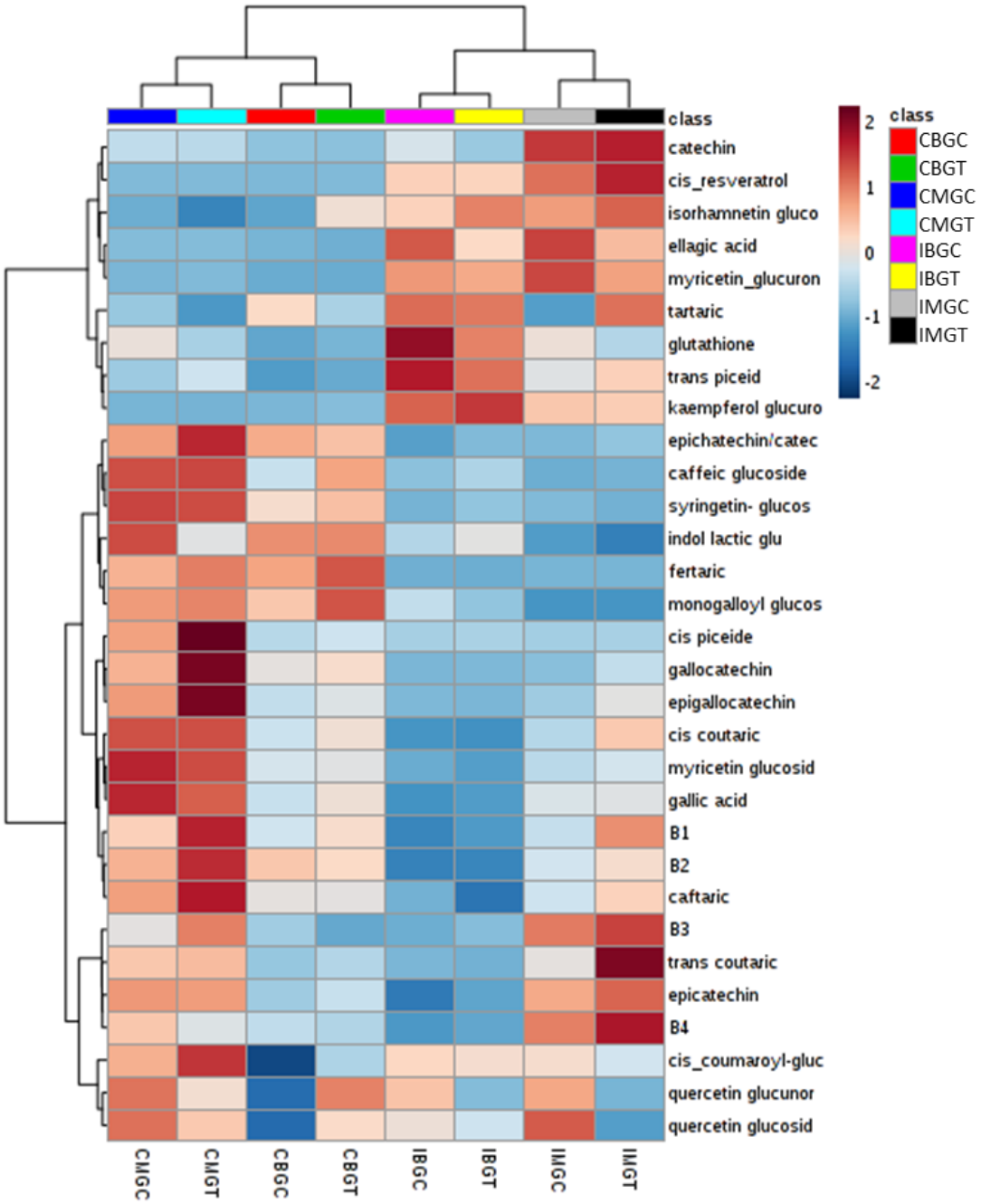

Figure SF2: Heatmap of non-anthocyanic compounds annotated on our study; C- Concord, I- Isabel Precoce; cultivated on South (BG) and Southeast region (MG) of Brazil; C- control group T - MeJa treatment.

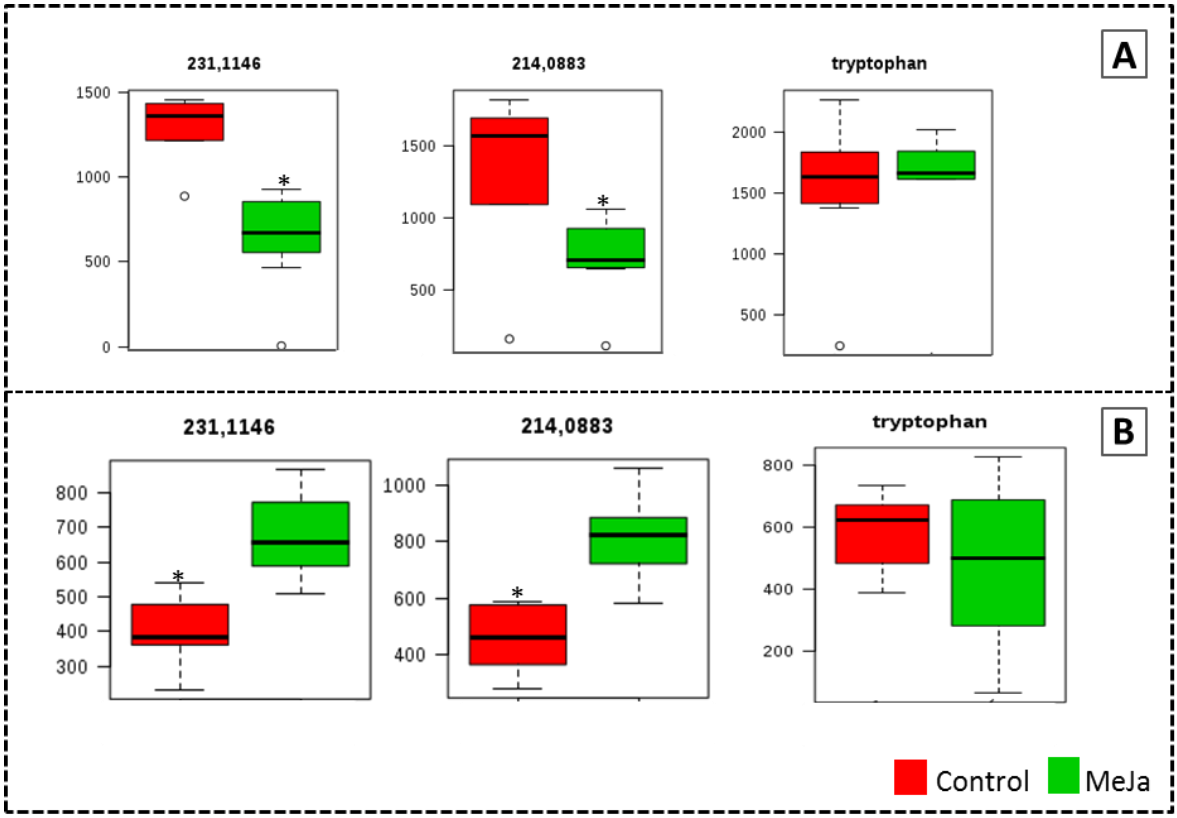

Figure SF3: Annotated compounds on ESI+: 1,2,3,4-tetrahydro-β-carbonile-3-carboxilic acid (m/z: 231.1126 and 214.1146) and tryptophan annotated for Concord grapes from south region (A) and Isabel Precoce grapes from southeast region (B); * indicate statistical difference for *p* value < 0.05.

Table S1. Tentative markers of MeJa treatment for Concord and Isabel Precoce grapes cultivated on south and southeast regions of Brazil;

ESI mode	RT (min)	Measured m/z	Theoretical mass	mass error (ppm)	Annotation	* level of identification
-	1,24	253,0566			unknown	
-	1,35	495,1122			unknown	
-	1,36	307,1086			uknown	
+	1,39	378,1419			unknown	
-	1,76	295,0670			unknown	
-	4,09	468,0937			unknown	
-	4,88	125,0246	125,0244	-0,19	gallic acid (fragment)	1
-	5,15	345,0826			unknown	
-	5,95	593,1317	593,1301	-0,28	dimeric proanthocyanidin	3
-	6,31	423,0610			cinnamic glucoside with phosphoric acid	3
-	7,01	179,0352	179,0344	-0,49	[H-M]-tartaric acid / caftaric acid (fragment)	1
-	7,47	153,0560	153,0566	0,39	vanillyl alcool glucoside	3
-	7,77	355,0662	355,0671	0,25	caffeoyl glucuronide derivative	3
-	8,13	165,0567			dihydroxy coumaroyl	3
-	8,54	439,9974			unknown	
-	8,54	341,0881	341,0878	-0,07	caffeic acid glucoside	1
-	8,54	203,0349	203,0344	-0,23	caffeic acid glucoside (fragment)	1
-	8,56	179,0354	179,0344	-0,59	caffeic acid glucoside	1
-	8,71	355,0666	355,0671	0,14	caffeoyl glucuronide derivative	3
-	9,50	425,1645			unknown	
+	9,88	387,1643			unknown	
+	10,56	91,0553	91,0541	-1,27	coumaric acid glucoside (fragment)	1
+	10,56	147,0457	147,0457	-0,02	coumaric acid glucoside	1
+	10,56	349,0916	349,0899	-0,49	coumaric acid glucoside sodiate	1
+	10,56	365,0621	365,0639	0,48	coumaric acid glucoside potassiate	1
+	10,56	119,0506	119,0506	0,00	coumaric acid glucoside (fragment)	1
+	10,56	185,0439	185,0431	-0,46	coumaric acid glucoside (fragment)	1
-	10,60	187,0407	187,0402	-0,25	coumaric acid glucoside (fragment)	1
-	10,60	117,0345	117,0340	-0,42	coumaric acid glucoside (fragment)	1
-	10,61	425,0168			coumaric acid glucoside (adduct)	3
+	10,76	214,0883	214,0851	-1,48	1,2,3,4-Tetrahydroharmane-3-carboxylic acid	1
+	10,76	231,1146	231,1204	2,51	1,2,3,4-Tetrahydroharmane-3-carboxylic acid	1
-	10,84	229,0979	229,1046	2,92	1,2,3,4-Tetrahydroharmane-3-carboxylic acid	1
+	11,25	655,1895	655,1869	-0,40	malvidin 3,5-diglucoside	1
-	12,05	382,1142			unknown	
+	12,51	218,0830			unknown	
-	12,55	270,0987			unknown	
-	13,83	786,1586	786,1888	3,85	IAA hexoside (adduct)	3
+	14,24	697,1995	697,1974	-0,31	malvidin 3-(6''-acetyl)-diglucoside	1
-	14,30	149,0452			caffeoyl derivative (fragment)	3
-	14,30	89,0234			unknown	
-	15,93	430,0943			unknown	

Table S1. Tentative markers of MeJa treatment for Concord and Isabel Precoce grapes cultivated on south and southeast regions of Brazil;

ESI mode	RT (min)	Measured m/z	Theoretical mass	mass error (ppm)	Annotation	* level of identification
-	17,56	605,0823			unknown	
-	18,67	817,2213			anthocyanin derivative	3
-	19,41	271,1545			unknown	
-	19,56	389,1237	389,1236	-0,03	*cis*-pideid	1
+	19,61	801,2284	801,2237	-0,58	malvidin 3,5-(6''-p-coumaroyl)-diglucoside	2
-	19,68	831,2376			anthocyanin derivative	3
-	19,94	315,0871			unknown	
-	20,32	605,1666			unknown	
-	20,32	289,0718			flavanol fragment	3
+	20,35	229,0878	229,0865	-0,56	resveratrol	1
-	20,38	227,0729	227,0707	-0,95	*cis*-resveratrol	1
-	20,44	463,0865			unknown	
-	20,54	537,2570			unknown	
-	20,64	759,1077			unknown	
-	21,05	641,1452			unknown	
-	21,28	435,2239			unknown	
+	21,30	351,2161			unknown	
+	21,30	275,2026			unknown	
-	21,34	327,2181			unknown	
*According to Sumner et al. (2007).						

Table S2. Identified markers for Concord and Isabel Precoce grapes cultivated on south and southeast regions of Brazil;

Compound	Level of identification*	HMDB	KEGG	Chemspider ID	Molecular Formula	RT (min)	Monoisotopic mass (Da)	m/z ESI (+)	Error (ppm)
anthocyanins									
delphinidin 3-glucoside	1		C12138	391783	$C_{21}H_{21}O_{12}$	11,38	465,1028	465,1024	0,76
cyanidin 3-glucoside	1		C08604	390284	$C_{21}H_{21}O_{11}$	12,75	449,1078	449,1088	-2,12
petunidin 3-glucoside	1		C12139	391784	$C_{22}H_{23}O_{12}$	13,30	479,1184	479,1177	1,84
peonidin 3-glucoside	1		C12141	391786	$C_{22}H_{23}O_{11}$	14,55	463,1235	463,1235	-0,06
malvidin 3-glucoside	1		C12140	391785	$C_{23}H_{25}O_{12}$	14,83	493,1341	493,1354	-2,71
delphinidin 3,5-diglucoside	1		C16312	8276439	$C_{27}H_{31}O_{17}$	7,60	627,1556	627,1575	-3,06
cyanidin 3,5-diglucoside	1		C06361	30777615	$C_{33}H_{31}O_{16}$	9,07	611,1607	611,1613	-0,98
petunidin 3,5-diglucoside	1			32697757	$C_{28}H_{33}O_{17}$	9,45	641,5509	641,5507	0,27
peonidin 3,5-diglucoside	1			4589999	$C_{28}H_{33}O_{16}$	10,86	625,1763	625,1765	-0,27
malvidin 3,5-diglucoside	1		C08718	390365	$C_{29}H_{35}O_{17}$	11,24	655,1869	655,1870	-0,17
delphinidin 3-(6''-acetyl)-glucoside	2			30777226	$C_{23}H_{23}O_{13}$	16,88	507,1133	507,1135	-0,37
cyanidin 3-(6''-acetyl)-glucoside	1			30780060	$C_{23}H_{23}O_{12}$	18,15	491,1184	491,1175	1,85
malvidin 3-(6''-acetyl)-glucoside	2	HMDB0038008		24842428	$C_{25}H_{27}O_{13}$	19,49	535,1446	535,1434	2,23
peonidin 3-(6''-acetyl)-glucoside	1			24842289	$C_{24}H_{25}O_{12}$	19,54	505,1341	505,1355	-2,84
petunidin 3-(6''-acetyl)-glucoside	1			30779241	$C_{24}H_{25}O_{13}$	18,30	521,1290	521,1299	-1,79
delphinidin 3-(6''-acetyl)-diglucoside	2				$C_{29}H_{33}O_{18}$	11,28	669,1661	669,1673	-1,79
cyanidin 3-(6''-acetyl)-diglucoside	2				$C_{29}H_{33}O_{17}$	12,40	653,5616	653,5617	-0,20
malvidin 3-(6''-acetyl)-diglucoside	2				$C_{31}H_{37}O_{18}$	14,20	697,1974	697,1940	4,88
peonidin 3-(6''-acetyl)-diglucoside	2				$C_{30}H_{35}O_{17}$	14,30	667,1869	667,1887	-2,70
petunidin 3-(6''-acetyl)-diglucoside	2				$C_{30}H_{35}O_{18}$	12,97	683,1818	683,1813	0,73
delphinidin 3-(6''-*p*-coumaroyl)-glucoside	1		C16370	26559505	$C_{30}H_{27}O_{14}$	19,97	611,1395	611,1414	-3,07
cyanidin 3-(6''-*p*-coumaroyl)-glucoside	1		C12095	4445294	$C_{30}H_{27}O_{13}$	20,18	595,1446	595,1441	0,83
petunidin 3-(6''-*p*-coumaroyl)-glucoside	1	HMDB0038100		30779240	$C_{31}H_{29}O_{14}$	20,19	625,1552	625,1577	-3,98
peonidin 3-(6''-*p*-coumaroyl)-glucoside	1			24842291	$C_{31}H_{29}O_{13}$	20,35	609,1603	609,1598	0,78
malvidin 3-(6''-*p*-coumaroyl)-glucoside	1			24842430	$C_{17}H_{15}O_7$	20,29	639,1708	639,1704	0,63
delphinidin 3-(6''-*p*-coumaroyl)-diglucoside	2				$C_{36}H_{37}O_{19}$	17,36	773,1924	773,1923	0,07
cyanidin 3-(6''-*p*-coumaroyl)-diglucoside	2				$C_{36}H_{37}O_{18}$	18,51	757,1974	757,1999	-3,25
petunidin 3-(6''-*p*-coumaroyl)-diglucoside	2				$C_{37}H_{39}O_{19}$	18,66	787,2080	787,2089	-1,14

Compound	Level of identification*	HMDB	KEGG	Chemspider ID	Molecular Formula	RT (min)	Monoisotopic mass (Da)	m/z ESI (+)	Error (ppm)
anthocyanins									
peonidin 3-(6''-*p* -coumaroyl)-diglucoside	2				$C_{31}H_{29}O_{18}$	19,67	609,1603	609,1601	0,33
malvidin 3-(6''-*p* -coumaroyl)-diglucoside	2				$C_{38}H_{41}O_{18}$	19,65	801,2237	801,2242	-0,62
cyanidin 3-(6''caffeoyl)- glucoside	2				$C_{30}H_{27}O_{14}$	19,99	611,1395	611,1403	-1,26
petunidin 3-(6''caffeoyl)- glucoside	2				$C_{31}H_{29}O_{15}$	20,32	641,1501	641,1660	-2,48
peonidin 3-(6'' caffeoyl)- glucoside	2				$C_{31}H_{29}O_{14}$	20,19	625,1552	625,1572	-3,23
malvidin 3-(6''caffeoyl)- glucoside	2				$C_{32}H_{31}O_{15}$	20,03	655,1657	655,1656	0,15
peonidin 3-(6'' caffeoyl)- diglucoside	2				$C_{37}H_{39}O_{19}$	18,63	787,2081	787,2094	-1,71
Amino acids									
arginine	1			6082	$C_6H_{14}N_4O_2$	1,20	174,1117	175,1171	14,16
threonine	1			6051	$C_4H_9NO_3$	1,26	119,0582	120,0669	-6,30
proline	1			594	$C_5H_9NO_2$	1,20	115,0633	116,0710	2,00
valine	1			6050	$C_5H_{11}NO_2$	1,38	117,0790	118,0870	-1,02
leucine	1			5880	$C_6H_{13}NO_2$	4,25	131,0946	132,1022	2,54
phenylalanine	1		C00079	5910	$C_9H_{11}NO_2$	5,97	165,0790	166,0869	-1,33
tryptophan	1		C00078	6066	$C_{11}H_{12}N_2O_2$	8,27	204,0899	205,0978	-0,13
tyrosine	1		C01536	1121	$C_9H_{11}NO_3$	4,08	181,0739	182,0833	-8,25
flavanols									
gallocatechin	1	HMDB38365	C12127	58594	$C_{15}H_{14}O_7$	6,45	306,0739		
procyanidin B3	1	HMDB33974		129476	$C_{30}H_{26}O_{12}$	8,06	578,1425		
procyanidin B1	1	HMDB29754		9425166	$C_{30}H_{26}O_{12}$	8,33	578,1425		
epigallocatechin	1	HMDB38361	C12136	65231	$C_{15}H_{14}O_7$	9,33	306,0739		
procyanidin B4	1	HMDB13690	C129882	129882	$C_{30}H_{26}O_{12}$	9,37	578,1425		
catechin	1	HMDB02780	C06562	8711	$C_{15}H_{14}O_6$	9,53	290,0790		
procyanidin B2	1	HMDB33973		4478723	$C_{30}H_{26}O_{12}$	10,54	578,1425		
epicatechin	1	HMDB01871	C65230	65230	$C_{15}H_{14}O_6$	12,55	290,0790		

Compound	Level of identification*	HMDB	KEGG	Chemspider ID	Molecular Formula	RT (min)	Monoisotopic mass (Da)	Theoretical mass (Da)	m/z ESI (-)	Error (ppm)
flavanols										
gallocatechin	1	HMDB38365	C12127	58594	$C_{15}H_{14}O_7$	6,45	306,0739	305,0660	305,0661	-0,18
procyanidin B3	1	HMDB33974		129476	$C_{30}H_{26}O_{12}$	8,06	578,1425	577,1346	577,1343	0,44
procyanidin B1	1	HMDB29754		9425166	$C_{30}H_{26}O_{12}$	8,33	578,1425	577,1346	577,1333	2,18
epigallocatechin	1	HMDB38361	C12136	65231	$C_{15}H_{14}O_7$	9,33	306,0739	305,0660	305,0661	-0,18
procyanidin B4	1	HMDB13690	C12988	129882	$C_{30}H_{26}O_{12}$	9,37	578,1425	577,1346	577,1361	-2,68
catechin	1	HMDB02780	C06562	8711	$C_{15}H_{14}O_6$	9,53	290,0790	289,0711	289,0714	-0,90
procyanidin B2	1	HMDB33973		4478723	$C_{30}H_{26}O_{12}$	10,54	578,1425	577,1346	577,1351	-0,94
epicatechin	1	HMDB01871	C65230	65230	$C_{15}H_{14}O_6$	12,55	290,0790	289,0711	289,0720	-2,97
flavonoids										
myricetin-3-glucoside	1	HMDB0034359		4588987	$C_{21}H_{20}O_{13}$	16,97	480,0904	479,0825	479,0810	3,12
myricetin-3-glucuronide	1			4589353	$C_{21}H_{18}O_{14}$	18,91	494,0696	493,0617	493,0616	0,29
quercetin-3-glucuronide	1			18699310	$C_{21}H_{18}O_{13}$	18,92	478,0747	477,0668	477,0658	2,18
quercetin 3-glucoside	1	HMDB00373	C05623	24773541	$C_{21}H_{20}O_{12}$	19,29	464,0955	463,0876	463,0860	3,43
syringetin-3-glucoside	1			16736532	$C_{23}H_{24}O_{13}$	20,32	508,1217	507,1138	507,1130	1,59
kaempferol 3-glucuronide	1	HMDB0029500		18699309	$C_{21}H_{18}O_{12}$	20,17	462,0798	461,0719	461,0725	-1,23
kaempferol 3-glucoside	1		C12249	4445311	$C_{21}H_{20}O_{11}$	20,23	448,1006	447,0927	447,0925	0,35
quercetin-3-rutinoside	1	HMDB0003249		4444362	$C_{27}H_{30}O_{16}$	20,02	610,1530	609,1451	609,1455	-0,66
stilbenoids										
trans-piceid	1	HMDB00305	C10275	4445034	$C_{20}H_{22}O_8$	15,73	390,1315	389,1236	389,1249	-3,42
cis-piceid	1	HMDB0031422		8353968	$C_{20}H_{22}O_8$	19,56	390,1315	389,1236	389,1224	3,01
cis-resveratrol	1	HMDB0034118		1265933	$C_{14}H_{12}O_3$	20,38	228,0786	227,0707	227,0707	0,19
phenolic acids										
gallic acid	1	HMDB05807	C01424	361	$C_7H_6O_5$	4,85	170,0215	169,0136	169,0131	3,14
ellagic acid	1	HMDB02899	C10788	4445149	$C_{14}H_6O_8$	19,84	302,0063	300,9984	300,9977	2,18

Compound	Level of identification*	HMDB	KEGG	Chemspider ID	Molecular Formula	RT (min)	Monoisotopic mass (Da)	Theoretical mass (Da)	m/z ESI (-)	Error (ppm)
cinnamics										
trans-caftaric acid	1	HMDB0013680		4944664	$C_{13}H_{12}O_9$	7,69	312,0481	311,0402	311,0394	2,66
cis-coutaric acid	1			35013748	$C_{13}H_{12}O_8$	9,11	296,0532	295,0453	295,0450	1,09
trans-coutaric acid	1			26325199	$C_{13}H_{12}O_8$	9,50	296,0532	295,0453	295,0458	-1,62
trans-fertaric acid	1	HMDB0029199		20058463	$C_{14}H_{14}O_9$	10,76	326,0638	325,0559	325,0563	-1,29
p-Coumaric acid glucoside	1		C04415	8016010	$C_{15}H_{18}O_8$	10,60	326,1002	325,0923	325,0918	1,41
caffeic acid 3-glucoside	1		C10431	4445073	$C_{15}H_{18}O_9$	8,56	342,0951	341,0872	341,0876	-1,19
ferulic acid O-glucoside	2			10212167	$C_{16}H_{20}O_9$	11,75	356,1107	355,1028	355,1044	-4,46

*According to Sumner et al. (2007).

Metabolomic profile of Brazilian grape juice after Methyl Jasmonate pre-harvest treatment

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1. Introduction

Brazilian grape juice production had increased 23,28% in the past year (2017/2018), based on major Brazilian state producer, (IBRAVIN, 2019), followed by a tendency to continuous increasing in the following years. In all the country, there is a high amount of small and family business (around 50.000 producers) that elaborate juice by a technique with low cost of implementation, the steam extraction system (Guerra et al 2016). The main grapes used for juice production are *Vitis labrusca* L. they present flavor and characteristics that reminds grape, appreciated by a significative portion of Brazilian consumers (Biasoto et al. 2014; Camarão et al. 2014).

These characteristics differ from *V. vinifera* in many aspects, e.g. aromas, anthocyanin and accumulation of flavan-3-ols (Flamini et al. 2010; Flamini, Mattivi, et al. 2013; Narduzzi et al. 2015). *V. labrusca* have a characteristic of "foxy" and fruity smell (Moro et al. 2019; Narduzzi et al. 2015). They often contain 3,5-*O*-diglucoside anthocyanins while *V. vinifera* contains most exclusively 3-*O*-monoglucoside ones (Flamini, Mattivi, et al. 2013; Narduzzi et al. 2015). *V*. *vinifera* contained a higher level of monomeric, dimeric, and trimeric procyanidins in the seeds, in comparison to other *Vitis* species except for *Vitis palmata,* while non *V. vinifera* grapes present elevated levels of catechin-gallate and epicatechin-gallate than *V. vinifera* (Narduzzi et al. 2015).

Grape juice is as a good source of phenolic compounds, such as flavonols, anthocyanins and stilbenes for the population, especially children, youngers and

elders, those that does not consume alcoholic beverages, as wine (Guerra et al 2016; Toaldo et al 2015). Studies related to the beneficial effects exerted by these compounds on health, have encouraged researches that aimed to increase their concentration in fruits, and their sub products. Among the alternatives to improve these metabolites content, different plants grow regulators have been studied, as methyl jasmonate (MeJa). MeJa, an ester of jasmonic acid, belongs to an important group of natural plant growth regulators, that is considered as generally regarded as safe compounds (GRASC). They are natural plant phytochemicals that activate plant defenses mechanism in different stress conditions and have a non-toxic mode of action, since is able to regulate the plant growth and development, and is immediately metabolized in plant cells, leaving no chemical residue (Asghari 2019).

Studies performed with MeJa in *V. vinifera* grapes aiming wine production, were very promising, they observed an increase on proanthocyanidins, flavonoids, stilbenes and anthocyanins, producing wines with higher color intensity and total phenolic content, than the wines produced without MeJa treated grapes (Gil-Muñoz et al. 2017; Portu et al. 2016; Ruiz-García et al. 2013). However, due to grape juice extraction, by the system of steam extraction, these results might not be reproduced. During wine production, there is an extend contact between grape skin, seed and the ethanol produced during alcoholic fermentation, that is as a good solvent for polyphenol extraction (Waterhouse, Andrew L. Gavin L. Sacks 2016).

On this behalf, against upcoming climate change and aiming to improve the content of anthocyanins and other non anthocyanic compound, MeJa treatment it presents a good alternative to improve juices elaborated with *Vitis labrusca* L. grapes. To better understand metabolites behavior and identify markers on juice elaborated with grapes treated with MeJa, metabolomic fingerprint is a very effective tool, with high sensitivity and selectivity, that allows to identify a wide range of compounds in just one analysis (Arapitsas, Ugliano, et al. 2016; Vrhovsek et al. 2012). On this contest, our research aims to better understand the impact of MeJa foliar application on juices produces with *V. labrusca* L. grapes grown on south and southeast of Brazil trough an UPLC-HRMS-QTOF approach.

2. Material and methods

2.1. Grape juice production

The grapes were cultivated, treated and harvested as described in Moro et al (2019). The juice extraction was made by steam juicer, followed by hot bottling in glass bottles previously sterilized, with closure with screw caps (Pinheiro et al., 2009). The juice samples were produced from the fruits of treated and control groups, being carried out triplicate of processing, generating 3 lots for each block. Juice samples were storage at -20 for 6 months, were transported at -20 to Italy, and kept at -80 until analysis.

2.2. Sample preparation

For the juice analysis, all the samples were analyzed according a sequence of random.org, to avoid bias. The juice was diluted 1:3 with Mili-Q water, centrifuge for 3 min at 9.000 g and filtered with 0.2 μm PTFE filters (Milipore®) into a 2 mL amber vial prior to LC/MS analysis.

2.3. UPLC–QTOF Analysis

A Waters Acquity UPLC coupled with an electrospray ionization (ESI) interface to a Synapt HDMS QTOF MS (Waters, Manchester, UK) operating in W-mode and controlled by MassLynx 4.1 software was used. The LC-MS conditions were described in (Arapitsas et al. 2014).

2.4. Marker detection and annotation

For markers detection, due to the big difference between cultivars, that it was larger than the treatment effect, we choose to made an alignment for each one, excluding outliers, and Quality Control (QC) samples. This way, it was possible diminish the probability of false negatives due to misalignment, because of this large variation between cultivars chromatographic profiles. Also, with this approach, we are able to see more clearly the groups when confronting MeJa and CnTr samples.

Marker annotation were done according to the 4 levels described by (Sumner et al. 2007). Annotation of the features was made base of internal database and retention time order given in (Arapitsas, Ugliano, et al. 2016) and/or external database (HMDB (http://www.hmdb.ca/), Chemspider (http://www.chemspider.com/), KEGG, etc). Metabolite identification was performed manually by comparing retention times and accurate mass spectra (mass difference of less than 5 ppm).

2.5. Data analysis

Data processing was done using Waters MassLynx 4.1 and TargetLynx software (Milford, MA), limited to the first 21 min of the chromatography, excluding clean up and equilibration of the column before the next injection. Raw data was collected, imported and analyzed with Progenesis QI (Version 2.0.0.0.0, nonlinear Dynamics) the workflow was composed of: run alignment, peak picking, deconvolution, and normalization.

For quality control during the runs and data analysis, we used PCA (Principal Component Analysis) plots generated by Progenesis, checking the distribution of the QC injections. Tentative markers were considered to be all features with max fold range > 2 and Annova (p value) < 0.001.

2.6. Physicochemical analyses

The pH and titratable acidity of the juices was measured according to the AOAC International (2006). Total soluble solids, (TSS) of juices was determined using a refractometer (ATAGO model Pal-1) according to the AOAC International (2006) and the results were expressed as °Brix. CIE-Lab color parameters (L*, a*, b*, C*, ΔE*) were measured using a Minolta Spectrophotometer CM 3500b (Konica Minolta, Osaka, Japan) and color differences were computed with the CIE76 formula. For the analysis the undiluted juice was filtered with a 0.45 µm PTFE filter and analyzed according to the OIV-MA-AS2-11 method with a 1 mm cuvette (Arapitsas et al. 2014).

3. Results and discussions

3.1. Physicochemical analyses

The results for quality parameters are important for grape juice, due to their influence on organoleptic properties, such flavor, color and aroma, affecting also the stability and shelf-life (Lima et al. 2014). It is known that the steam grape juice, is a method for juice extraction very used for small and medium agrobusiness on Brazil. However, the juice elaborated with this method might have 8 to 17% more water than other methods for grape juice extraction (GUERRA 2016). When comparing results obtained with the respective grapes, which originated the juices, lower soluble solids and higher titratable acidity were observed in the juices (Moro et al. 2019b). For juices elaborated on southeast, lower content of TSS were observed on all varieties,

regardless treatment application. Do not reaching the minimum required by Brazilian patters of identity and quality law.

Regarding other physicochemical parameters, our data corroborated with the observations made for the grape juice from the same *Vitis labrusca* L. grape juice (da Mota et al. 2018; Margraf et al. 2016).

Table 1: Physicochemical parameters for Brazilian grape juice

	CSC	CST	ISC	IST	CSEC	CSET	ISEC	ISET
pH	3,45± 0,01	3,47± 0,01	3,44± 0,02	3,49± 0,01	3,39± 0,06	3,30± 0,00	3,35± 0,02	3,30± 0,02
TSS	14,50± 0,14	10,25± 0,35	14,03± 0,68	13,90± 0,36	11,20± 0,14	9,80± 0,14	10,33± 0,31	10,67± 0,21
AT (Meq/L)	1,26±0,43	1,00±0,32	0,94±0,66	0,82±0,37	0,79±0,43	0,79±0,21	0,69±0,04	0,70±0,13
L*	75,76 ±1,95	78,79±4,68	91,27±1,06	89,36±1,85	75,96±1,43	78,26±9,29	86,64±5,79	90,05±1,56
a*	24,41±1,65	24,55±3,92	10,98±1,48	13,80±2,57	27,92±1,54	29,34±11,23	19,42±8,43	15,10±2,34
b*	-6,86±0,74	-3,37±1,47	0,49±0,26	-0,54±0,169	-5,64±0,52	-2,81±0,89	-2,50±1,07	-1,78±0,36
C*	25,36±1,71	24,79±4,05	10,99±1,47	13,82±2,58	28,48±1,61	29,48±11,25	19,58±8,49	15,20±2,36
h	344,30±1,31	352,37±2,40	2,71±1,60	357,74±0,51	348,59±0,55	354,36±1,30	352,64±0,55	353,32±0,61

CSC - Concord South Control; CST – Concord South Treated; ISC - Isabel Precoce South Control; IST - Isabel Precoce South Treated; CSEC - Concord Southeast Control; CSET – Concord Southeast Treated; ISEC - Isabel Precoce Southeast Control; ISET - Isabel Precoce Southeast Treated; TSS - Measured as °Brix. AT - Expressed as % w/v of tartaric acid equivalents. *Absorbance originally measured in cuvettes of 1 mm of optical path. Results are expressed as mean ± standard deviation (independent samples, N=3).

Colorimetric analysis are important for grape juice, since color is associated with quality and consumer acceptance. Colorimetric analysis showed higher differences between cultivars than treatments response. L* values (which ranges from 0 for black to 100 for white) were higher (darkening) for Isabel Precoce juice in comparison to Concord ones. We observed more red tones (a*) for Concord grape juices (Fig. 1), while Isabel Precoce grapes presents more blue tones (b*).

These differences might be due also to the anthocyanins profile, it is known that *V. labrusca* L. grapes contain 3,5-*O*-diglucoside, that present higher stability than 3-*O*-monoglucoside (Flamini, Mattivi, et al. 2013). Besides the profile between varieties, Concord grapes present derivatives of cyanidin and delphinidin (coumaroyl glucoside) and petunidin and delphinidin (acetyl glucoside) in comparison with Isabel Precoce grapes (Moro et al 2019b). We also were unable to identify cyanidin and petunidin diglucoside in Isabel Precoce grape juices (Figure 3). Combined with that, it is known that several factors including viticultural practice and geographical region exert a significant influence on phenolic composition of juices (Lima et al. 2014).

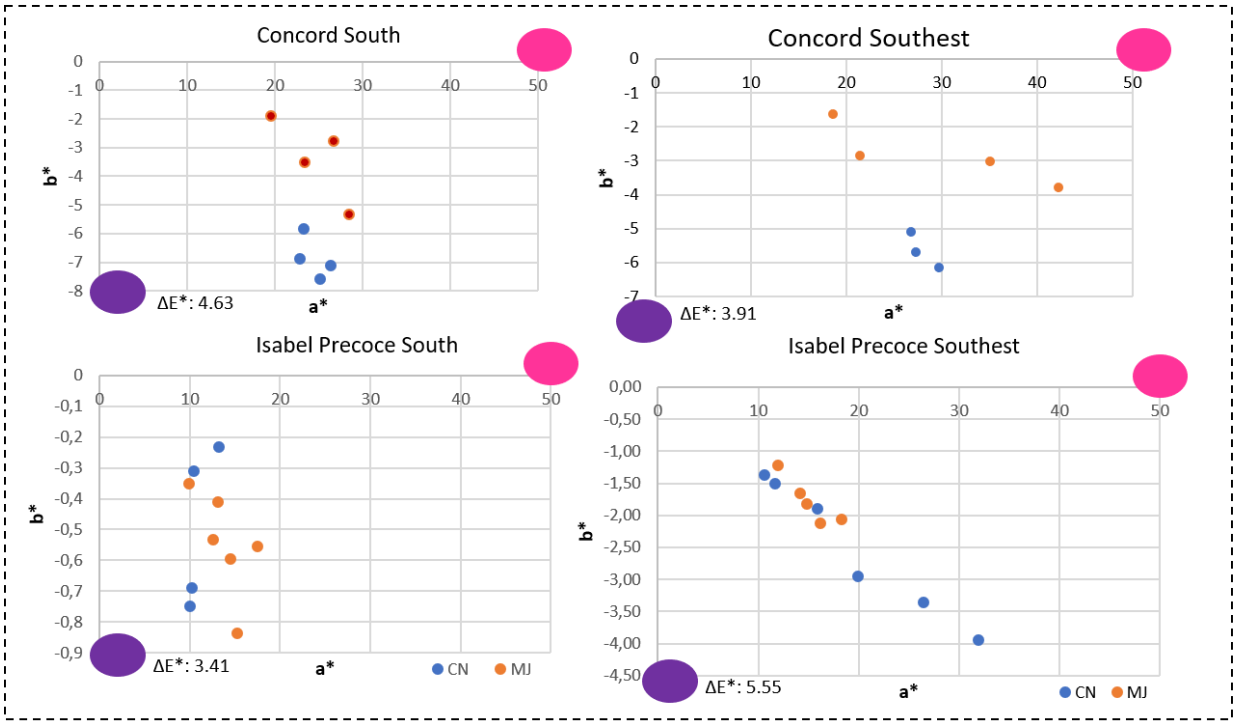

Figure 1. Colorimetric analysis of grape juice produced with MeJa treated grapes (MJ) and Control group (CN) cultivated on South and Southeast of Brazil.

The global colorimetric difference (ΔE*) between control and treated samples might be clearly distinguish by the human eye, since we should be able to distinguish samples with a ΔE* value greater than 1, while for wine its around 3-5 (Figueiredo-González et al. 2013). The average ΔE* between control and treated samples was higher than 3 for all the samples (Fig 1.), highlighting the chromatically particularity between regions and cultivars. ΔE*calculated between average CnTr and MeJa samples were, in decreasing order: Isabel P. (SE), Concord (S), Concord (SE) and Isabel P. (S).

3.2. Untarget Approach

According to preliminary data analysis, we were able to observed the good quality of the data. The tide cluster of the QC samples in the middle of all samples at the PCA plots of Fig. 2, was a first demonstration of the data set quality.

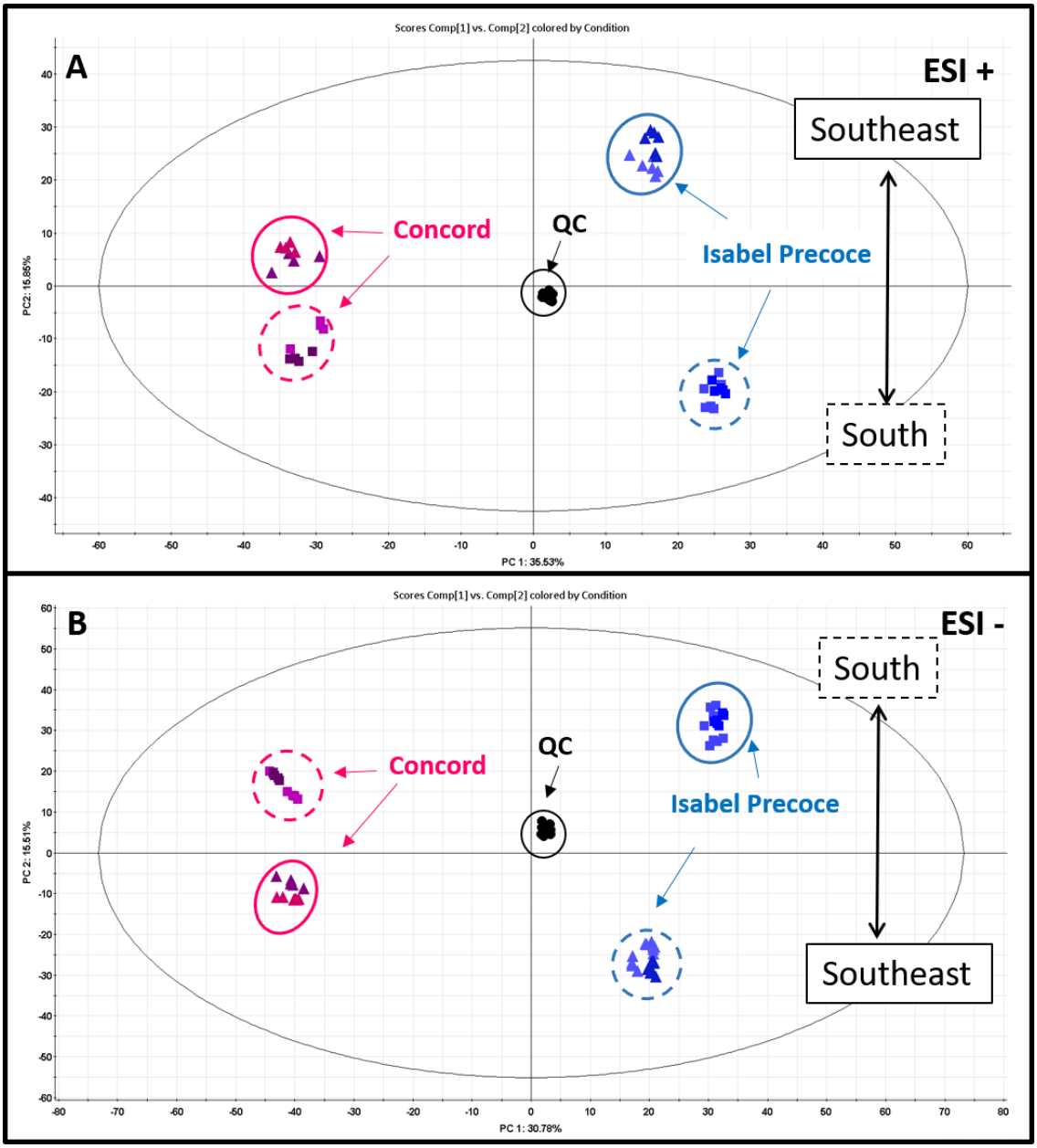

Fig 2. PCA plots of the ESI+ mode dataset (A) and ESI- mode (B). QC injections create a tide cluster, while in both cases PC1 separate the cultivars and PC2 the regions

Tentative biomarkers were considered as “compounds”, according Progenesis QI, as a group of isotopic and adducts features belonging to the same metabolite. At first sight, both PCA plots were based on the 2.632 “compounds” detected of ESI+ mode (Fig. 2A) or the 1.532 “compounds” registered at ESI- mode (Fig. 2B). Fig 2. show 4 clusters (plus the QC cluster), thus clear separation between the two cultivars

and the two regions. Therefore, we observed that the cultivar and region were by far the main factors affecting the unsupervised sample distribution, pointing out the suitability of metabolomics to support cultivar and regional identification for grape juice, as it was detected on the grapes.

3.3. Anthocyanins in grape juices MeJa treated

Anthocyanins are mainly responsible for the color of grape juice. A critical factor of quality which affects consumer acceptance and preference, high-quality grape is associated with a typical purple-red color (Danişman et al. 2015; Muche et al. 2018). On ESI positive mode we were able to identify 32 anthocyanins (Fig 3.), such cyaniding (Cy), peonidin (Pn), delphinidin (Dp), malvidin (Mv) and petunidin (Pt) in the caffeoylglucoside (CF-GLC) acetyl (AC) and *p*-coumaroyl (CM) mono (GLC) and diglucoside (DI-GLC) forms. We identified as strong biomarkers between grape juice, petunidin and cyanidin derivatives (Supplementary Table ST1).

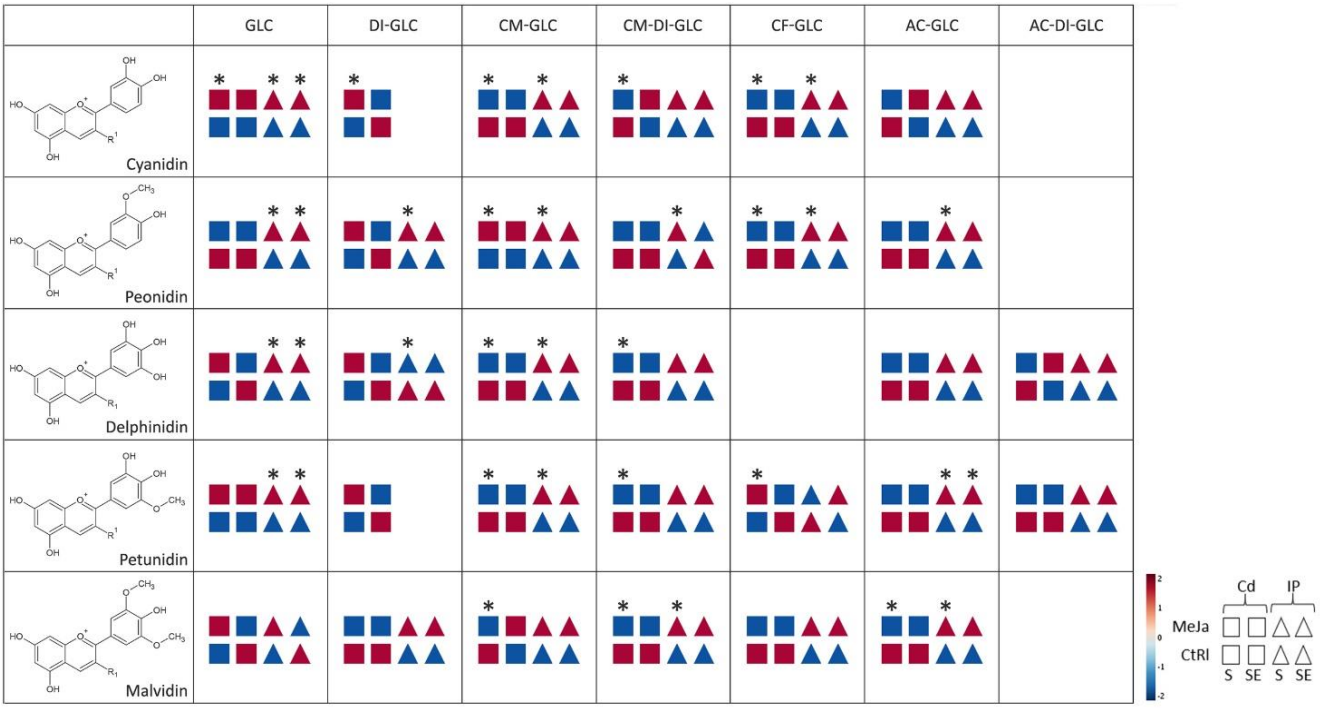

Figure 3. Annotated anthocyanins (cyanidin; peonidin; delphinidin; petunidin and malvidin) behavior in our experiment. GLC: glucoside; DI-GLC: diglucoside; AC: acetyl; CM: *p*-coumaroyl; CF: caffeoyl. MeJA: treatment; CtRl: control group; Cd: Concord; IP: Isabel Precoce; S: south region; and SE: Southeast. **p* value < 0.05.

Grape juice anthocyanins were found mainly as the 3-glucosides (86,78% of all identified anthocyanins), followed by coumaroyl forms (32% of all identified anthocyanins). The most abundant anthocyanins on Concord grapes were delphinidin,

cyanidin and petunidin, while for Isabel Precoce grapes, predominates malvidin, petunidin and peonidin. However, the profile varies within the region, e.g. cyanidin represent 39,71% of total anthocyanin detected on Concord grapes grown on the south while for southeast, it represents only 15,31%, being delphinidin the main anthocyanin detected on Concord juice from southeast (38,42%).

Higher amounts of 3' hydroxylated anthocyanins (composed by delphinidin, petunidin and malvidin, were observed on Isabel Precoce grape juices, in comparison with Concord ones, that present more hydroxylated anthocyanins (cyanidin and peonidin). This could be a variety response, through the activity of the enzyme flavonoid 3'5' hydroxylase (F3'5'OH EC: 1.14.14.81). Evaluating the ratio between petunidin and cyanidin; and malvidin and delphinidin we were able to observed the activity of the enzymes F3'H (flavonoid 3'-hydroxylase) and 5'OMT (flavonoid 5'-methyltransferase) respectively (Mattivi et al. 2006). The ratio calculated indicates higher activities with significant difference in the juice elaborated with control grapes cultivated on south region.

It is known that 3,5-diglucosides anthocyanins are a very strong characteristic of these varieties (*V. labrusca* L.) (Flamini, Mattivi, et al. 2013; Keller 2010) however, we weren't able to quantify cyanidin and petunidin diglucosides in Isabel Precoce grapes independently of the grown region (Fig 3). In our previous study with grapes (Moro et al 2019b) we were able to quantify these anthocyanins in Isabel Precoce grapes. This response might be due to the temperature used during the juice extraction. Since high temperature can cause changes in the color of the product, due to the formation of compounds as chalcones (colorless) causing loss of color due to anthocyanin degradation (Danişman et al. 2015; Lambri et al. 2015)

Beside the importance of anthocyanins when it comes to health, were is associated to biological activities as antioxidant capacity and cardiovascular disease prevention (Lima et al. 2014; Toaldo et al. 2015), they are an important quality factor for consumer acceptance and buying choice. The anthocyanin profiles found in our study were in accordance with literature reported for *V. labrusca* L. grapes (Flamini et al. 2010; Nixdorf and Hermosín-Gutiérrez 2010).

3.4. Non-anthocyanic flavonoids in grape juices MeJa treated

Flavonoids are responsible to provide important organoleptic properties such as astringency, bitterness, and body (Gil et al. 2018; Keller 2010). Among the flavanols generally related to the antioxidant activity in grape juices, compounds such as (+)-catechin, (-)-epicatechin, kaempferol, quercetin and myricetin have gained attention (Lima et al. 2015; Toaldo et al. 2015).

We were able to identify 16 non - anthocyaninc flavonoids on our study, the flavonols gallocatechin, epigallocatechin, epicatechin, catechin and 3 procyanidin and some flavonoids, including 2 myricetin, 3 quercetin, 2 kaempferol, and syringetin, besides the phenolic acid, gallic acid (Figure 4 and Supplementary Table ST2).

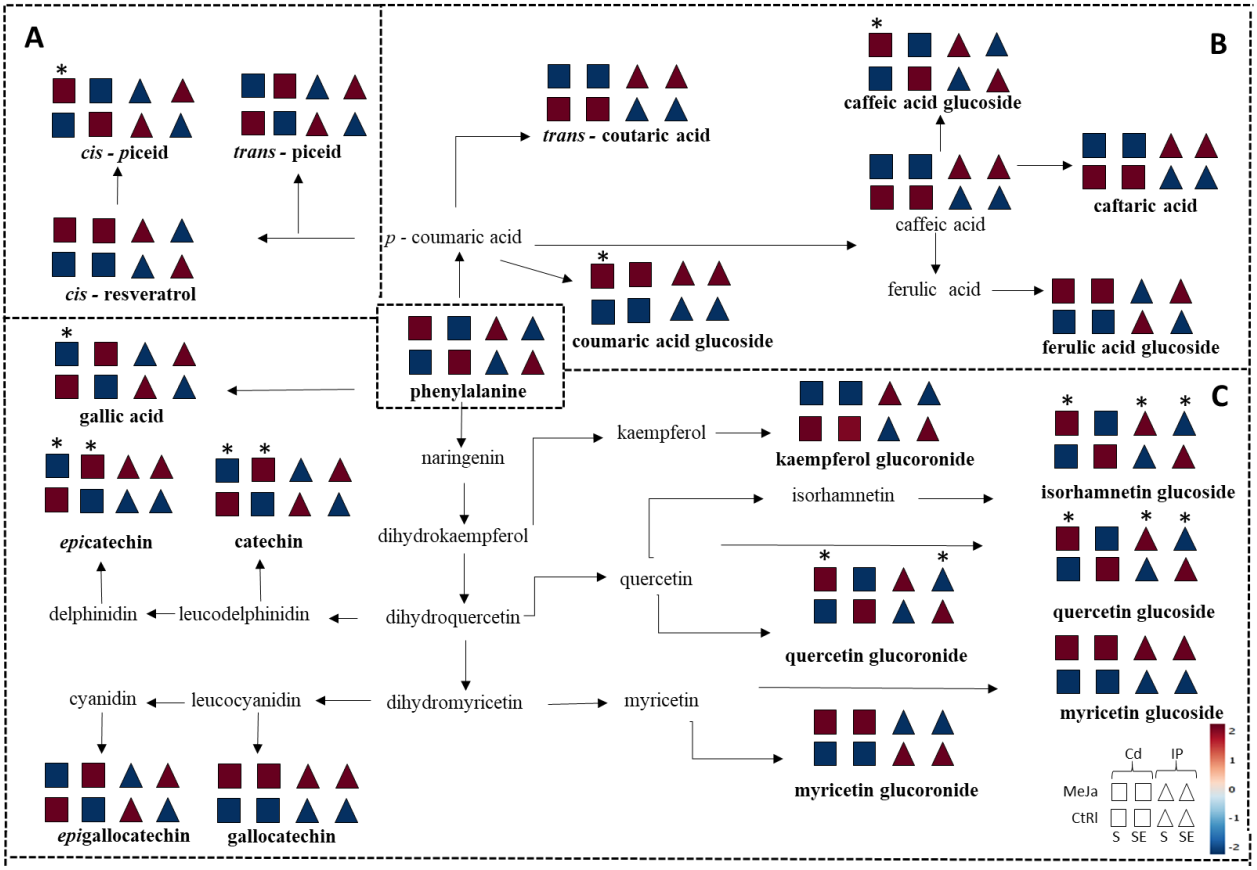

Figure 4. General metabolic pathway of non-anthocyanic phenolic compounds detected on grape juices MeJA: treatment; CtRl: control group; divided into Stilbenes (A), hydroxycinnamates (B) and flavonoids; Cd: Concord; IP: Isabel Precoce; S: southern region; and SE: Southeast. *p value < 0.05.

We observed a better response regarding MeJa treatment, in juice elaborated with Concord grapes grown on Southeast, especially regarding catechin, epicatechin

and quercetin derivatives. Interestingly, it was detected as strong biomarkers on MeJa treated juices, catechin, epicatechin and quercetin (Supplementary Table ST1).

MeJa treatment doesn't seem to affect flavonol's in studies with *V. vinifera* grapes cultivated on Europe for wine production (Portu et al. 2015, 2016; Ruiz-garc et al. 2012). It seems, that anthocyanin biosynthesis is preferentially activated, since they share most of the pathway, it might be a deviation at the pathway and an increase in the activity of enzymes upstream in the flavonoid biosynthetic pathway. Since agronomic and environmental factors strongly affect the amount and profile of flavanols in grapes (Flamini, Mattivi, et al. 2013; Portu et al. 2016; Ruiz-García et al. 2013).

3.5. Stilbenes

The main expected result following MeJa treatment it was an increase in stilbenes content. Since it was observed an increase on stilbenes content on *V. vinifera* L. grapes (Javier Portu, Pilar Santamaría, Isabel López-Alfaro, Rosa López 2015; Portu et al. 2016, 2018). Despite the thermal process used to juice production, higher stilbenes levels were observed on juices elaborated with MeJa treated grapes. We detected *cis*-resveratrol as biomarkers of MeJa Treatment (Fig 4A and Supplementary table ST1). *Cis*-resveratrol levels were higher on majority of the MeJa juices except, in Isabel Precoce grapes grown on southeast. For this cultivar higher levels of piceid were observed on MeJa treated juices, since glycosylation is a functional form to storage and translocate stilbenes (Flamini, Mattivi, et al. 2013), we believe that lower levels of *cis*-resveratrol is a consequence of the higher levels of piceid found on these juices.

3.6. Hydroxycinnamic acids

Hydroxycinnamic acid are major phenolic constituents of grape juices and white wine. Studies demonstrate its potential properties regarding heart disease-decreasing (Keller 2010). Hydroxycinnamic derivatives as caffeoyl derivatives were detected among the biomarkers (Supplementary Table ST1). We also were able to identify caffeic acid, caftaric acid and *trans*-coutaric acid (Fig 4 and Supplementary Table ST2), which presented similar patterns. In our previous study, we were able to observe that hydroxycinnamic derivatives it presents as strong biomarkers for MeJa pre-harvest treatment on grapes, it seems, that these compounds were preserved and transfer to the grape juice. For coumaric acid glucoside, we observe statistical difference for Concord grapes (Moro et al 2019b) and juice after MeJa treatment (Figure 4).

5. Conclusion

In summary, this work has evidenced that grape juice elaborated after MeJa foliar application, had a different profile regarding several compounds in comparison with non-treated ones. UPLC-HRMS-QTOF approach were very effective to differentiate grape juice from different Brazilian region, and after a supervised data analysis, we were able to identify markers on grape juice, as stilbenes (resveratrol), anthocyanin (cyanidin and petunidin derivatives) and flavanols (catechin and epicatechin) in *V. labrusca* L. grape grown on south and southeast of Brazil. It was observed that many compounds detected on grapes were effective extracted, and even after the thermal treatment, maintained as strong markers for MeJa treatment, especially for Concord grapes grown on Southeast.

Conflict of interest statement: The authors have declared no conflict of interest.

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Supplementary Material

Table S1. Tentative markers of MeJa treatment for Concord and Isabel Precoce grape juice elaborated on south and southeast regions of Brazil;

Anova (p)	Max Fold Change	CS	CSE	IS	ISE	ESI mode	m/z	Rt (min)	Annotations
8,03E-04	3,55	X		X		-	175,0243	1,22	
3,15E-06	2,01	X	X			+	274,1051	1,68	
3,20E-02	2,45	X	X			+	686,2020	1,75	
0,038221	2,21	X	X			+	686,2005	1,77	
3,74E-02	Infinity	X			X	+	233,0608	2,94	
1,98E-02	Infinity			X	X	+	308,0927	2,95	
1,22E-07	2,79			X	X	+	244,0699	3,93	
5,26E-05	2,14		X		X	+	326,0863	4,81	
5,90E-03	2,15		X		X	-	616,1091	7,60	
8,40E-04	31,28	X		X		-	355,0643	7,87	caffeoyl glucuronide derivative
3,56E-03	2,08			X	X	+	273,0784	9,68	catechin
3,30E-05	2,66	X	X			+	170,0621	10,33	
4,75E-06	3,73	X	X			-	325,0932	11,49	
0,03356	5,06	X		X		-	729,1480	11,80	dímer procyanidin gallate (mono galloylated dimer)
7,70E-04	4,48		X		X	+	475,1433	12,28	cianidin glucoside
1,83E-04	3,75		X	X		+	317,1037	12,40	
4,29E-03	3,96	X	X			-	287,0568	12,79	epicatechin
8,24E-03	2,52		X		X	+	312,0546	13,70	petunidin glucoside
4,32E-05	2,73	X			X	+	162,0433	14,52	same compound
1,88E-02	2,93			X	X	+	436,1485	14,53	same compound
1,65E-03	2,52	X	X			+	487,2170	17,85	vitisin B - malvidin-acetaldehyde
7,88E-05	2,34	X			X	-	463,0871	19,17	quercetin glucoronide
3,32E-04	2,60	X	X		X	+	403,0478	19,44	
3,13E-02	7,81	X	X		X	+	951,1838	19,44	
2,05E-08	Infinity			X	X	-	562,0047	19,47	quercetin glucoside
3,74E-05	2,54			X	X	+	383,1487	20,04	rutin
5,87E-05	3,17	X			X	+	471,0912	20,06	
1,69E-04	3,80	X			X	-	447,0923	20,11	Kaempferol-related
3,27E-03	2,39	X		X		-	641,1512	20,20	
6,69E-08	2,13	X			X	+	471,0925	20,22	epigallocatechin_catechin? Gallocatechin_epicatechin
1,64E-02	2,08	X	X			+	229,0873	20,39	**resveratrol**
2,11E-03	2,68			X	X	-	227,0719	20,44	**resveratrol**
0,00E+00	Infinity	X	X			-	345,2278	20,84	
8,84E-05	2,70			X	X	+	295,2286	20,95	
2,78E-03	36,93			X	X	+	371,2419	20,95	
3,20E-07	2,95	X		X	X	+	275,2021	21,32	Unknown same marker id found in grapes
2,37E-07	2,37			X	X	+	351,2151	21,32	Unknown same marker id found in grapes
3,03E-06	2,51	X	X			-	327,2176	21,35	Unknown same marker id found in grapes
3,59E-06	2,15	X			X	-	329,2333	21,48	Unknown same marker id found in grapes

Table S2. Identified markers for Concord and Isabel Precoce grape juice elaborated on south and southeast regions of Brazil;

Compound	Level of identification*	HMDB	KEGG	Chemspider ID	Molecular Formula	RT (min)	Monoisotopic mass (Da)	m/z ESI (+)	Error (ppm)
Anthocyanins									
delphinidin 3-glucoside	1		C12138	391783	$C_{21}H_{21}O_{12}$	11,30	465,1028	465,1025	0,54
cyanidin 3-glucoside	1		C08604	390284	$C_{21}H_{21}O_{11}$	12,73	449,1078	449,1084	-1,23
petunidin 3-glucoside	1		C12139	391784	$C_{22}H_{23}O_{12}$	13,28	479,1184	479,1184	0,02
peonidin 3-glucoside	1		C12141	391786	$C_{22}H_{23}O_{11}$	14,56	463,1235	463,1215	4,26
malvidin 3-glucoside	1		C12140	391785	$C_{23}H_{25}O_{12}$	14,80	493,1341	493,1343	-0,48
delphinidin 3,5-diglucoside	1		C16312	8276439	$C_{27}H_{31}O_{17}$	7,66	627,1556	627,1582	-4,18
cyanidin 3,5-diglucoside	1		C06361	30777615	$C_{33}H_{31}O_{16}$	9,13	611,1607	611,1624	-2,78
petunidin 3,5-diglucoside	1			32697757	$C_{28}H_{33}O_{17}$	9,64	641,1712	641,1727	-2,34
peonidin 3,5-diglucoside	1			4589999	$C_{28}H_{33}O_{16}$	10,95	625,1763	625,1783	-3,15
malvidin 3,5-diglucoside	1		C08718	390365	$C_{29}H_{35}O_{17}$	11,32	655,1869	655,1890	-3,22
delphinidin 3-(6''-acetyl)-glucoside	2			30777226	$C_{23}H_{23}O_{13}$	16,83	507,1133	507,1113	3,97
cyanidin 3-(6''-acetyl)-glucoside	1			30780060	$C_{23}H_{23}O_{12}$	18,21	491,1184	491,1191	-1,41
malvidin 3-(6''-acetyl)-glucoside	2	HMDB0038008		24842428	$C_{25}H_{27}O_{13}$	19,51	535,1446	535,1467	-3,94
peonidin 3-(6''-acetyl)-glucoside	1			24842289	$C_{24}H_{25}O_{12}$	19,56	505,1341	505,1344	-0,67
petunidin 3-(6''-acetyl)-glucoside	1			30779241	$C_{24}H_{25}O_{13}$	18,40	521,1290	521,1290	-0,06
delphinidin 3-(6''-acetyl)-diglucoside	2				$C_{29}H_{33}O_{18}$	11,36	669,1661	669,1669	-1,20
petunidin 3-(6''-acetyl)-diglucoside	2				$C_{30}H_{35}O_{18}$	13,13	683,1818	683,1833	-2,20
delphinidin 3-(6''-*p*-coumaroyl)-glucoside	1		C16370	26559505	$C_{30}H_{27}O_{14}$	19,96	611,1395	611,1408	-2,08
cyanidin 3-(6''-*p*-coumaroyl)-glucoside	1		C12095	4445294	$C_{30}H_{27}O_{13}$	20,15	595,1446	595,1463	-2,87
petunidin 3-(6''-*p*-coumaroyl)-glucoside	1	HMDB0038100		30779240	$C_{31}H_{29}O_{14}$	20,16	625,1552	625,1542	1,62
peonidin 3-(6''-*p*-coumaroyl)-glucoside	1			24842291	$C_{31}H_{29}O_{13}$	20,32	609,1603	609,1612	-1,51
malvidin 3-(6''-*p*-coumaroyl)-glucoside	1			24842430	$C_{17}H_{15}O_{7}$	20,26	639,1708	639,1707	0,16
delphinidin 3-(6''-*p*-coumaroyl)-diglucoside	2				$C_{36}H_{37}O_{19}$	17,38	773,1924	773,1906	2,27
cyanidin 3-(6''-*p*-coumaroyl)-diglucoside	2				$C_{36}H_{37}O_{18}$	18,63	757,1974	757,1964	1,37
petunidin 3-(6''-*p*-coumaroyl)-diglucoside	2				$C_{37}H_{39}O_{19}$	18,72	787,2080	787,2083	-0,37

Table S2. Identified markers for Concord and Isabel Precoce grape juice elaborated on south and southeast regions of Brazil;										
Compound	***Level of identification**	**HMDB**	**KEGG**	**Chemspider ID**	**Molecular Formula**	**RT (min)**	**Monoisotopic mass (Da)**	**m/z ESI (+)**	**Error (ppm)**	**Theoretical mass (Da)**
Anthocyanins										
peonidin 3-(6''-*p*-coumaroyl)-diglucoside	2				$C_{31}H_{29}O_{18}$	19,71	609,1603	609,1583	3,28	
malvidin 3-(6''-*p*-coumaroyl)-diglucoside	2				$C_{38}H_{41}O_{18}$	19,67	801,2237	801,2252	-1,87	
cyanidin 3-(6''caffeoyl)- glucoside	2				$C_{30}H_{27}O_{14}$	19,95	611,1395	611,1415	-3,22	
petunidin 3-(6''caffeoyl)- glucoside	2				$C_{31}H_{29}O_{15}$	20,05	641,1501	641,1518	-2,66	
peonidin 3-(6'' caffeoyl)- glucoside	2				$C_{31}H_{29}O_{14}$	20,16	625,1552	625,1553	-0,19	
malvidin 3-(6''caffeoyl)- glucoside	2				$C_{32}H_{31}O_{15}$	20,00	655,1657	655,1682	-3,82	
Amino acids										
arginine	1			6082	$C_6H_{14}N_4O_2$	1,15	174,1117	175,1198	-1,14	175,1196
proline	1			594	$C_5H_9NO_2$	1,15	115,0633	116,0718	-4,31	116,0713
phenylalanine	1		C00079	5910	$C_9H_{11}NO_2$	6,00	165,0790	166,0865	2,41	166,0869
tryptophan	1		C00078	6066	$C_{11}H_{12}N_2O_2$	8,33	204,0899	205,0980	-0,98	205,0978

Table S2. Identified markers for Concord and Isabel Precoce grape juice elaborated on south and southeast regions of Brazil;

Compound	*Level of identification	HMDB	KEGG	Chemspider ID	Molecular Formula	RT (min)	Monoisotopic mass (Da)	Theoretical mass (Da)	m/z ESI (-)	Error (ppm)
Flavanols										
gallocatechin	1	HMDB38365	C12127	58594	$C_{15}H_{14}O_7$	6,45	306,0739	305,0660	305,0670	-3,13
procyanidin B1	1	HMDB29754		9425166	$C_{30}H_{26}O_{12}$	8,30	578,1425	577,1346	577,1356	-1,81
epigallocatechin	1	HMDB38361	C12136	65231	$C_{15}H_{14}O_7$	9,51	306,0739	305,0660	305,0651	3,09
procyanidin B4	1	HMDB13690	C129882	129882	$C_{30}H_{26}O_{12}$	12,30	578,1425	577,1346	577,1333	2,18
catechin	1	HMDB02780	C06562	8711	$C_{15}H_{14}O_6$	9,70	290,0790	289,0711	289,0718	-2,28
procyanidin B2	1	HMDB33973		4478723	$C_{30}H_{26}O_{12}$	10,57	578,1425	577,1346	577,1349	-0,60
epicatechin	1	HMDB01871	C65230	65230	$C_{15}H_{14}O_6$	12,70	290,0790	289,0711	289,0720	-2,97
Flavonoids										
myricetin-3-glucoside	1	HMDB0034359		4588987	$C_{21}H_{20}O_{13}$	17,12	480,0904	479,0825	479,0804	4,37
myricetin-3-glucuronide	1			4589353	$C_{21}H_{18}O_{14}$	16,65	494,0696	493,0617	493,0626	-1,74
quercetin-3-glucuronide	1			18699310	$C_{21}H_{18}O_{13}$	19,10	478,0747	477,0668	477,0665	0,71
quercetin 3-glucoside	1	HMDB0037362	C05623	24773541	$C_{21}H_{20}O_{12}$	19,48	464,0955	463,0876	463,0854	4,73
syringetin-3-glucoside	1			16736532	$C_{23}H_{24}O_{13}$	20,34	508,1217	507,1138	507,1158	-3,94
kaempferol 3-glucuronide	1	HMDB0029500		18699309	$C_{21}H_{18}O_{12}$	20,20	462,0798	461,0719	461,0699	4,41
kaempferol 3-glucoside	1		C12249	4445311	$C_{21}H_{20}O_{11}$	20,25	448,1006	447,0927	447,0928	-0,32
quercetin-3-rutinoside	1	HMDB0003249		4444362	$C_{27}H_{30}O_{16}$	20,35	610,1530	609,1451	609,1438	2,13
Stilbenoids										
trans-piceid	1	HMDB0030564	C10275	4445034	$C_{20}H_{22}O_8$	15,85	390,1315	389,1236	389,1241	-1,36
cis-piceid	1	HMDB0031422		8353968	$C_{20}H_{22}O_8$	19,75	390,1315	389,1236	389,1231	1,21
cis-resveratrol	1	HMDB0034118		1265933	$C_{14}H_{12}O_3$	20,44	228,0786	227,0707	227,0714	-2,89

Table S2. Identified markers for Concord and Isabel Precoce grape juice elaborated on south and southeast regions of Brazil;

Compound	*Level of identification	HMDB	KEGG	Chemspider ID	Molecular Formula	RT (min)	Monoisotopic mass (Da)	Theoretical mass (Da)	m/z ESI (-)	Error (ppm)
Phenolic acids										
gallic acid	1	HMDB05807	C01424	361	$C_7H_6O_5$	4,85	170,0215	169,0136	169,0144	-4,56
Cinnamics										
caffeic acid	1	HMDB03501		600426	$C_9H_8O_4$	11,83	180,0423	179,0344	179,0347	-1,68
trans-caftaric acid	1	HMDB0013680		4944664	$C_{13}H_{12}O_9$	7,70	312,0481	311,0402	311,0410	-2,49
cis-coutaric acid	1			35013748	$C_{13}H_{12}O_8$	9,32	296,0532	295,0453	295,0464	-3,65
trans-coutaric acid	1			26325199	$C_{13}H_{12}O_8$	9,70	296,0532	295,0453	295,0453	0,08
p-Coumaric acid glucoside	1		C04415	8016010	$C_{15}H_{18}O_8$	10,74	326,1002	325,0923	325,0926	-1,05
caffeic acid 3-glucoside	1		C10431	4445073	$C_{15}H_{18}O_9$	8,70	342,0951	341,0872	341,0879	-2,07
ferulic acid O-glucoside	2			10212167	$C_{16}H_{20}O_9$	11,83	356,1107	355,1028	355,1019	2,59
*According to Sumner et al. (2007).										

SESSÃO 3

Improving stilbenes content on grape and grape juice trough Methyl jasmonate pre-harvest treatment on Brazilian regions

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1. Introduction

Brazilian grape production is developed in many states, particularly in Rio Grande do Sul state at Southern region, that concentrate about 90% of total production (Toaldo et al. 2015). However, many other regions are being described by their potential for grapevine cultivation (Alves Filho et al. 2019; da Silva et al. 2019). The southeast region has been highlighted on this contest, with wine and grape juice production (da Mota et al. 2018; da Silva et al. 2019; Regina, M.A., Mota, R.V., Souza, C.R. and Favero 2011). On this scenario, non vinifera grapes (*Vitis labrusca* and its hybrids [*V. vinifera* x *V. labrusca*]) represent an important part of the total processed grapes, e.g. grape juice represents 49% of the total grape processed, whose main varieties are 'Isabel', 'Concord' and ''Ives' (Antonio et al. 2020; da Mota et al. 2018; Toaldo et al. 2015).

Grape juice consumption increased 23.28% on the past year (2017/2018) in Brazil, being natural/integral the major form produced (IBRAVIN, 2019). Grape juice is considered a very rich source of polyphenols, such as flavonoids, tannins, and stilbenes. Among these compounds, we highlight stilbenes, for being associated with the beneficial effects of drinking wine. On grapes, the main stilbenes are *cis*- and *trans*-resveratrol (3,5,4'-trihydroxystilbene), it's glucoside derivative, piceid (resveratrol-3-O-β-D-glucopyranoside), piceatannol (3,4,3',5'-tetrahydroxy-trans-stilbene) and resveratrol dimers (viniferins) (Bavaresco et al. 2016; Flamini, Mattivi, et al. 2013). However, grape biproducts phenolic composition is affected by many factors, as cultivar, vintage, environment, cultural practices and method of processing (Flamini, Mattivi, et al. 2013; Toaldo et al. 2015; Waterhouse, Andrew L. Gavin L. Sacks 2016).

The increase in public awareness on food safety and environment health, encourage researches independent of GMO's (genetically modified organism) and heavy chemical applications to increase and stimulate plant secondary metabolism. On this behalf, methyl jasmonate (MJ) are a natural plant phytochemical, considered as generally regarded as safe compounds (GRASC) (Asghari 2019).

In previous studies with MJ application on *V. vinifera* grapes cultivated on Europe a positive response was observed regarding grape secondary metabolism (Portu et al. 2015; Ruiz-García et al. 2013; Vezzulli et al. 2007). Our previous work demonstrates that a similar response was observed regarding volatile compounds on Brazilian subtropical climate (Moro et al. 2019).

We speculate that these compounds highlighted by MJ treatment might be transferred to grape biproducts, as grape juice, resulting on a beverage with higher stilbene content, a non-alcoholic beverage that might be consume by youngers, elders and those who doesn't like, want or can't drink wine.

2. Material and Methods

2.1. Plant material and experimental design

MJ application was conducted in 2016/2017 and 2018/2019 in Isabel Precoce and Concord (*V. labrusca* L.) grapes grafted onto *1103-Paulsen*, into two different Brazilian regions. On the south region, the experiments were performed in Bento Gonçalves (RS) (29° 10′ 26″ S e 51° 31′ 7″ W, 671 meters above sea level) in a commercial vineyard, trained on tendone system with a between row and within-row spacing of 2.8 × 1.5 m, respectively. In the southeast region, the experiment was conducted in Caldas (MG) (21° 55' 23'' S e 46° 23' 15'' W, 1.131 meters above sea level), vines were trained on vertical shoot positioning system with a between-row and within-row spacing of 2.5 × 1.5 m.

Weather conditions were recorded and collected by the Instituto Nacional de Meteorologia (http://www.inmet.gov.br/portal/).

For each region/cultivar two blocks were used, control versus treatment. For the south region, each block comprised 10 vines, while for southeast 18 vines. Each plant represented a biological replicate. The MeJa solution was prepared and applied as described in Moro et al (2019), a chronogram with the treatment application is presented on Figure 1.

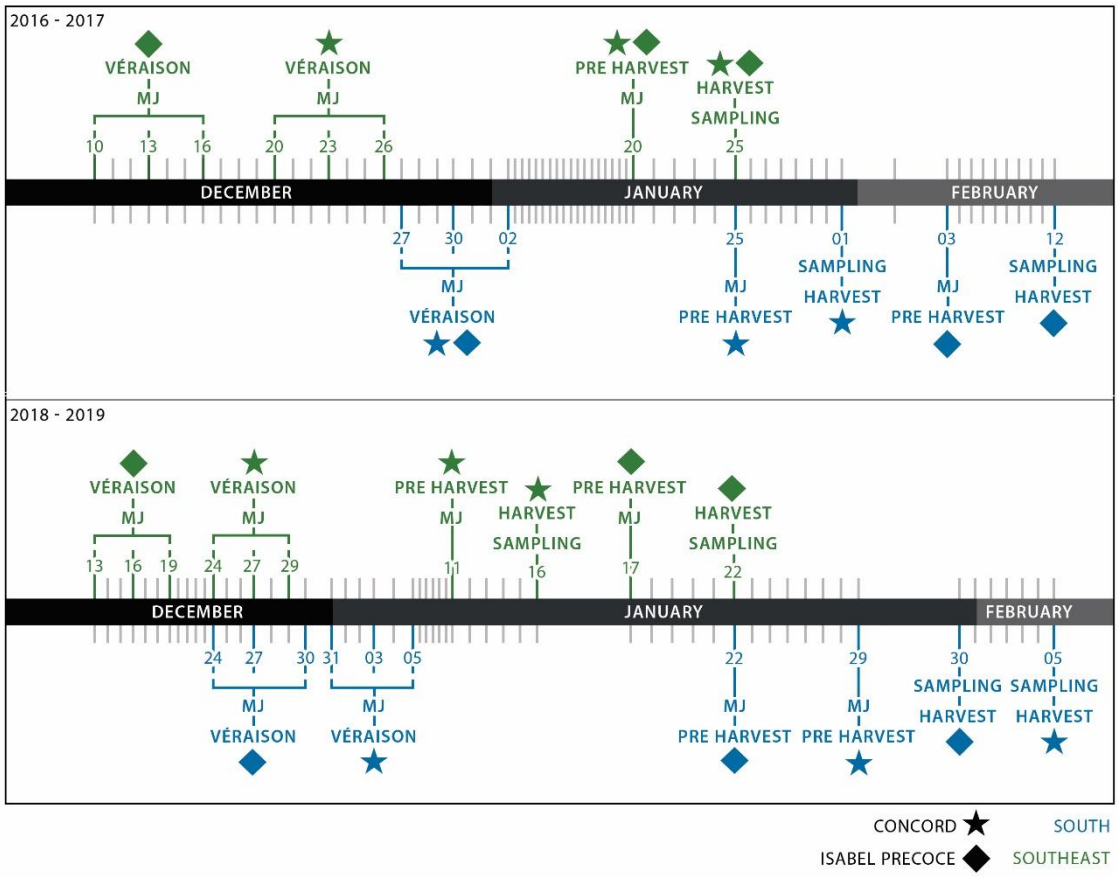

Figure 1: Chronogram of treatment application on Concord and Isabel Precoce grapes grown on South (Bento Gonçalves/RS) and Southeast (Caldas/MG) Brazil.

Random grapes (total of 100 berries) were harvested at maturity for each cultivar, treatment, and region. Each sample was hand pressed and the musts were analyzed for pH, titratable acidity and soluble solids (Amerine and Ough 1980). Another randomized samples of 40 berries were collected. Skins and seeds were separated manually, weighed, crushed in liquid nitrogen and stored at -80 °C for further analyses.

2.2. Grape juice processing

The juice extraction was made by steam juice extractor cooker (Stamp Inox, Caxias do Sul, RS, Brazil). The juice samples were produced from grapes MJ treated and control groups, the grapes were hand stripped, and 10 kg of berries were placed in the extraction chamber. Juice extraction was accomplished with water vapor during 75 minutes (Motta et al., 2018). The juice was bottled at 80 °C with no addition of preservatives, in glass bottles previously sterilized, with closure with screw caps, being carried out triplicate of processing, generating 3 lots for each block. Juice samples

were storage and evaluated per 6 months after processing during both years (2017 and 2019 harvest).

2.3. Basic oenological analysis

The titratable acidity, pH, and total soluble solids (SS) of juices were measured according to the (Amerine and Ough 1980).

2.4. Stilbenes extraction and quantification

Stilbenes were extracted from grapes peels and seeds as described by (Liu et al. 2013). For grape juice, the extraction was performed according to Liu et al (2013) with some modifications. Grape juice (2 mL) were extracted with methanol:ethyl acetate (50/50; v/v) three times, and centrifuged for 5 minutes at 7000 RPM, the supernatant were combined and completely dry under vacuum (Rotavapor, RE 120, Büchi, Flawil, Suíça) at 40 ºC. Afterwards, it was resuspended with 1 mL of methanol and filtered through a 0.45 mm Millipore filter. *Trans*-resveratrol and piceid were quantified by analytical reversed-phase liquid chromatography (Agilent Technologies, model 1260 Infinity) equipped with a DAD. The column used was Prodigy 5 ODS3 reversed-phase silica (250 mm x 4.6 mm, i.d., 5 µm, Phenomenex Ltd., Torrance, CA), using a gradient program as described by Liu et al (2013). Phenolic compounds were identified according to the retention times and the UV–Vis data obtained from authentic standards of trans-resveratrol and piceid (Extrasynthese, Genay, France). The results were expressed as mg/100g grape fresh weight (f.w).

2.5. Statistical Analysis

Grape stilbene content and basic enological juice analysis were expressed by means ± standard deviation (SD, n=6). Statistical analysis was performed using Graph Pad Prism software version 5.01 (GraphPad Software, CA, USA). Differences among treatments were analyzed by ANOVA and the Student–Newman–Keuls test, assuming a significance level of 0.05

3. Results and discussion

Grapes were harvested at commercial maturity, according to the viticultural parameter used at the growing region (Supplementary Table ST1). In general, 2019 harvest present better-quality grapes than 2017, being the mean of total soluble solids (SS) for 2019 harvest, a slightly higher than 2017. Since our study was conducted on

the same vineyard on both regions for both years, the vines and the soil can be considered constant. These variation in the grapevine behavior and grape ripening strongly reflect the effect of weather, being strongly responsible for the “vintage effect” (de Oliveira et al. 2019).

During both harvests, temperature, relative humidity and rainfall were monitored (Table 1).

Table 1: Climatic conditions during 2016 to 2017; and 2018 to 2019, on of South (Bento Gonçalves/RS) and Southeast (Caldas/MG), Brazil

	Temperature (ºC)				**Relative Humidity (%)**				**Precipitation (mm)**			
	South		Southeast		South		Southeast		South		Southeast	
	2017	2019	2017	2019	2017	2019	2017	2019	2017	2019	2017	2019
OT	17,2	17,2	19,6	20,1	76,1	78,4	76,0	81,2	373	221	231	189
NV	18,6	20,1	19,5	19,7	70,9	71,6	80,2	83,4	104	191	204	171
DE	21,6	21,2	20,8	20,6	73,2	72,4	79,1	79,5	81	134	150	15
JA	22,2	23,6	20,8	21,4	77,7	77,1	84,2	79,5	147	138	91	146
FE		21,1		20,9		76,9		82,5		128		246

Table 1: Mean temperature (ºC), mean relative humidity (RU %), mean precipitation (mm), of South (Bento Gonçalves/RS) and Southeast (Caldas/MG), Brazil, from 2016 to 2017; and 2018 to 2019. Ot-October, Nv-November, De-December. Ja-January, Fe-February.

On both regions budding started on September, followed by flowering on October, berry development in November and *véraison* in December. During the treatment application (Fig. 1), at *véraison*, it can be observed a minor decrease on temperature for both regions, while rainfall present an opposite behavior in 2019 harvest in comparison with 2017, higher rainfall were observed on south region (2019). However, during ripening (December - January) 2019 presented higher temperatures on both regions. This data is very important for better understanding the berry behavior, especially because climate is an environmental aspect that directly impacts the fruit during ripening to its optimum quality to produce a desirable product (Gerós and Delrot 2016).

3.1. Grape juice basic oenological analysis

The physico-chemical characteristics of juices prepared with grapes from different cultivars are indicated on Table 2. The highest soluble solids (SS) values were obtained from grapes cultivated on south on both harvests. Grape juices produced from grapes grown on southeast present lower levels of SS, above the minimum limit of 14.0 °Brix, established by Brazilian regulations for integral grape juices (Brasil,

2000). This might be a response due the grape juice processing, by stream extraction, since with this method, between 8 and 17% more water might be transferred to the juice, comparing to other processing methods. Besides, plasmolysis of the membrane and rupture on the berry cell wall might occur, as a result of the higher temperatures used, facilitating the liberation of water (da Mota et al. 2018; Guerra 2016).

Grape juice organoleptic characteristics are strongly inflected by SS, pH, and acidity. The main component of volatile acidity is acetic acid, it's an indicator of grape quality procedure since higher concentrations are typically a result of bacterial spoilage (Waterhouse, Andrew L. Gavin L. Sacks 2016).

Our results are in agreement with literature reports for *Vitis labrusca* L. varieties (da Mota et al. 2018; Margraf et al. 2016). Regarding MeJa treatment influence on physicochemical characteristics, we didn't observe any significative difference, being the main variation due to harvest and growing region.

Table 2: Grapes juices physicochemical characteristics on 2017 and 2019 harvest.

			2017 Harvest				***2019 Harvest***			
Region	**Cultivar**	**Treatment**	**SS**	**pH**	**Acidity**	**AT (g/L)**	**SS**	**pH**	**Acidity**	**AT (g/L)**
South	Concord	CT	14.50± 0.14	3.45± 0.01	8.30± 0.28	12.57± 0.43	14.72 ± 0.15	3.47 ± 0.01	6.90 ± 0.01	5.85± 0.13
		MJ	10.25± 0.35	3.47± 0.01	6.60± 0.21	10.00± 0.32	13.53 ± 0.10	3.38 ± 0.02	6.15 ± 0.01	6.15± 0.30
	Isabel P.	CT	14.03± 0.68	3.44± 0.02	6.21± 0.44	9.40± 0.66	14.20 ± 0.20	3.43 ± 0.01	5.93 ± 0.11	5.93 ± 0.11
		MJ	13.90± 0.36	3.49± 0.01	5.42± 0.25	8.21± 0.37	14.28 ± 0.39	3.45 ± 0.03	6.15 ± 0.40	6.15± 0.40
Southeast	Concord	CT	11.20± 0.14	3.39± 0.06	5.20± 0.28	7.88± 0.43	10.60 ± 0.12	3.33 ± 0.01	5.25 ± 0.03	7.95 ± 0.13
		MJ	9.80± 0.14	3.30± 0.00	5.20± 0.14	7.88± 0.21	10.00 ± 0.51	3.29 ± 0.04	5.05 ± 0.60	7.65 ± 0.91
	Isabel P.	CT	10.33± 0.31	3.35± 0.02	4.58± 0.03	6.94± 0.04	9.00 ± 0.74	3.27 ± 0.03	3.75 ± 0.26	5.68 ± 0.40
		MJ	10.67± 0.21	3.30± 0.02	4.60± 0.09	6.97± 0.13	9.00 ± 0.46	3.36 ± 0.01	3.55 ± 0.13	5.78 ± 0.20

Table 2: Physicochemical characteristics (soluble solids, pH, acidity, titratable acidity) of grapes juices on Concord and Isabel Precoce grapes grown on South (Bento Gonçalves/RS) and Southeast (Caldas/MG) Brazil.

3.2. Stilbene analysis

Stilbene content, was analyzed separately for the seed and peel of grapes, since they are the main source of stilbenes (Santos et al. 2011). Seeds content of *trans*-resveratrol is presented on Table 2. We observed that Concord grapes present higher content in comparison with Isabel Precoce, independently of the growing region.

Table 3: Concord and Isabel Precoce grapes seed *trans*-resveratrol quantification

Region	Cultivar	Treatment	2017 harvest	2019 harvest
South	Concord	CT	0.29 ± 0.08	1,57 ± 0,42
		MJ	0.30 ± 0.08	0,64 ± 0,13
	Isabel P.	CT	0.07 ± 0.04	0,15 ± 0,05
		MJ	0.14 ± 0.01 *	0,18 ± 0,02
Southeast	Concord	CT	0.26 ± 0.02	0,83 ± 0,12
		MJ	0.37 ± 0.08	1,03 ± 0,31
	Isabel P.	CT	0.16 ± 0.04	0,16 ± 0,05
		MJ	0.21 ± 0.05	0,19 ± 0,05

Table 3: *trans*-resveratrol quantification on grapes seed of Concord and Isabel Precoce grapes grown on South (Bento Gonçalves/RS) and Southeast (Caldas/MG) of Brazil. Results expressed as mean (mg of *trans*-reveratrol/100g seed f.w) ± standard deviation. CT: control; MJ: Methyl jasmonate (n=6); '*' represent significative difference between samples * $p < 0.05$, in comparison with CT group.

Besides the discrete increase of *trans*- resveratrol content at some groups, for 2017 harvest, we observed a significative difference for Isabel Precoce grapes grown on south, while for 2019 harvest, it was not observed any significant difference between control and treated grapes.

Since the peel present a larger concentration of stilbenes (Santos et al. 2011), we evaluated *trans*-resveratrol and their glucoside derivative piceid content (Table 4). For Concord grapes from both regions, we weren't able to detect piceid on 2017 harvest, while for Isabel Precoce grapes, a lower content was detected on 2019 in comparison with the previous harvest.

Considering *trans*-resveratrol quantification of the seed (table 3), peel results are in agreement, presenting higher content on MJ treated grapes in comparison with CT group.

2017 harvest

Region	Cv.	Treatment	*trans*-resveratrol		*piceid*
South	Concord	CT	0.12 ± 0.01		a.l.d
		MJ	0.57 ± 0.09	**	a.l.d
	Isabel P.	CT	0.26 ± 0.10		1.45 ± 0.08
		MJ	2.22 ± 0.25	***	1.57 ± 0.10
Southeast	Concord	CT	2.46 ± 0.15		a.l.d
		MJ	1.81 ± 0.54	**	a.l.d
	Isabel P.	CT	2.93 ± 0.18		1.20 ± 0.54
		MJ	3.00 ± 0.43		0.96 ± 0.16

2019 harvest

Region	Cv.	Treatment	*trans*-resveratrol		*piceid*
South	Concord	CT	1.70 ± 0.72		1.09 ± 0.71
		MJ	2.02 ± 0.87		0.99 ± 0.25
	Isabel P.	CT	1.37 ± 0.04		0.68 ± 0.06
		MJ	2.16 ± 0.27		0.11 ± 0.05
Southeast	Concord	CT	0.67 ± 0.08		1.24 ± 0.13
		MJ	0.93 ± 0.08	*	0.55 ± 0.19
	Isabel P.	CT	0.14 ± 0.05		0.04 ± 0.02
		MJ	0.41 ± 0.25		0.12 ± 0.02

Table 4: Stilbenes quantification on peel grapes of Concord and Isabel Precoce grapes grown on South (Bento Gonçalves/RS) and Southeast (Caldas/MG) Brazil harvest on 2017 and 2019. Results expressed as mean (mg/100g peel f.w) ± standard deviation. CT: control; MJ: Methyl jasmonate (n=6); '*' represent significative difference between samples * $p < 0.05$, in comparison with CT group.

The peel, of grapes cultivated on southeast (2017 harvest), presented higher content of *trans*-resveratrol than those cultivated at the south region, however, the opposite behavior was perceived for 2019 harvest.

After evaluating the climatic conditions data, we speculate that these profiles might be correlated with the precipitation levels observed on these years. Since stilbenes are phytoalexins, their content increases from *véraison* to ripening, being directly related to the plants defense system used against abiotic and biotic stresses (Flamini, Mattivi, et al. 2013; Romero et al. 2018). Therefore, higher stilbenes concentrations were expected, were the climatic condition were favorable for the development of infection and pathogens. This stimulus it will not be present on a microclimate with lower precipitation, as it was detected in December on 2017 and 2019, with only 81 and 15 mm of rainfall, respectively. Since many infections are stimulated, and fungus germinate when relative humidity exceeds 90%(Keller 2010).

Regarding MJ treatment response, the results agree with the positive results observed on *Vitis vinifera* L. cultivars in Europe (Javier Portu, Pilar Santamaría, Isabel

López-Alfaro, Rosa López 2015; Portu, Santamaría, et al. 2015; Vezzulli et al. 2007). Our study suggests, that this response is preserved between species, and besides the difference belonging to the characteristics of the studied regions, the treatment was effective to enhance stilbene content on Concord and Isabel Precoce grapes.

Aiming at elaborating a non-alcoholic beverage with higher stilbene content, we extracted the juice from grapes MJ treated and CT group harvested on 2017. To better understand stilbenes behavior during grape juice storage, we evaluated their content after the production (Initial), after 3 and 6 months of storage. The juices were kept in the dark, at room temperature during this period.

South (Fig 2-A) and Southeast (Fig 2-B) grape juice show different *trans*-resveratrol profiles between regions. While higher *trans*-resveratrol content is observed on initial evaluation for juices produced from grapes cultivated on south, those juices elaborated with grapes cultivated on southeast presents higher levels after storage. This behavior was observed even on the same variety e.g. the juice elaborated with Concord grapes cultivated on the south, had their content diminished during storage, while for southeast ones, higher content is observed even after 3 months of storage.

Altogether, Isabel Precoce grape juice presented higher levels of *trans*-resveratrol than those produced with *Concord* (Fig 2.), and a better maintenance during storage was observed, especially for the juice elaborated with southeast grapes.

The positive effect of MJ treatment in *trans*-resveratrol content was detected mostly on the peel of the grapes (Table 4) and maintained even after the thermal treatment and 6 months of storage.

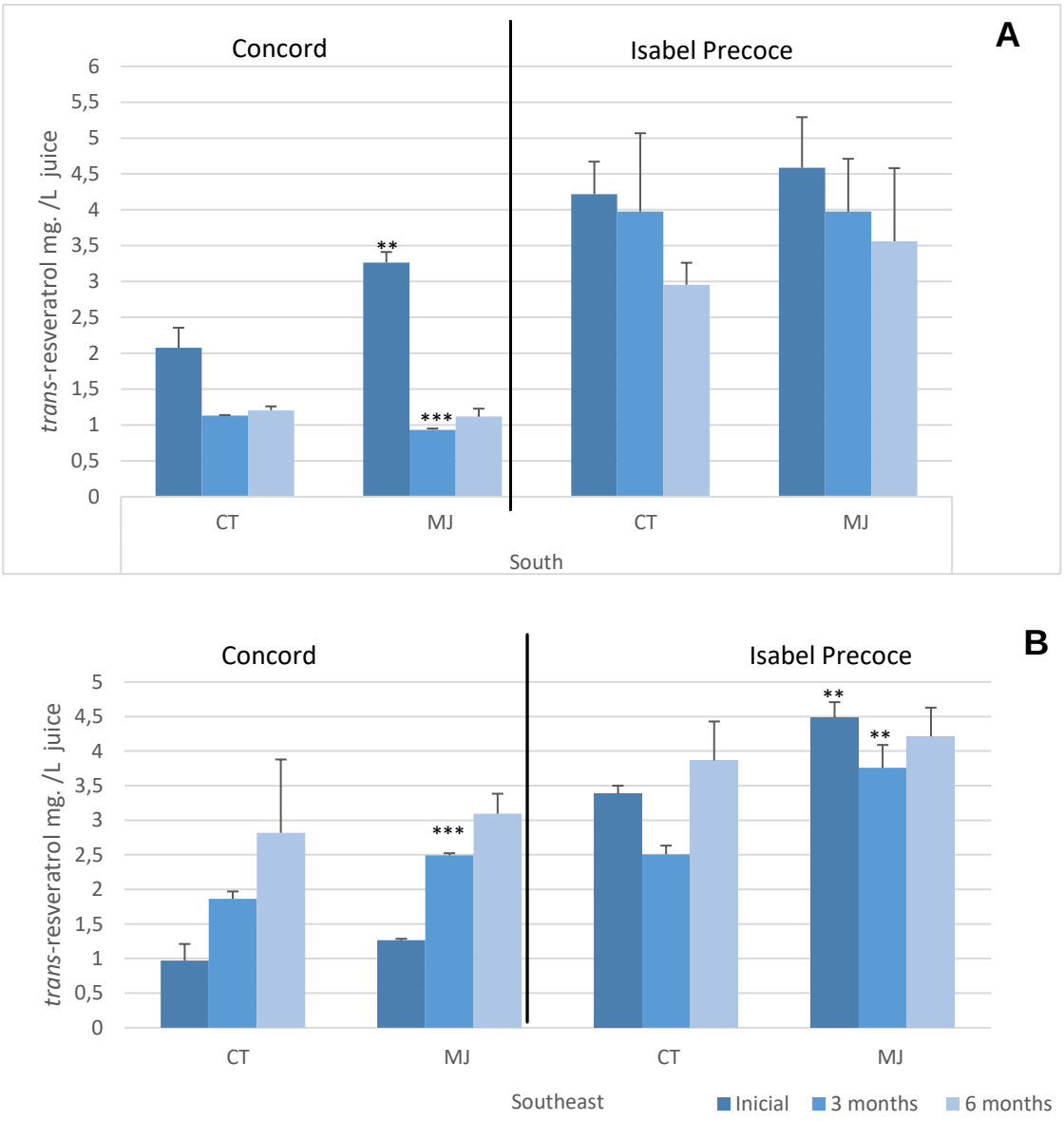

Figure 2: *Trans*-resveratrol quantification on grapes juices of Concord and Isabel Precoce grapes grown on South (A) and Southeast (B) Brazil. CT: control; MJ: Methyl jasmonate (n=6); '*' represent significative difference between samples * $p < 0.05$, in comparison with CT group

Resveratrol glycosylated derivative, piceid (resveratrol-3-O-β-D-glucopyranoside), is also one of the main stilbenes found in grapes (Flamini, Mattivi, et al. 2013). As well as resveratrol, piceid formation is affected by many factors, including grape variety, the clone, meteorological conditions, soil type, cultural practices, and is a functional form to storage, translocate and protect resveratrol from oxidative degradation (Bavaresco et al. 2016; Flamini, De Rosso, et al. 2013). Piceid also appears to promote several beneficial effects on the cardiovascular system (Jeffrey A. Stuart • Ellen L. Robb 2013), therefore, due to its importance, we evaluate its content on the studied grape juice.

Piceid content on *Concord* grape juices, was above the limit of detection on both studied regions (Fig 3), and they reflect the higher *trans*-resveratrol levels detected (Fig 2.)

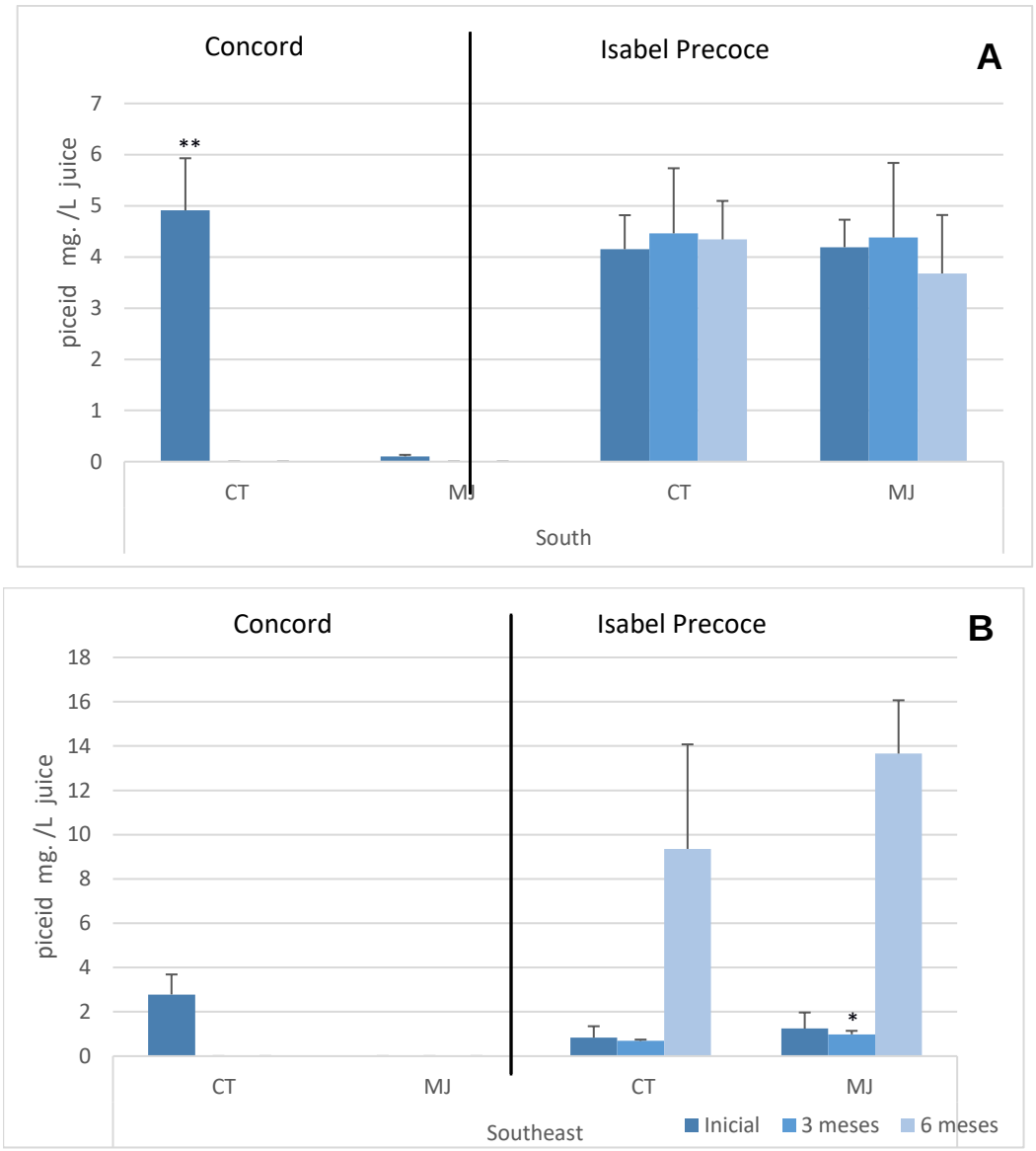

Figure 3: Piceid content on grapes juices of Concord and Isabel Precoce grapes grown on South (A) and Southeast (B) of Brazil. CT: control; MJ: Methyl jasmonate (n=6); '*' represent significative difference between samples * $p < 0.05$, in comparison with CT group.

Deposits were found at the bottom of the bottles after 6 months of the storage period. In red wines, they usually are tannin-stained tartrate deposits, and they might appear also on juices. It is known that colder temperatures decrease potassium bitartrate solubility and accelerate crystal growth, and the potential for visible crystal formation increases upon chilling (Ribéreau-Gayon P., Glories Y., Maujean A. 2006; Waterhouse, Andrew L. Gavin L. Sacks 2016). Aiming better understand if stilbenes

might also be in the inferior part of the bottles. We quantified the stilbene content of this part (bottom) and higher levels of piceid were observed on juices produced with Isabel Precoce grapes grown on southeast (Supplementary ST2).

Evaluating the total stilbene content after 6 months (Fig 3.), including the precipitated part, our results points to the potential of MJ treatment to increase stilbene content on grape juice produced with Isabel Precoce grapes cultivated on southeast region.

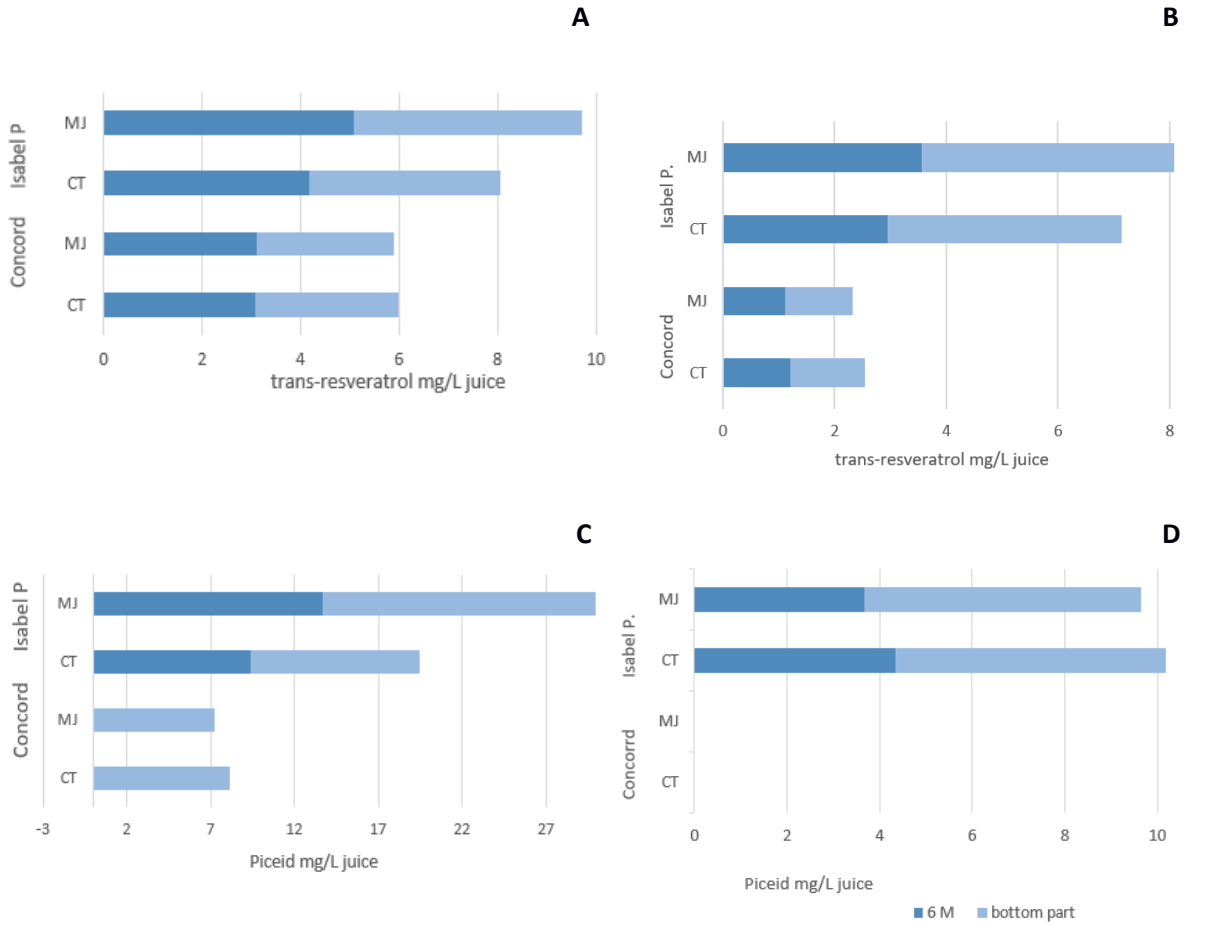

Figure 3: Total stilbenes content on grapes juices of Concord and Isabel Precoce grapes grown on South (B-D) and Southeast (A-D) of Brazil after 6 months of storage. CT: control; MJ: Methyl jasmonate.

The rupture of the berry cell, due to the high temperatures on grape steam extraction (da Mota et al. 2018), provides an efficient extraction of the stilbenes present on the peel, it is important to emphasize that wasn't performed any kind of filtration or tartaric stabilization on the grape juice. These characteristics, associated with MJ treatment and southeast '*terroir*' might positively affect the results.

4. Conclusion

In summary this work has evidenced the potential of MJ in increasing the stilbenes content on *Vitis labrusca* L. grapes during two consecutive harvests, on different Brazilian regions. The juice produced by these grapes, also present higher levels of stilbenes, in comparison with non-treated grapes. A better maintenance of these higher levels was observed on grapes cultivated on Southeast region. This might be a '*terroir'* response, however, further studies on 2019 grape juice are being performed to confirm this hypothesis. The results indicate that higher stilbene content, was present on MJ grape juice (elaborated with southeast Isabel Precoce grapes), even after 6 months of storage, however, to consume all the amount of stilbenes present on the juice, we should emphasize the future consumers to shake the product, in case of precipitated or potassium bitartrate crystal are seen on the bottom of the bottles.

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Supplementary Material

ST1: Physicochemical characteristics (soluble solids, pH, titratable acidity) of grapes grown on Concord and Isabel Precoce grapes grown on South (Bento Gonçalves/RS) and Southeast (Caldas/MG) of Brazil.

2017 harvest						
Region	**Cultivar**	**Treatment**	**Soluble Sugar**	**pH**	**Acidity**	**tartaric (g/L)**
South	Concord	CT	14.5			
		MJ	12.75			
	Isabel P.	CT	13.5			
		MJ	13.7			
Southeast	Concord	CT	13.2	3.29	3.35	5,08
		MJ	13.5	3.19	3.6	5,45
	Isabel P.	CT	13.8	3.32	4.45	6,74
		MJ	13.1	3.26	5.45	8,26
2019 harvest						
Region	**Cultivar**	**Treatment**	**Soluble Sugar**	**pH**	**Acidity**	**tartaric (g/L)**
South	Concord	CT	13.50 ± 0.50	3.45 ± 0.03	3.98 ± 0.23	5.96 ± 0.34
		MJ	14.10 ± 0.30	3.57 ± 0.04	3.75 ± 0.60	5.62 ± 0.90
	Isabel P.	CT	15.30 ± 0.50	3.35 ± 0.01	5.63 ± 0.83	8.44 ± 1.24
		MJ	14.10 ± 0.49	3.41 ± 0.03	4.35 ± 0.15	6.53 ± 0.23
Southeast	Concord	CT	14.92 ± 0.16	3.30 ± 0.01	4.40 ± 0.53	6.60 ± 0.80
		MJ	14.33 ± 0.09	3.28 ± 0.01	4.50 ± 0.03	6.75 ± 0.04
	Isabel P.	CT	14.21 ± 0.28	3.21 ± 0.02	3.40 ± 0.15	5.00 ± 0.22
		MJ	13.97 ± 0.36	3.24 ± 0.03	3.35 ± 0.20	4.93 ± 0.30

ST2: Stilbenes content on bottom of grape juice produced with Concord and Isabel Precoce grapes grown on South and Southeast of Brazil, after 6 months of storage.

Region	**Treat***	***trans*-resveratrol (mg/L juice)**		**piceid (mg/L juice)**	
		Mean	SD	Mean	SD
South	CT	1,34	0,06	0,00	0,00
	MJ	1,21	0,13	0,00	0,00
	CT	4,18	0,65	5,85	2,45
	MJ	4,52	1,30	5,98	2,02
Southeast	CT	3,08	0,44	8,16	4,02
	MJ	3,12	0,22	7,21	4,34
	CT	4,18	0,39	10,14	5,64
	MJ	5,07	0,43	16,25	1,05

Treat*: treatment applied; CT: control; MJ: Methyl jasmonate (n=3).

Considerações finais

Durante este trabalho foi possível otimizar o método de aplicação de metil-jasmonato (MeJa) para uvas *Vitis labrusca* L., sendo possível observar que período de aplicação do tratamento é fundamental para a efetividade do mesmo, devendo ser aplicado durante a mudança de cor dos frutos (*véraison*) e no período de pré-colheita. A aplicação exógena de MeJa em uvas da cultivar Concord e Isabel Precoce, influenciou a biossíntese de compostos aromáticos, em uvas cultivadas na região sul, e o conteúdo de estilbenos em uvas e suco de uva, cultivadas nas regiões Sul (Bento Gonçalves, RS) e Sudeste (Caldas, MG).

Suco de uva Isabel Precose cultivadas na região sudeste, tratadas com MeJa apresentam maiores teores de estilbenos até o período de 6 meses de armazenamento, no escuro sob temperatura ambiente.

Após a análise de *fingerprinting* foi possível detectar a influência positiva do tratamento com MeJa em outras vias metabólicas além das dos estilbenos, como a das antocianinas, outros flavonoides não ciânicos e hidroxicinamatos nas uvas e suco, apesar da grande diferença encontradas entre as regiões.