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**Taísa Ceratti Treptow**

# EFEITO DA TECNOLOGIA *THERMAL PEST CONTROL* SOBRE UVAS E VINHOS

**Santa Maria, RS**
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Tese apresentada ao Curso de Pós-Graduação em Ciência e Tecnologia dos Alimentos, Área de Concentração em Ciência e Tecnologia dos Alimentos, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do grau de **Doutor em Ciência e Tecnologia dos Alimentos.**

**Orientadora: Profª. Drª. Cláudia Kaehler Sautter**

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Santa Maria, RS
2016

# DEDICATÓRIA

***Aos meus pais,***

***Vinicius e Suzana***

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À Deus por me dar forças para enfrentar minhas dificuldades
Aos meus amáveis pais Suzana Maris Ceratti Treptow e Vinícius Treptow, pelos princípios e valores, pela presença e apoio em todos os momentos.
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Se eu gosto de poesia?
Gosto de gente, bichos, plantas, lugares, chocolate, vinho, papos amenos, amizade, amor. Acho que a poesia está contida nisso tudo.

(Carlos Drummond de Andrade)

# RESUMO

## EFEITO DA TECNOLOGIA *THERMAL PEST CONTROL* SOBRE UVAS E VINHOS

AUTORA: Taísa Ceratti Treptow
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*Thermal Pest Control* (TPC) é uma tecnologia alternativa de aquecimento em vinhedos, e que foi desenvolvida para proteger as plantas das geadas e posteriormente doenças fúngicas. Além disso, existem indícios de que esta tecnologia possa desencadear alterações no metabolismo de defesa estimulando a produção dos compostos fenólicos. Em vista disso, o objetivo do trabalho foi avaliar o efeito da tecnologia TPC em uvas e o impacto sobre a composição fenólica e qualidade dos vinhos. Os vinhedos das cultivares 'Tannat' receberam 4, 5 e 21 aplicações do tratamento térmico nas safras 2011/12, 2012/13 e 2013/14, respectivamente; a 'Cabernet Sauvignon' recebeu 21 aplicações na safra 2013/14. As uvas foram analisadas quanto à maturação industrial e fenólica, seguida da vinificação em escala laboratorial. Os vinhos produzidos foram armazenados por 1 e 12 meses para as análises físico-químicas, cromatográficas e sensoriais. O tratamento térmico influenciou na maturação industrial da uva 'Tannat' mas sem alterar a extratibilidade. Para a uva 'Cabernet Sauvingon' o TPC não alterou a maturação industrial, reduzindo os taninos das cascas e aumentando na semente. A intensidade de cor foi incrementada no vinho 'Cabernet Sauvignon' permanecendo elevada até 12 meses. No perfil antociânico dos vinhos elaborados a partir de uvas 'Tannat' que receberam 4 aplicações na safra 2011/12 houve um aumento de 49,8% nas antocianinas monoméricas, 51,4% em piranoantocianinas, 7,0% produtos de condensação direta e 39,6% produtos de condensação mediados por acetaldeído. Nos vinhos com uvas da mesma cultivar na safra 2012/13, que receberam 5 aplicações de TPC não houve diferença significativa nas antocianinas e derivados, devido ao elevado índice pluviométrico. Na safra 2013/14, os vinhos produzidos com uvas 'Tannat' que receberam 21 aplicações do TPC apresentaram um aumento de 30,6% nas antocianinas monoméricas e 11,5% nos produtos de condensação direta, os demais compostos reduziram. No vinho 'Cabernet Sauvignon' elaborado com uvas tratadas com TPC houve um aumento de 4,5% nas antocianinas monoméricas, 29,4% nas piranoantocianinas e 29,5% nos produtos de condensação direta, sem a formação de compostos oriundos do acetaldeído. Sensorialmente, a cor vermelha ($p \leq 0,10$) e o sabor amadeirado ($p \leq 0,05$) foram mais intensos nos vinhos de uvas tratadas com TPC. Mas, olfativamente o atributo de frutas vermelhas foi menos intenso com o tratamento térmico. Visualmente, houve um aumento na intensidade das lágrimas ($p \leq 0,05$), no entanto, não sendo relacionado ao teor alcoólico. Quanto à aceitabilidade ambos os vinhos foram descritos como "gostei moderadamente". Dessa forma, o tratamento térmico TPC depende do número de aplicações, do manejo frente às condições meteorológicas para promover a resposta fisiológica adequada nas uvas e no vinho quanto à sua qualidade fitoquímica e sensorial.



**Palavras-chave: *Thermoculture, Vitis vinifera,* potencial fenólico, antocianinas poliméricas, análise descritiva quantitativa.**

# ABSTRACT

## EFFECT OF THERMAL PEST CONTROL TECHNOLOGY ON GRAPES AND WINES

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Thermal Pest Control (TPC) is an alternative thermal technology in vineyards, which was developed for protecting plants from frost and subsequent fungal diseases. Moreover, there are indications that this technology can trigger changes in defensive metabolism by stimulating the production of phenolic compounds. In view of this, the objective of this study was to evaluate the effect of TPC technology in grapes and its impact on the phenolic composition and quality of wines. The vineyards of the cultivars 'Tannat' received 4, 5 and 19 applications of thermic treatment in the 2011/12, 2012/13 and 2013/14 crops, respectively; the Cabernet Sauvignon received 19 applications in the 2013/14 crop. The grapes were analyzed for industrial and phenolic maturation, followed by vinification in laboratory scale. The wines produced were stored for 1 and 12 months for physicochemical, chromatographic and sensory analysis. Thermic treatment influenced the industrial ripening of the ‘Tannat’ grapes but without changing the extractability. For the Cabernet Sauvignon grape TPC did not alter the industrial maturity, reducing the tannins of the shells and increasing the ones of the seed. The color intensity was increased in Cabernet Sauvignon wine remaining high up to 12 months. In the anthocyanin profile of wines produced from 'Tannat' grapes which received four applications in the 2011/12 crop, there was an increase of 49.8% in monomeric anthocyanin, 51.4% in pyranoanthocyanins, 7.0% direct condensation products and 39.6% condensation product mediated by acetaldehyde. In wines from grapes of the same variety in the 2012/13 crop, which received 5 applications of TPC there was no significant difference in anthocyanins and derivatives due to the high rainfall. In the 2013/14 crop, the wines produced with 'Tannat' grapes which received 19 TPC applications increased by 30.6% in monomeric anthocyanins and 11.5% in the direct condensation products, other compounds reduced. In the Cabernet Sauvignon wine made from grapes treated with TPC there was an increase of 4.5% in monomeric anthocyanin, 29.4% in pyranoanthocianins and 29.5% in the direct condensation products, without the formation of acetaldehyde derived compounds. In sensory, red color ($p \leq 0.10$) and woodsy flavor ($p \leq 0.05$) were more intense in wine grapes treated with TPC. Nevertheless, the olfactory red fruits attribute was less intense with the thermic treatment. Visually, there was an increase in the intensity of tears ($p \leq 0.05$), however, not being related to alcohol. As to the acceptability, both wines were described as "moderately liked". Thus, the TPC treatment depends on the number of applications, the management on different weather conditions to promote proper physiological response in grapes and wine for their phytochemical and sensory quality.



**Key-words: Thermoculture, *Vitis vinifera*, potencial phenolic, anthocyanins polimerics, analysis descriptive quantitative**

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# 1. APRESENTAÇÃO

## 1.1 INTRODUÇÃO

O equipamento *Thermal Pest Control* (TPC) é uma tecnologia alternativa que libera ar aquecido diretamente na planta e nos frutos de videiras, sendo lançada no Brasil com a perspectiva de reduzir ou eliminar o uso dos agrotóxicos, e também devido às condições climáticas, com o intuito de reduzir as contaminações fúngicas nas plantações, visto que, o equipamento tem a capacidade de inativar as enzimas dos patógenos causadores de doenças, especialmente fungos e insetos (IBRAVIN, 2009). Contudo, existem indícios de que a tecnologia TPC pode atuar como elicitor abiótico nas uvas causando estresse, e desse modo, estimulando o metabolismo secundário que contribuiria no aumento da proteção às plantas e produção de algumas fitoalexinas, como os compostos fenólicos (FISCHER, 2012). Foi observado que a aplicação do tratamento térmico em videiras promove em uvas um estímulo na biossíntese dos flavonóides, como nas antocianinas poliméricas, ácido gálico, ácido caftárico, catequina, epicatequina e taninos (UNITED STATES PATENT, 2014).

Estudos pós-colheita revelam que o efeito estressor abiótico por choque térmico promove um acúmulo de compostos fenólicos (KANG; SALTVEIT, 2003), e quando aplicado em uvas viníferas brancas na pré-colheita originou um mosto com maior concentração de compostos fenólicos e atividade antioxidante (DULLIUS et al., 2011). O sistema TPC quando aplicado em uvas viníferas tintas também favoreceu a qualidade do vinho produzido, com maior atividade antioxidante e um estímulo de 20,6% dos polifenóis totais, sem alterar a acidez (LOPES et al., 2013). Contudo, as temperaturas aplicadas pelo TPC ocorrem por um curto período de tempo, deste modo, o estresse causado deve originar o mínimo de Espécies Reativas de Oxigênio (EROs) suficiente apenas para estimular o metabolismo secundário, atingindo rapidamente a homeostase. O mosto produzido a partir de uvas que receberam o tratamento térmico TPC apresentou maior eficiência em capturar os radicais livres, estabilizando em 80,64% do radical DPPH (DULLIUS et al., 2011).

As características na maturação fenólica das uvas que receberam tratamento TPC e os efeitos na composição antociânica e sensorial dos vinhos ainda não foram relatados cientificamente. As pesquisas enfatizam o controle térmico na fase de pós-colheita, sendo raras as referências desta aplicação diretamente na planta. Em vista disso, a aplicação do TPC poderia diminuir os custos energéticos e químicos à campo, pois reduziria a aplicação dos resíduos de

pesticidas em uvas, além de possivelmente agregar fitoquímicos benéficos à saúde humana e contribuir nas características sensoriais.

## 1.2 REFERENCIAL TEÓRICO

### 1.2.1 Uvas

#### *1.2.1.1 Histórico e adaptação*

No Rio Grande do Sul a facilidade de adaptação, proporcionou a inserção das videiras em 1626 com as missões jesuítas (POMMER, 2003). As primeiras vinhas *Vitis vinifera* foram trazidas pela colonização espanhola, e em seguida as portuguesas, francesas, italianas e alemãs. As cultivares Tannat e Cabernet Sauvignon se propagaram no início de 1980 juntamente com outras cultivares viníferas nas propriedades da região da Serra e Campanha Gaúcha (CAMARGO, 2003a).

A cultivar Tannat é proveniente de Madiran, na região sul da França, sendo trazida no século 19 para o Uruguai por colonos bascos (ZOECKLEIN, 2002). No Rio Grande do Sul a cultivar é uma variedade vigorosa e bastante produtiva (CAMARGO, 2008), além disto, produz um vinho caracterizado pela coloração intensa e longevidade, como o próprio nome sugere, é excepcionalmente tânico, rico em antocianinas e adstringente, portanto, necessita de envelhecimento (ZOECKLEIN, 2002; CAMARGO, 2008).

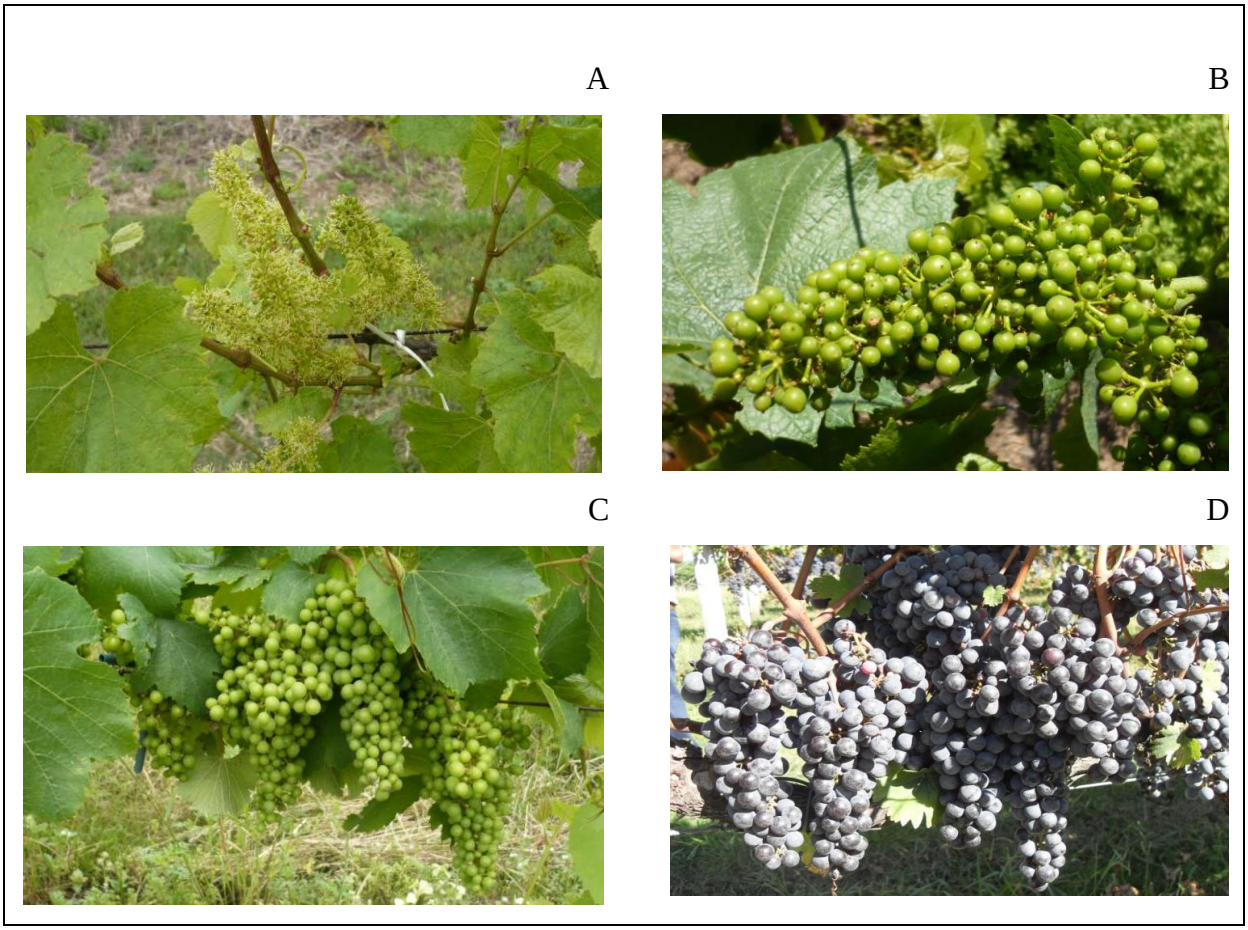
A ‘Cabernet Sauvignon’ foi cultivada inicialmente em parte do Império Romano na região de Bordeaux, no sudoeste da França, sendo referida como a mais nobre casta francesa (SWEET, 2008; GALET, 1998). No Rio Grande do Sul, com características de uma planta vigorosa e medianamente produtiva, apresenta uma boa adaptação, contudo em anos com invernos amenos apresenta brotação irregular e deficiente. O vinho ‘Cabernet Sauvignon’ é amplamente consumido devido sua intensa coloração, aroma e sabores complexos. A concentração dos compostos fenólicos complexos e tânicos exige envelhecimento em barril ou garrafa (SWEET, 2008; ROBINSON, 2006; GUERRA, et al. 2009; CAMARGO, 2003b).

#### *1.2.1.2 Maturação Industrial*

Os viticultores identificaram três estádios de desenvolvimento da baga: o primeiro é logo após florescimento e nesse período a baga ainda está verde tendo como característica o crescimento da baga, aumentando principalmente o diâmetro (Figura 1B). O segundo estádio

é a interrupção do desenvolvimento da baga verde com pausa antes de iniciar a maturação (Figura 1C) e o terceiro estádio conhecido como *veraison* ou amadurecimento (Figura 1D). Durante o *veraison* são transportadas substâncias essenciais, como a água, açúcares e compostos nitrogenados via floema, considerados imprescindíveis para o desenvolvimento da baga. Em seguida, ocorrem algumas alterações fisiológicas como a hidrólise da sacarose em glicose e frutose na baga, e a síntese de compostos que conferem sabor e aroma (BISSON, 2001). Segundo a mesma autora, ainda não está definido se a regulação do *veraison* é hormonal ou se ocorre independentemente à sinalizações da videira. Porém, estudos *in vitro* indicam que a biossíntese de antocianinas é dependente do teor de açúcares (ZHENG, et al 2009).

Figura 1 – Florescimento (A) e três estádios de desenvolvimento de uvas 'Tannat': estádio 1 (B), estádio 2 (C) e estádio 3 (D).




Fonte: (DOMINGUES, 2016).

A maturidade enológica nas uvas se refere ao potencial para produzir vinhos de alta qualidade, no entanto, diversos fatores devem estar em equilíbrio. A maturação tecnológica

(proporção açúcar/ ácido) e a maturação fenólica são independentes, porém devem ser consideradas, pois auxiliam na determinação do momento ideal da colheita (RIBÉREAU-GAYON, 2006). No Brasil, a maturação ótima para a colheita das uvas destinadas para a produção de vinhos varia conforme a região de produção, o tipo de vinho elaborado e as condições naturais do clima referente ao ano da safra (GUERRA; ZANUS, 2003).

A maturação pode ser considerada como o estádio de desenvolvimento em que o fruto atinge os requisitos ou atributos para a colheita, sendo conhecido como "maturidade comercial", "de colheita" (CHITARRA; CHITARRA, 2005), "maturação tecnológica" ou "maturação industrial".

A maturação industrial é uma das técnicas utilizadas nas vinícolas para determinar o período de colheita. Quantifica-se o teor de açúcares nas amostras de bagas representativas de todo vinhedo através de um refratômetro que conferem resultados na escala graus Brix (°Brix) ou com um densímetro (mostímetro) graduado em graus babo (°Babo), ambas as técnicas representam o teor de sólidos solúveis totais. Além desta técnica também é acompanhado o teor de ácidos na uva, visto que, está diretamente relacionado à qualidade dos vinhos (GUERRA; ZANUS, 2003). Através da maturação industrial ideal é possível garantir a fermentação alcoólica e malolática satisfatórias, mas não garante totalmente a qualidade do vinho que depende também da maturação fenólica.

*1.2.1.3 Maturação Fenólica*

A maturação fenólica abrange a concentração global das substâncias, além da estrutura e capacidade de extração das uvas durante a vinificação (RIBÉREAU-GAYON, 2006). A composição fenólica dos vinhos é composta pelos taninos e antocianinas que provém das partes sólidas da uva e são extraídos durante o período de fermentação e maceração (SAINT-CRIQ; VIVAS; GLORIES, 1998). Monitorar a maturação fenólica contribui para determinar o período em que as uvas podem alcançar um conteúdo máximo de compostos fenólicos (taninos e antocianinas) (STATION DE VITICULTURE ET D'ENOLOGIE, 2005).

O princípio do método consiste na extração das antocianinas das cascas lentamente, e posteriormente sob condições extremas. A acidez é utilizada para facilitar a extração, pois o meio ácido rompe a membrana fosfolipídica quebrando as proteínas e liberando o conteúdo dos vacúolos. As soluções ácidas são aquosas pH 1,0 (HCl N10) com a extração de todas as antocianinas e pH 3,2 (solução com 5 g $L^{-1}$ de ácido tartárico neutralizado), que representa a extração comparável às cubas de fermentação. Além disso, a trituração das bagas também

favorece o esmagamento das sementes que resultam em uma extração parcial dos taninos (RIBÉREAU-GAYON et al., 2006).

O potencial de extração ou extratibilidade varia nas safras (CAGNASSO et al., 2008), especialmente quanto às condições de maturação (STATION DE VITICULTURE ET D'ENOLOGIE, 2005), e variedade da uva (MORI et al., 2007; ORTEGA-REGULES et al., 2008; GIL-MUÑOZ et al., 2009). Além disso, diversos fatores climáticos como a precipitação pluviométrica excessiva juntamente com elevadas temperaturas podem reduzir os componentes que determinam a qualidade das uvas e causar inibição na biossíntese dos compostos antociânicos (HARBERTSON; KENNEDY; ADAMS, 2002; DOWNEY; DOKOOZLIAN; KRSTIC, 2006; TIAN; PANG; DIXON, 2008).

O potencial das antocianinas (ApH) é observado no ApH 1,0 que varia de 500 a 2000 mg $L^{-1}$ conforme a variedade da uva (RIBÉREAU-GAYON et al., 2006). Em um estudo sobre a avaliação do conteúdo fenólico de uvas em quatro safras com diferentes condições climáticas foi observado que a cultivar Tannat apresentou valores mais elevados para o ApH 1,0 e ApH 3,2 em relação a cultivar Cabernet Sauvignon em todas as safras, especialmente na safra em que o clima foi moderadamente seco (GONZÁLEZ-NEVES; FERRER; GIL, 2012).

A determinação do potencial fenólico de uvas é realizado pela metodologia de Glories e Augustin (1993). Nesta análise as bagas das uvas são trituradas e maceradas independentes em duas soluções (ApH 1,0) e (ApH 3,2) durante 4 horas, sendo então filtrados e centrifugados por 3 minutos à 1559,6 *g*. O extrato com ApH 3,2 é diluído 1:100, e determinado o índice de polifenóis totais (IPT) em absorbância 280 nm, através de um espectrofotômetro. E, em ambos os extratos são determinadas as antocianinas pelo método de branqueamento (RIBERÉAU-GAYON et al., 2006). Os resultados dos índices são utilizados nas equações (Quadro 1) afim de calcular em percentuais a extratibilidade das antocianinas (EA), taninos das sementes (Mp) e taninos das cascas (dpell). (GONZÁLEZ-NEVES et al., 2004).

Quadro 1 – Equações para o cálculo em porcentagem da extratibilidade das antocianinas (EA), taninos das sementes (Mp) e taninos das cascas (dpell)

$$EA\ (\%) = \frac{ApH\ 1{,}0 - ApH\ 3{,}2}{ApH\ 1{,}0} \times 100 \quad (1)$$

$$Mp\ (\%) = \frac{IPT\ ApH\ 3{,}2 - (ApH\ 3{,}2 \times 40)/\ 1000}{IPT\ ApH\ 3{,}2} \times 100 \quad (2)$$

$$dpell\ (\%) = \frac{(ApH\ 3{,}2 \times 40)/\ 1000}{IPT\ ApH\ 3{,}2} \times 100 \quad (3)$$

Fonte: (GONZÁLES-NEVES et al., 2004, p. 192).

A extratibilidade das antocianinas (EA) é também conhecida como índice de maturação celular (IMC). Os valores de referência (20-70) dependem da variedade e da maturação das uvas. Quanto menores os valores, menos diferença haverá entre as medições (ApH 1,0) e (ApH 3,2), e as antocianinas serão mais facilmente extraíveis, o que demonstra uma maior fragilidade da parede celular. Além disso, a EA diminui com algumas técnicas de cultivo (desbaste dos cachos e remoção de folhas), assim como ocorre naturalmente durante o período de maturação. A cultivar Cabernet Sauvignon apresenta casca mais resistente, sendo assim apresenta uma elevada extratibilidade das antocianinas (RIBÉREAU-GAYON et al., 2006). Entretanto, estudos sobre a diferenciação do potencial fenólico entre variedades de uva reportam que a cultivar Tannat apresenta valores superiores na EA quando comparado à cultivar Cabernet Sauvignon, ou seja, na uva ‘Tannat’ as antocianinas são difíceis de extrair, e na ‘Cabernet Sauvignon’ mais facilmente extraíveis (GONZÁLEZ-NEVES; FERRER; GIL, 2012).

Os níveis de taninos das sementes das uvas (dTpep) podem ser calculados pela absorbância, ou através da proporção relativa (Mp%). Os valores de referência variam de 60-0 conforme a variedade da uva, número de sementes e maturação. Uvas com valores elevados na Mp, terão maior teor de taninos nas sementes, no entanto, existe uma grande probabilidade

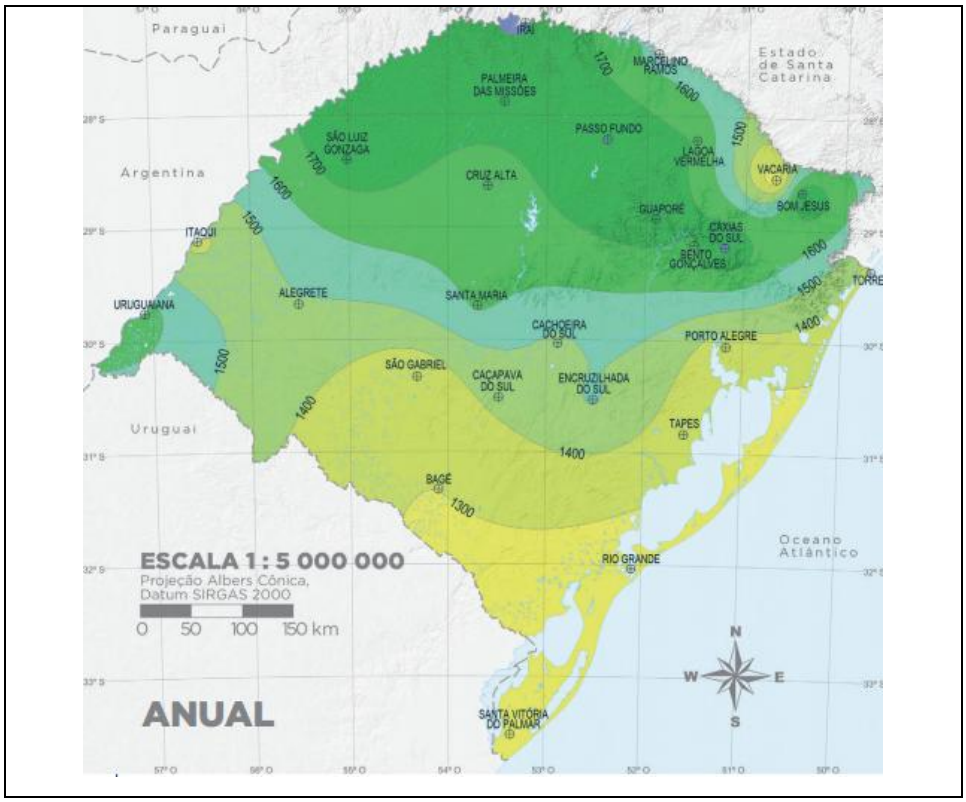
do efeito negativo sobre o *flavor* do vinho (RIBÉREAU-GAYON et al., 2006). E os níveis de taninos das cascas das uvas (dpell) são calculados através da absorbância, ou através da proporção relativa (dpell%).

A colheita das uvas, 10 a 14 dias após a determinação do maior teor de antocianinas parece permitir a obtenção de vinhos aromáticos, mais tânicos e estruturados (STATION DE VITICULTURE ET D'ENOLOGIE, 2005).

### *1.2.2 Condições Climáticas*

Nas regiões vitivinícolas, as condições térmicas, chuvas e insolação exercem influência sobre a composição das uvas e características sensoriais da cor, do aroma e do sabor dos vinhos (GARRIDO; SÔNEGO, 2003). No Rio Grande do Sul, a média da precipitação pluviométrica (Figura 2) e da temperatura (Figura 3) varia entre as regiões vitivinícolas. Além disto, as variáveis climáticas como a insolação e os índices climáticos de ambas as regiões também apresentam comportamento diferenciado durante o ano (MONTEIRO; TONIETTO, 2013).

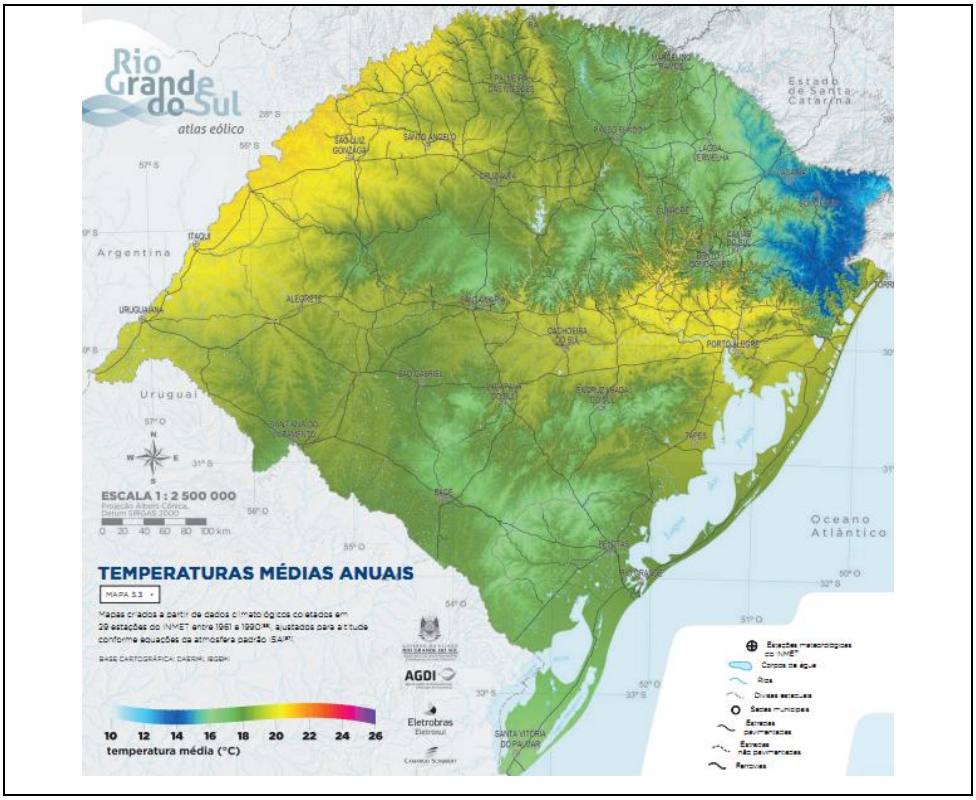
Figura 2 – Precipitação média anual nas regiões do Rio Grande do Sul.




Fonte: (CAMARGO-SCHUBERT, 2014, p.23)

Na Região da Campanha Gaúcha, o mesoclima se caracteriza por ser quente e subúmido com precipitações inferiores à 150 mm, as noites são temperadas com temperaturas que variam de 15 à 16 °C. A insolação é maior, sendo assim apresentam uma colheita mais precoce (TONIETTO, CARBONNEAU, 1999; TONIETTO, MANDELLI, 2003).

Figura 3 – Temperatura média anual nas regiões do Rio Grande do Sul.




Fonte: (CAMARGO-SCHUBERT, 2014, p.25)

As cultivares *Vitis Vinifera* são amplamente conhecidas pela alta sensibilidade ao desenvolvimento de doenças fúngicas devido às condições climáticas. A incidência de chuvas, mesmo sendo regulares, adicionadas a períodos úmidos com temperaturas elevadas, favorecem a germinação de esporos e propiciam infecções na planta (GARRIDO; SÔNEGO, 2003).

### 1.2.3 Tecnologia Thermal Pest Control

O tratamento térmico *Thermal Pest Control*, também conhecido como Tratamento Térmico de Plantas (TTP) ou *thermoculture* é uma tecnologia alternativa desenvolvida no Chile por Florêncio Lazo Barra para atender às necessidades climáticas, e inicialmente visava

proteger as plantas das geadas pelo aquecimento, evitando a cristalização do orvalho sobre o vinhedo e em culturas cítricas (UNITED STATES PATENT, 1999).

A cultura térmica foi lançada prometendo reduzir ou eliminar o uso dos agrotóxicos (IBRAVIN, 2009), ou seja, estimulando a produção de uvas orgânicas. Além disso, a tecnologia também demonstrou eficiência no controle dos insetos atuando na queima das asas, ruptura do exoesqueleto, desidratação e morte dos insetos, e no controle dos fungos pela secagem das folhas, diminuindo a umidade necessária para germinação dos esporos, inativando enzimas dos micélios, principalmente em locais de cultivo com elevadas precipitações pluviométricas (UNITED STATES PATENT, 2003; FISCHER, 2012). No Rio Grande do Sul foi implementado para o controle de fungos (IBRAVIN, 2009), devido ao clima das regiões da campanha e serra gaúcha, de temperado à quente, e de úmido à subúmido.

Em um estudo sobre o efeito do tratamento térmico TPC em doenças fúngicas de uvas *Vitis vinifera* e *Vitis labrusca* foi observado que a 'Cabernet Sauvignon' e 'Bordô' submetidas à aplicação de fluxo de ar quente simulando a aplicação do TPC com inoculação do agente causador do míldio na videira foi possível observar que o ar aquecido em tecidos foliares favoreceu a dispersão do míldio, porém reduziu a severidade deste fungo em cachos da cultivar Chardonnay. Neste mesmo estudo quando o TPC foi suplementado com controle químico foi observada uma redução nas lesões por míldio quando as aplicações procederam desde o período de florescimento (CAVALCANTI et al., 2014).

No Brasil, são poucos os trabalhos desenvolvidos para avaliar o efeito do tratamento térmico em vinhedos. Um estudo realizado na região Nordeste, mais especificamente no município de Petrolina com uma proposta diferenciada avaliou o efeito fisiológico e físico-químico em espécies *Vitis labrusca* da variedade 'Benitaka' que receberam aplicações do *Thermal Pest Control* na temperatura de 180 ºC durante todo ciclo vegetativo e observaram que o TPC não diferiu significativamente quanto aos hormônios ácido abcísico, ácido jasmônico e ácido salicílico, além de também não alterar os sólidos solúveis tampouco a firmeza das bagas. No entanto foi observada uma redução na coloração avermelhada das uvas (DOMINGUES, 2013).

A metodologia da utilização do TPC nos vinhedos ocorre com o equipamento que é rebocado em um trator (Figura 1A), e que se move de 2,5 a 4,0 km $h^{-1}$ expelindo ar aquecido por combustão de gás liquefeito de petróleo (GLP) que aquece o ar à 160 ºC e lança a uma velocidade de 160 a 200 km $h^{-1}$, a uma distância de 20 cm das linhas da espaldeira (IBRAVIN, 2009; FISCHER, 2012) (Figura 1B e 1C). As recomendações para as aplicações

no Brasil são após a formação da quinta folha, com duas aplicações semanais e intervalos de cinco dias entre as aplicações seguindo até uma semana antes da colheita. E no período de floração são recomendadas três aplicações semanais com intervalo de dois dias entre cada aplicação e apenas uma intervenção química (RECOMENDAÇÕES TÉCNICAS, 2013).

Figura 4 – Tecnologia *Thermal Pest Control* em vinhedos. A) Máquina TPC rebocada no trator. B) TPC entrando no vinhedo. C) Aplicação do TPC durante ciclo vegetativo. Santana do Livramento, 2013 e Dom Pedrito, 2014

A

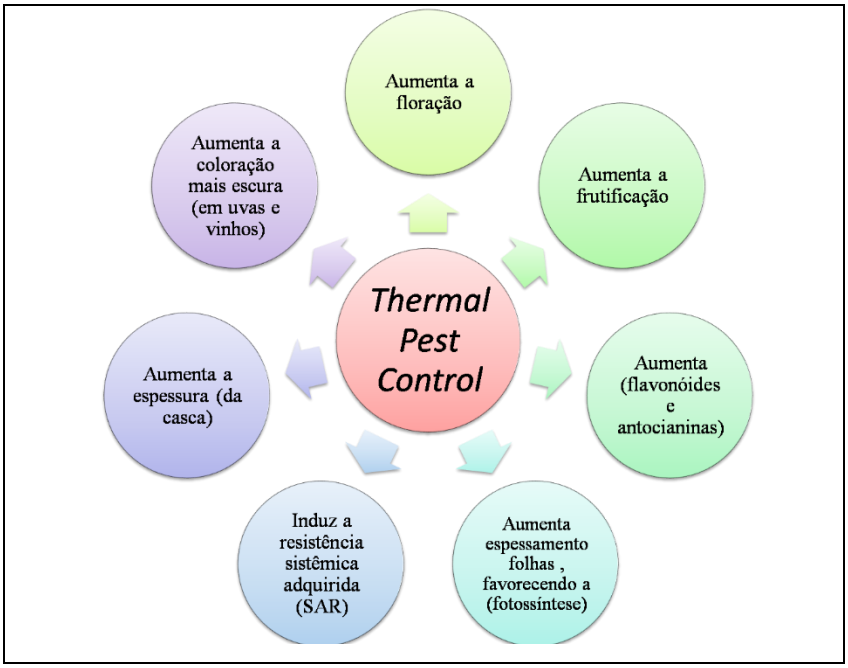
B


C


Fonte: (DOMINGUES, 2013; AUTOR, 2014).

O tratamento térmico TPC também pode desencadear alterações biológicas na planta e causar impactos nos seus frutos, além de contribuir com benefícios econômicos (Figura 5).

Figura 5 – Efeitos do tratamento térmico *Thermal Pest Control* em uvas




Fonte: Adaptado UNITED STATES PATENT, 2014.

Em estudo com o fluxo de ar quente simulando o tratamento térmico TPC foram observadas que as cultivares Cabernet Sauvignon e Bordô apresentaram respostas discretas à

Resistência Sistêmica Adquirida (SAR), e sensíveis alterações nas enzimas Polifenoloxidase (PPO) e Peroxidase de monolignóis (GPX) (CAVALCANTI et al., 2014).

O choque térmico aumenta a polinização e a produção das uvas 'Pinot Noir', proporcionando a auto-defesa e frutos com cascas mais grossas que protegem da umidade. As uvas que recebem o tratamento térmico também apresentam um aumento na coloração, açúcares, atividade antioxidante e polifenóis totais (FISCHER, 2012). Em trabalhos com vinhos da cultivar Tannat elaborados a partir de uvas que receberam o TPC foi observado um estímulo dos polifenóis totais e antocianinas (LOPES et al., 2014).

#### 1.2.3.1 Metabolismo secundário no tratamento térmico

As plantas ao receberem exposições breves e periódicas de temperaturas que poderiam ser letais desenvolvem a termo-tolerância e para suportar o estresse térmico são produzidas as proteínas de choque térmico (PCT), conhecidas também como HSPs (*heat-shock proteins*) (TAIZ; ZEIGER, 2009). O tratamento térmico TPC pode induzir potencialmente estas HSPs (UNITED STATES PATENT, 2014). Em bagas de uvas *Vitis vinifera* que receberam pré-tratamento com calor foi observado o acúmulo específico da HSP70 (SUN et al., 2010), nas plantas esta classe de proteínas tem localização celular nas mitocôndrias e cloroplastos (TAIZ; ZEIGER, 2009).

Os tratamentos térmicos na pós-colheita de frutas elevam a expressão gênica da catalase e conferem um aumento na atividade enzimática da célula (WANG, 1995). O choque térmico também na pós-colheita de uvas *Vitis vinifera* favoreceu um aumento de quase 30% da atividade antioxidante (PINELO et al., 2005). Todavia, quando o tratamento térmico foi aplicado no período da pré-colheita houve um aumento de 56,56% na atividade antioxidante da cultivar Chardonnay (DULLIUS et al., 2011).

#### 1.2.3.2 Atividade antioxidante no tratamento térmico

O estresse abiótico por choque térmico promove a indução de enzimas com atividade chave no metabolismo secundário (DIXON; PAIVA, 1995). Especialmente, o aumento da atividade da enzima fenilalanina amônia-liase (FAL EC 4.3.1.5) (KANG; SALTVEIT, 2003) que desencadeia a formação de polifenóis, os quais atuam como antioxidantes além da ação estrutural na formação de lignina e de defesa. A formação de flavonóides e antocianinas são desencadeadas pela chalcona sintase (CHS, EC 2.3.1.74); e a formação dos estilbenos é

catalisada pela estilbeno sintase (STS, EC 2.3.1.95, 2.3.1.146) em ambas as vias são ativadas pela temperatura em uvas, especialmente na cv. Isabel (BAKHSHI; ARAKAMA, 2006; SAUTTER, 2003).

O estresse térmico por *Thermal Pest Control* na pré-colheita da cultivar Chardonnay favoreceu o aumento de 34,8% dos polifenóis totais no mosto (DULLIUS et al., 2011). O tratamento térmico em uvas aumenta a concentração dos compostos fenólicos que também pode ser afetado pelas condições do tempo e da temperatura (KIM et al., 2006). Em uvas qsão submetidas a diferentes tratamentos térmicos pós-colheita são detectados diversos compostos fenólicos tais como, ácido gálico, galocatequina, procianidina B1, catequina, epigalocatequina, procianidina B2, epigalocatequina galato, epicatequina e epicatequina galato (DAVIDOV-PARDO et al., 2011).

### 1.2.4 Compostos Fenólicos

Os compostos fenólicos não são produzidos para o crescimento e desenvolvimento das células vegetais, como os carboidratos, proteínas, lipídios e ácidos nucléicos. No entanto, desempenham importantes funções na defesa das plantas, frutos e flores na coloração, sabor e equilíbrio hormonal sendo, portanto caracterizados como produtos do metabolismo secundário (LADANIYA, 2008), e formados através de aminoácidos aromáticos como triptofano, tirosina, fenilalanina e produtos do seu metabolismo (NEISH, 1964).

Em vinhos, os polifenóis são importantes, pois influenciam nas características da cor, adstringência, sabor (MONAGAS; BARTOLOMÉ; GÓMEZ-CORDOVÉS, 2005) e podem ser agrupados em duas categorias: não-flavonóides e flavonóides (WATERHOUSE, 2002). As duas classes de não-flavonóides mais encontradas em vinhos são os hidroxicinamatos e os estilbenos. Um dos principais polifenóis em vinhos brancos são os hidroxicinamatos, que estão localizados na polpa da uva e esterificados pelo ácido tartárico. Em vinhos são conhecidos como potentes antioxidantes, no entanto, sem impacto sensorial, exceto quando oxidados podem formar pigmentos castanhos que eventualmente se precipitam (WATERHOUSE, 2002; HARBERTSON, 2007). Os estilbenos são descritos como uma classe menor, e o principal é o resveratrol que pode ser estimulado em videiras pelos fungos (ROLDÁN et al., 2003). Apesar dos estilbenos não contribuírem notavelmente na cor ou nas propriedades sensoriais dos vinhos, possuem efeito protetor antioxidante contra as doenças cardiovasculares (MORENO; PEINADO, 2012).

Os flavonóides são compostos fenólicos com vários anéis aromáticos, especificamente três anéis compreendem a maioria dos fenóis no vinho tinto e são derivados da extração das cascas e sementes das uvas durante a fermentação, principalmente durante o processo de maceração (WATERHOUSE, 2002). A classe dos flavonóides em vinhos é representada pelas catequinas, flavanols, taninos e antocianinas (HARBERTSON, 2007).

As catequinas ou flavan-3-ols são compostos encontrados em cascas e sementes e, em vinhos o amargor é devido a formação destes compostos. Uma grande parte dos compostos fenólicos em vinhos é gerada a partir da condensação de unidades de flavan-3-ol que originam os oligômeros, representados pelas proantocianidinas e polímeros pelos taninos condensados (VERMERRIS; NICHOLSON, 2006). Os flavonols são encontrados na epiderme de uva, e são co-fatores para o fenômeno de aumento de cor (copigmentação) e os taninos encontrados em cascas e sementes de uvas, são responsáveis pela adstringência em vinhos (HARBERTSON, 2007).

As antocianidinas são flavonóides representados por estruturas químicas sem a glicose, também conhecidas como agliconas. As antocianinas em uvas são representadas pela ligação de uma aglicona (antocianidina) com uma glicose, e devido à estas ligações glicosídicas se tornam estruturas com maior estabilidade química e solubilidade. Além disto, podem ser complexadas/aciladas, normalmente no C6 por uma ligação de glicose com um ácido acético, ácido cumárico ou ácido caféico (JACKSON, 2000). As alterações de glicosilação e acilação das antocianinas podem impactar diretamente na cor ou indiretamente na copigmentação (GLOVER; MARTIN, 2012).

A biossíntese das antocianinas é catalisada por uma via de núcleo com as enzimas: Fenilalanina amônia-liase, cinamato 4-hidroxilase, p-coumaroil 4-CoA ligase na via fenilpropanóide geral, além da chalcona sintase, chalcona isomerase, flavonona 3-hidroxilase, dihidroflavonol 4-redutase e antocianidina sintase (KOES; QUATTROCCHIO; MOL, 1994).

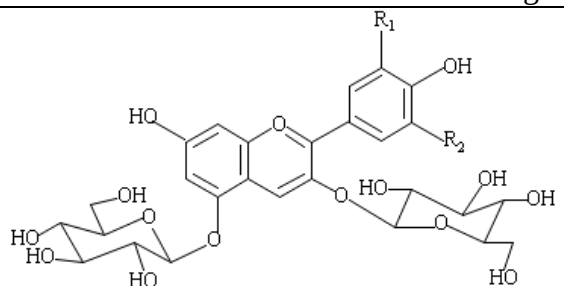
O acúmulo de pigmentos é o processo mais familiar do metabolismo secundário e também o mais entendido na bioquímica da síntese de antocianinas. Além disso, estudos sugerem que as antocianinas possuem propriedades de promoção à saúde humana e atividades antioxidantes (MARTIN et al., 2012).

### 1.2.5 Antocianinas em uvas

A classificação das antocianinas é baseada na posição dos grupos hidroxil e metil do anel B das antocianidinas. Sendo divididas em seis classes: pelargonidina, cianidina,

peonidina, delfinidina, petunidina e malvidina (YOSHIDA; OYAMA; KONDO, 2012) (Figura 6). Segundo Wenzel, Dittrich e Heimfarth (1987), a proporção de cada classe pode variar conforme a cultivar e as condições do clima.

Figure 6 – Estrutura das antocianinas e suas agliconas




| Antocianina | Aglicona | R1 | R2 |
|---|---|---|---|
| Pelargonina | Pelargonidina | H | H |
| Cianina | Cianidina | OH | H |
| Peonina | Peonidina | $OCH_3$ | H |
| Delfinina | Delfinidina | OH | OH |
| Petunina | Petunidina | $OCH_3$ | OH |
| Malvina | Malvidina | $OCH_3$ | $OCH_3$ |

Fonte: (YOSHIDA; OYAMA; KONDO, 2012)

As antocianinas estão localizadas principalmente nas cascas das uvas, com exceção das variedades *teinturier* (do francês “para tingir” ou “para manchar”) que também possuem antocianinas nas polpas (PATTERSON, 2011; MORENO-ARRIBAS; POLO, 2009). As antocianinas variam conforme o pH, sendo vermelhas em meio ácido, violeta em meio neutro e azuis em solução alcalina (PIETTA; MINOGGIO; BRAMATI, 2003). A mudança na cor das antocianinas de acordo com o pH pode existir em quatro formas: base quinoidal (pH 6.0 à 6.5) na cor azul, cátion flavílio (pH 1.0 à 2.0) na cor vermelha, carbinol pseudo-base (pH 4.0 à 5.0) e chalcona (pH > 7.0), ambos na coloração incolor (MARTIN et al., 2012).

Os glicosídeos das antocianidinas não-metiladas (cianidina, delfinidina e pelargonidina) são os mais amplamente distribuídos na natureza, e estão presentes em 69% das frutas (KONG et al., 2003). Na maioria das frutas existe uma grande concentração de antocianinas, e em uvas *Vitis vinifera* são observadas todas as classes de antocianinas, sendo que a malvidina está presente em maiores concentrações (MARGALIT, 2012). Em

comparação com as demais antocianinas, a pelargonidina praticamente não é observada, no entanto, em um trabalho sobre o perfil antociânico de uvas ‘Cabernet Sauvignon’ e ‘Pinot Noir’ foram detectadas por HPLC-ESI-MS a presença de 17 antocianinas, e entre estas a confirmação da presença de níveis traços da pelargonidina-3-glicosídeo (HE et al., 2010).

As antocianinas nos vacúolos das células das plantas migram das células vegetais das uvas para o vinho, a partir da difusão que é um processo pelo qual um composto se move de uma região de alta concentração para uma região de concentração inferior (KENNEDY, 2008).

### 1.2.6 Antocianinas na fermentação/ envelhecimento

As antocianinas e seus pigmentos poliméricos derivados por condensação com outros flavonóides são responsáveis pela coloração vermelha no vinho tinto (PIETTA; MINOGGIO; BRAMATI, 2003). Durante o processo de fermentação e envelhecimento ocorrem reações químicas com a formação de compostos derivados das antocianinas que envolvem reações enzimáticas gerando: O-quinonas, piranoantocianinas, reações de condensação antocianinas-antocianinas e reações diretas antocianinas-flavanol mediadas por acetaldeído (MORENO-ARRIBAS; POLO, 2009).

As piranoantocianinas são pigmentos de vinho tinto derivados das antocianinas com um novo anel de pirano com hidroxilas nos carbonos 4 e 5 das antocianinas devido a adição de hidroxicinamato, piruvato, acetaldeído, vinilflavanols e vitisins (ANGELOSANTE, 2015), nos vinhos estes pigmentos contribuem na estabilidade e cor (RENTZSCH; SCHWARZ; WINTERHALTER, 2007). Estes pigmentos se formam a partir de reações, como a vitisin tipo A através da reação do ácido pirúvico em antocianinas, vitisin B pela cicloadição de acetaldeído em uma antocianina, metil piranoantocianinas pela reação da acetona em antocianina e pinotin pela reação entre ácido hidroxicinâmico e antocianina (MARQUEZ; SERRATOSA; MERIDA, 2013).

Os pigmentos derivados das antocianinas, representados pelas piranoantocianinas são pouco conhecidos e estudados, portanto, poucos trabalhos foram encontrados, a maioria evidenciando o aumento dos pigmentos quando foram utilizadas leveduras que produzem maior concentração de acetaldeído e piruvato (MORATA et al., 2003), na fermentação malolática atrasada devido o esgotamento de acetaldeído e piruvato (BURNS E OSBORNE 2014), e em algumas técnicas de vinificação para aumentar flavonóides e antocianinas (RENTZSCH et al. 2007). Devem ser realizadas mais pesquisas sobre a formação destes

pigmentos, e seus efeitos nas técnicas de vinificação e na cor do vinho tinto (ANGELOSANTE, 2015).

As reações com os produtos de condensação direta/ antocianinas-flavonol ou antocianinas-antocianinas apresenta como precursores as antocianinas e flavonols que atuam como eletrófilos e nucleófilos. Nesta reação são propostos dois tipos de reações antocianinas-flavonol (A-F) e flavonol-antocianinas (F-A) (MORENO-ARRIBAS; POLO, 2009). Os produtos de condensação direta são descritos como ligações que podem ocorrer entre catequina ou galocatequina com delfinidina, petunidina, peonidina, ou malvidina-3-glicosídeo. E, entre catequina e malvidina-3-acetil glicosídeo e malvidina-3-(p-coumaroil) glicosídeo (BOIDO et al., 2006). Os produtos de condensação mediados por acetaldeído são formados a partir da fermentação com as leveduras (NAVE et al., 2010), e são características de pigmentos etilados de malvidina-3-glicosídeo condensado com catequina ou epicatequina (BOIDO et al., 2006). Em vinhos, os produtos de condensação direta e acetaldeído mediados por produtos de condensação já foram observados nos trabalhos de Alcade-Eon et al. (2006), Boido et al. (2006) e Gordillo et al. (2012).

## 1.3 OBJETIVOS

### 1.1.1 Objetivo geral

Avaliar o efeito da tecnologia TPC em uvas e o impacto sobre a composição fenólica e qualidade dos vinhos.

### 1.1.2 Objetivos específicos

- Estudar o efeito do tratamento térmico sobre a maturação industrial e fenólica de uvas ‘Tannat’ e ‘Cabernet Sauvignon’, assim como a composição fenólica dos vinhos;
- Avaliar o efeito do TPC sobre a composição antociânica dos vinhos ‘Tannat’ em diferentes safras;
- Avaliar o efeito do TPC sobre o perfil antociânico e sensorial dos vinhos ‘Cabernet Sauvignon’

## 1.4 MATERIAIS E MÉTODOS

Os materiais e métodos estão descritos nos artigos 1, 2 e 3. Os artigos foram divididos em experimentos, conforme a tabela 1.

Tabela 1 – Separação dos artigos da tese

| Artigo | Experimento | | |
|---|---|---|---|
| *Artigo 1* | *Experimento I* | *Experimento II* | |
| | Uva e vinho<br>‘Tannat’<br>Safra 2013/14. | Uva e vinho<br>‘Cabernet Sauvignon’<br>Safra 2013/14. | |
| *Artigo 2* | *Experimento I* | *Experimento II* | *Experimento III* |
| | Vinho<br>‘Tannat’<br>Safra 2011/12. | Vinho<br>‘Tannat’<br>Safra 2012/13. | Vinho<br>‘Tannat’<br>Safra 2013/14. |
| *Artigo 3* | *Experimento I* | | |
| | Vinho<br>‘Cabernet Sauvignon’<br>Safra 2013/14. | | |

# 2 ARTIGO 1 – Effect of thermal treatment in potential phenolic of grapes and wines

(Manuscrito submetido para a Revista Brasileira De Fruticultura)

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**RESUMO -** *Thermal Pest Control* é uma tecnologia alternativa que emprega calor nas vinhas para reduzir insetos e doenças fúngicas. No entanto, também poderia estar alterando o metabolismo secundário de uvas, e consequentemente nos vinhos. O objetivo desta pesquisa foi determinar o efeito do tratamento térmico no processo de maturação industrial e fenólica das uvas e composição fenólica de vinhos de 1 e 12 meses de armazenamento. Os experimentos foram realizados na safra 2013/14, em vinhas 'Tannat' e 'Cabernet Sauvignon' com cultivares de Dom Pedrito, RS. Cada vinhedo foi dividido em dois blocos para tratamentos: controle e térmico (*Thermal Pest Control* – TPC). Após a colheita uma parte das uvas foi analisada em relação à maturação industrial e fenólica e outra parte para vinificação e armazenamento de 1 e 12 meses para análises de polifenóis totais, procianidinas, antocianinas monoméricas e intensidade de cor. Na 'Tannat', o tratamento térmico TPC reduziu a acidez total, teor de polifenóis totais em uvas. Já nos vinhos, não foram observadas alterações nos polifenóis totais, procianidinas e antocianinas monoméricas. Em 'Cabernet Sauvignon', o TPC estimulou o índice de polifenóis totais e reduziu os taninos da casca das uvas, sem alterar a composição do vinho. Portanto, a utilização do TPC em videiras pode induzir o metabolismo secundário nas uvas, no vinho entretanto foi observado um efeito elicitor na intensidade de cor.

**Termos de indexação:** *Vitis vinifera*; *Thermal Pest Control*; vinificação; armazenamento, compostos fenólicos

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## ABSTRACT

Thermal Pest Control is an alternative technology that employs heat in vineyards to reduce insects and fungal diseases. However, it could also be altering secondary metabolism of grapes and hence wines. The aim of these research was to determine the effect of thermal treatment in industrial and phenolic ripening of grapes and phenolic composition of wines at 1 and 12 months of storage. The experiments were conducted in 2013/14 harvest in vineyards with 'Tannat' and 'Cabernet Sauvignon' cultivars of Dom Pedrito, RS. Each vineyard has been divided into two blocks for treatments: control and thermal (Thermal Pest Control - TPC). After harvest a part of grapes was analyzed regarding to industrial and phenolic maturation and another part was for vinification and storage for 1 and 12 months for analyzes of total polyphenols, procyanidins, monomeric anthocyanins and color intensity. In 'Tannat', TPC treatment reduces total acidity and content of total polyphenols in grapes. Already in wine, no changes were observed in total polyphenols, procyanidins and monomeric anthocyanins levels. In 'Cabernet Sauvignon', TPC stimulated total polyphenol index and reduced the bark tannins in grapes, without changing wine composition. Therefore, using the TPC field of heat treatment on grapevines can induce secondary metabolism in response grapes, wine however was observed elicitor effect in the color intensity.


**Index terms**: *Vitis vinifera*; Thermal Pest Control; vinification; storage, phenolic compounds.

# INTRODUCTION

*Vitis vinifera* cultivars are widely known to be highly susceptible to development of fungal diseases due to climatic conditions. The rain incidence, even regular, associated with wet periods and high temperatures contributes to germination of bacterial spores and provides infections in plants (GARRIDO; SÔNEGO, 2003).

An alternative technology implemented in fungal control of fields is the thermal treatment (Thermal Pest Control – TPC), initially developed in Chile by Florêncio Lazo Barra, to meet climate requirements (UNITED STATES PATIENT, 2003). This technology is efficient in drying the leaves, reducing the moisture necessary for germination of the bacterial spores, besides inactivating mycelia enzymes (FISCHER, 2012). In addition, this technology also supports insects control by burning the wings, exoskeleton breaking and dehydration leading to these pest's death.

TPC treatment, also known as Thermal Treatment Plants (TTP) can trigger biological changes in plants such as the induction of systemic acquired resistance, and in fruits the applications can increase-fruitification, skin thickening, coloration, sugars, total polyphenol and antioxidant activity (UNITED STATES PATENT, 2014). In California *Vitis vinifera* cultivars, thermal shock increased pollination and grapes production, besides providing self-defense and fruit with thicker peels that protect from moisture (FISCHER, 2012).

Abiotic stress by thermal shock promotes induction of enzymes with key activity in secondary metabolism (DIXON; PAIVA, 1995). Especially, increase in phenylalanine ammonia-lyase enzyme activity (KANG; SALTVEIT, 2003) triggers polyphenols formation, which act as antioxidants, in addition to structural action in lignin formation and the defense. Thermal treatment in grapes increases phenolic concentration, but may be affected by weather conditions and temperature (KIM et al., 2006).

Thus, based on proposal that the thermal treatment in *Vitis vinifera* plants may influence secondary metabolism of vines and consequently, the produced wine, this study aimed to determine the effect of thermal treatment in industrial and phenolic ripening of 'Tannat' and 'Cabernet Sauvignon' grapes and in wine phenolic composition with 1 and 12 months of storage.

## MATERIAL AND METHODS

Experiments were conducted in the harvest of 2013/14, in 'Tannat' and 'Cabernet Sauvignon' vineyards in Dom Pedrito city - RS (30°58´54´´S, 54°40´39´´W and altitude of 230 m). Each vineyard was divided into two blocks for the treatments: control and thermal (Thermal Pest Control - TPC), the cultural practices were the same. The experimental design was completely randomized with two treatments and four replications in each of the independent experiments.

In 'Tannat' and 'Cabernet Sauvignon' vineyards the row spacing of 3.3 m and 1.0 m between plants, totaling 3.030 plants per hectare, conducted in espalier system, and simple guyot pruning. But in 'Tannat' vineyard, grafted with rootstock (Paulsen 1103) and vineyard 'Cabernet Sauvignon' grape grafted in rootstock ($SO_4$) and pruned plants in simple royat cordon.

Equipment TPC used for 19 heat applications was tow by a trator in speed of 4 km $h^{-1}$, air at a temperature 130°C was applied by a distance of 20 cm from the vines in the flowering period until harvest. Grapes were harvested (March 12, 2014) and after, the grapes of both experiments were conducted in the laboratory of fermentation and beverage technology from Federal University of Santa Maria (UFSM).

To determine industrial maturity were quantified soluble solids (SS, °Brix), pH, total acidity (TA, g of tartaric acid 100 $mL^{-1}$) according to the methods of the OIV (2010), and calculated by the ratio soluble solids/acidity (SS/A, °Brix/g of tartaric acid 100 $mL^{-1}$). Phenolic potential were analysed according to Glories and Augustin (1993) and González-Neves et al. (2003). In order to determine the total potential in anthocyanin (ApH 1.0, mg $L^{-1}$ malvidin glucoside), potential extractable anthocyanin (ApH 3.2, mg $L^{-1}$ malvidin glucoside) and total polyphenol index by absorbance reading at 280 nm. The tannic components of the skins and seeds, their relative proportions (dpell% and Mp%) and the cellular maturity index (EA%) were calculed by Ribéreau-Gayon et al. (2006).

The winemaking was performed according to Pszczólkowski and Lecco (2011) with amendments. The destemming was initially made, manual crushing of berries and separation in 4 replicates each treatment. The must was placed into micro fermenters

(2 liters) and the sulfur dioxide addition was performed. Subsequently, enzymes were added (Lafase® He Grand Cru and Lafase® fruit, Laffort®). Then the yeast (Zymaflore® FX10, Laffort®) with probiotic additives (Superstart®, Laffort®).

The micro fermenters were sealed with water seal and sealable access for sample collection with a syringe to monitor the anaerobic fermentation. Micro-fermentation occurred in an environment with controlled temperature of 25 °C, and after one day of the beginning of fermentation, the probiotic additives (Bioactiv®, Laffort®) was added at a concentration of 40 g $hL^{-1}$. With two daily pumpings being carried out. After the maceration of eight days was carried out and monitored alcoholic fermentation by colorimetric measurement of total sugars, following Somogyi and Nelson (Nelson, 1944). Subsequently, the malolactic fermentation monitoring was performed through paper chromatography according to Daudt (1971). After malolatic fermentation, wines were filtered and bottled to be storage for 1 and 12 months.

In wines were analyzed procyanidins content (PRO, g of cyanidin chloride per $L^{-1}$) through dilutions, environment acidification and sample heated to 100°C in a water bath, and color intensity (CI) measured in absorbance units and reading in spectrophotometer of wine in cuvette of 1mm of OD, at 420, 520 and 620 nm, both methods by Ribéreau-gayon et al. (2006). Total phenolics (TP, mg of gallic acid per $L^{-1}$) was obtained by Singleton and Rossi (1965). Total monomeric anthocyanins (MA, mg of malvidin glucoside per $L^{-1}$) were determined by pH deferential method proposed by Giusti and Wrolstad (2005).

The physicochemical analyses were performed in triplicate and data were submitted to analysis of variance (ANOVA) by factorial followed by the Tukey test at 5% probability of error through the Statistica® 9.0 software.

## RESULTS AND DISCUSSION

In 'Tannat' grape berry, industrial maturation results demonstrate a reduction in TA for the thermal treatment, which reflects in the increase of the ratio SS/A, as there was no change in the SS. These crop parameters in cultivar Tannat with TPC favor a correct vinification, without need of sugar addition for ethanol production. Moreover,

this reduction in TA did not change the pH, which can give a greater stability to the wine.

In 'Tannat' phenolic maturation, a TPI reduction in grapes with thermal treatment is observed (Table 1), possibly due to the interference of other non-phenolic compounds which also have benzene rings in their structure as the amino acids phenylalanine and tryptophan. In another parameters of phenolic maturity there were no changes in extractabilities of anthocyanins (ApH 1.0) and in anthocyanins that simulate the fermentation tanks (ApH 3.2). Cell walls integrity, represented by EA% was also not affected by TPC, thus providing a good mechanical strength of the berries.

TPC treatment did not influence phenolic composition of wines (Table 2). However, with storage there was an increase of total polyphenols among them procyanidins. The anthocyanins reduced during wine storage of 1 month to 12 months, suggesting that it is probably related to polymeric pigments formation. In accordance with present work were García-Falcón et al. (2007) and Marquez et al. (2013) who also observed a decrease in anthocyanin during the storage period of wine.

‘Cabernet Sauvignon’ grapes, it is noted that the thermal treatment did not influence in any of industrial maturation parameters. It can be inferred that climate has possibly influenced them, it is noted that in the harvest in February there was a greater rainfall volume (Figure 1), and unlike the 'Tannat', it stimulated the TPI (Table 1). But this difference of TP was not observed in wine, except the color intensity increased with TPC. As in 'Tannat', an increase of TP with storage of 1 month to 12 months, but without addition procyanidins (Table 2).

By observing the reduction of skin tannins (Table 1), it can be assumed that they were oxidized with thermal treatment. However, they do not seem to have affected quality, since total polyphenols in wine were not changed (Table 2).

The total polyphenol analysis performed in this work quantifies all the phenolic structures with hydroxyls, including procyanidins and anthocyanins. However, it is noted that the total polyphenols stimulation in wines with TPC at 12 months does not seem to be related to procyanidins and anthocyanins, because they do not show

alterations, possibly because they are related to seed tannins (Table 1), or the tannin and stilbene, which were not quantified in this work.

The TPC thermal treatment also favored increase of color intensity (Table 2) in both cultivars after 12 months stored. Increased color of red wine to consumers is associated with rise in quality and acceptability (Parpinello et al., 2009; Marquez et al., 2014).

## CONCLUSIONS

Therefore, TPC treatment influences the industrial maturity of 'Tannat' grapes reducing the total polyphenol content, without changing extractability. In addition also changes phenolic maturity of 'Cabernet Sauvignon' grapes, as it stimulated total polyphenol index and tannins in the seeds.

In wines, thermic treatment had little influence on the physicochemical parameters, except color intensity. During the storage there was an increase in total polyphenols and reduced monomeric anthocyanin. In view of this, studies are needed on the profile of anthocyanins detailing the effect on the wine, since there are influences on grapes.

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Table 1 – Industrial and Phenolic maturation produced with grapes, under effect of thermal treatment (TPC), in Dom Pedrito, RS, harvest 2013/14.

| Analysis | 'Tannat' | | 'Cabernet Sauvignon' | |
|---|---|---|---|---|
| | Control | Thermal | Control | Thermal |
| Industrial maturity | | | | |
| TA* | 0.73[a*] | 0.69[b] | 0.56[a] | 0.56[a] |
| pH | 3.63[a] | 3.63[a] | 3.94[a] | 3.98[a] |
| SS | 19.5[a] | 20.2[a] | 20.2[a] | 20.2[a] |
| SS/A | 26.7[b] | 29.1[a] | 36.0[a] | 36.0[a] |
| Phenolic maturity | | | | |
| TPI** | 173.8[a] | 113.8[b] | 50.0[b] | 118.0[a] |
| ApH 1.0 | 1441.6[a] | 1383.6[a] | 749.2[a] | 749.0[a] |
| ApH 3.2 | 401.2[a] | 472.8[a] | 546.2[a] | 486.4[a] |
| EA% | 72.2[a] | 65.8[a] | 27.0[a] | 35.0[a] |
| dpell% | 9.5[a] | 16.6[a] | 43.6[a] | 16.7[b] |
| Mp% | 90.4[a] | 83.3[a] | 56.3[b] | 83.2[a] |

*Equal letters between treatments in the same cultivar and parameter do not differ according to the Tukey test at 5% probability of error. TA, total acidity (g $100mL^{-1}$); SS, soluble solid (°brix); SS/A, soluble solid/ acidity ratio; TPI, total polyphenol index; ApH 1,0, potential anthocyanin; ApH 3,2, extractable anthocyanin; EA, extratibility anthocyanin; dpell, skin tannin; Mp, seed tannin

Table 2 – Phenolic composition of the wines produced with grapes, under effect of thermal treatment (TPC), in Dom Pedrito, RS, harvest 2013/14.

| Analysis | 'Tannat' | | 'Cabernet Sauvignon' | |
|---|---|---|---|---|
| | Control | Thermal | Control | Thermal |
| Wine (1 month) | | | | |
| TP | $1599.0^{aB}$ | $1527.8^{aB*}$ | $1097.8^{aB}$ | $1155.7^{aB}$ |
| PRO | $0.55^{aB}$ | $0.33^{aB}$ | $1.19^{aA}$ | $1.26^{aA}$ |
| MA | $465.2^{aA}$ | $442.5^{aA}$ | $270.7^{aA}$ | $228.9^{bA}$ |
| CI | $1.39^{aA}$ | $1.08^{bA}$ | $0.33^{bB}$ | $0.44^{aA}$ |
| Wine (12 months) | | | | |
| TP | $2377.6^{aA**}$ | $2377.6^{aA}$ | $1353.2^{aA}$ | $1359.4^{aA}$ |
| PRO | $2.78^{aA}$ | $2.77^{aA}$ | $1.32^{aA}$ | $1.40^{aA}$ |
| MA | $175.9^{aB}$ | $241.9^{aB}$ | $135.0^{aB}$ | $144.6^{aB}$ |
| CI | $1.21^{aB}$ | $1.18^{aA}$ | $0.42^{bA}$ | $0.49^{aA}$ |

*Averages with the same small letters did not differ between treatments in the same cultivar by Tukey test ($p < 0.05$). **Averages with the same capital letter do not differ between months in the same cultivar by Tukey test ($p < 0.05$). TP, total polyphenols (mg $L^{-1}$); PRO, procyanidins (g $L^{-1}$); MA, monomeric anthocyanins (mg $L^{-1}$); CI, color intensity (units of absorbance).

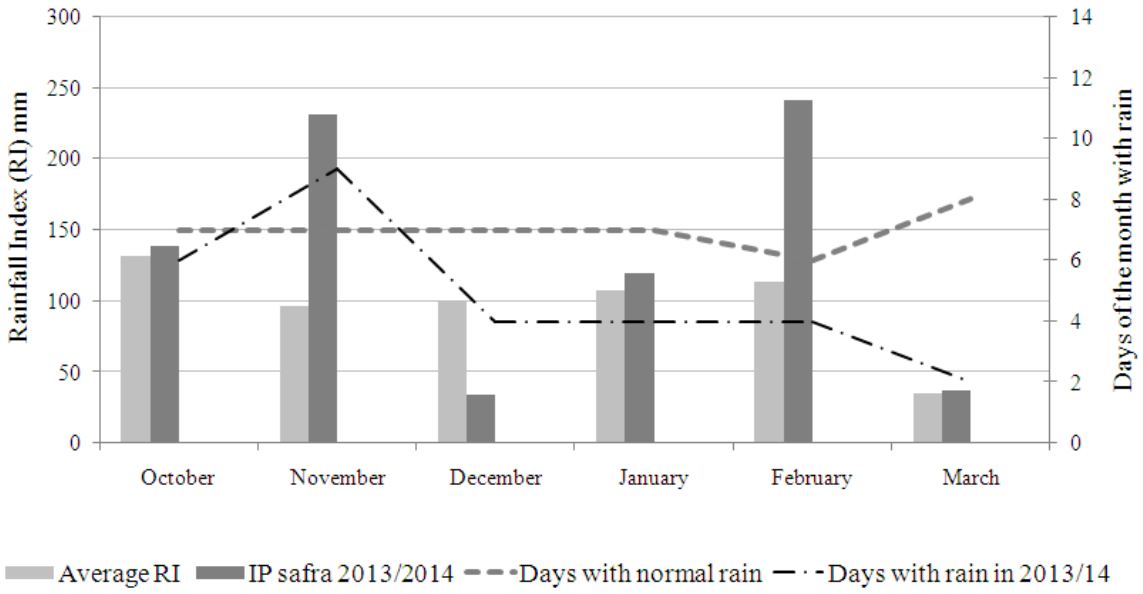


Figure 1 - Normal Rainfall Index and of the 2013/14 in flowering at harvest in Dom Pedrito, RS (INMET, 2014).

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# 3 ARTIGO 2 - Thermal Pest Control in 'Tannat' grapes: effect on anthocyanins, sensorial and color of one year old wines.

(Manuscrito submetido à Revista *Journal of Food Science and Technology*)

**Abbreviated running title:**

**Thermoculture on grapes: effect in Tannat wine anthocyanins and sensorial**

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**Research highlights**

1. Thermal Pest Control (TPC) effect on wine quality is evaluated for the first time.
2. TPC with 4 applications was able to increase anthocyanins content and improve sensory parameters.
3. 19 applications had increased anthocyanins without improvement in sensorial quality and color.
4. Climate changes seem affect TPC results on wine quality.

**Abstract**

TPC is a technology that applies heated air in the vineyard to reduce pests. However, it also could alter anthocyanins synthesis in answer to temperature increase. This work aimed to determine the impact on wine anthocyanins, sensorial attributes, and color due to the application of TPC in 'Tannat' grape vineyards. HPLC-PDA-$MS^n$ was used to characterize wine pigments, sensorial quantitative descriptive analysis was performed using a trained panel, and color was determined spectrophotometrically. In experiment I, 20 peaks were detected and TPC was able to increase all pigment families and color ($p \leq 0.05$). Sensorial was also affected by TPC in visual descriptor of translucent and the gustatory descriptors of woodsy and body. Five TPC applications (Experiment II) were not able to significantly alter wine pigments which could be due to the climate conditions during this harvesting year. Even with a high rain incidence, 19 applications from flowering to harvesting (Experiment III) was able to increase anthocyanin content (30.6%) and direct condensation products (11.5%). Finally, TPC was able to alter anthocyanins in wines produced with treated 'Tannat' grapes even when only 4 applications from flowering to fruit set were used. Nonetheless, results are dependent of weather conditions and when TPC has resulted in the greatest anthocyanin increments some wine sensorial descriptors were also improved.

**Keywords:** grape management, thermoculture, tandem mass spectrometry, pyranoanthocyanins, condensation products, descriptive sensorial analysis.

## Introduction

Thermal Pest Control (TPC) is a technology that applies heated air in high speed to the vineyard to reduce contamination by fungus and pests (Fischer 2012). As the major advantage of this technology is the possibility to develop a green manage practice in vine that reduces the usage of pesticides (Barra 2006). This practice is also referred as Plant Thermic Treatment (PTT) or Thermoculture and it was developed and patented by Florêncio Lazo Barra (Barra 1999) in Chile.

Nowadays, this technology is being tested in some commercial vineyards in south of Brazil. 'Tannat' grape has robust growing and high productivity in climate conditions of south Brazilian region (Miele et al. 2003). However, the wet climate is this region also increases the possibility of pest diseases by fungus, as *Glomerella cingulate* (Chavarria et al. 2007). Thus, TPC treatment conditions were studied (Fischer 2012) and are being tested in commercial vineyard to reduce crop losses in the intent for organic grape production. In reviewed literature there are no reports about the impact of this management practices in relation to wine quality and in target compounds linked to color and flavor characteristics.

Adverse environment conditions could induce plant metabolic pathways to endurance, increasing target compounds linked plant defense (Bartwal et al. 2013). Physical variations that are able to positive modulate the synthesis of defense metabolites are called as abiotic elicitors (Radman et al. 2003)(Naik and Al-Khayri 2016) . It is already known that thermic stress is able to stimulate secondary metabolism, increasing the synthesis of flavonoids (Kang and Saltveit 2003).

Anthocyanins belong to flavonoid class of polyphenols produced in secondary metabolism. In grapes, these compounds are the major natural pigments responsible by color hue and intensity, having also a significant impact in fruit astringency (Flamini et al. 2013). For vine development, they also play an important role in plant defense against temperature increment resulting in

grapes with greater skin thickness and color intensity (Fong et al. 1974). During winemaking, the initial levels of anthocyanins and the chemical diversity in relation to composition have significant impact in pigment transformation reactions and in the final product color and sensorial characteristics(Moreno-Arribas and Polo 2013).

Plant stress due to the increase in temperature ranges was already related with increased levels of anthocyanins in Cabernet Sauvignon and Grenache grape cultivars (Bergqvist et al. 2001). Same behavior was also observed for Merlot grape cultivars as reported (Cohen et al. 2008). Thus, TPC treatment could induce to a short-time thermic stress in the vineyards increasing target compounds for fruit defense, as anthocyanins.

The company that developed an equipment for TPC treatment (Agrothermal Systems, New Zealand) have already reported the effect of this technique on total polyphenols and anthocyanins for Cabernet Franc wine (Winemaker 2015). This work has shown that thermic treatment was able to increase total levels of this compounds mainly for free anthocyanin in grapes. These results reinforces the hypothesis that TPC treatment could induce plant to a thermic stress resulting in greater amounts of some secondary metabolites enrolled in plant defense to adverse conditions. However, there is no work on literature that has determined if the incremented levels of fruit anthocyanins have a significant impact in pigment transformations during wine production and if it could also affect the final product quality in relation to color characteristics. These questions could only be answered using modern analytical tools as high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-$MS^n$). Studies like this could determine if this pest control technique could also produce grapes with greater winemaking characteristics.

Thus, this work has aimed to determine the impact of *Thermal Pest Control* treatments during crop management of different ‘Tannat’ grape vintages on anthocyanin composition and sensorial wine quality of one year bottled aged wines.

## Materials and methods

### Grapes and treatments

'Tannat' grapes were cultivated in Brazil southwest regions with similar edaphoclimatic conditions at crops years of 2011/12, 2012/13, and 2013/14. Every treatment was applied with a distance of 2 blocks in three separate regions of the Vineyard. Since there were differences among climate conditions of each year in the vineyard regions, every date was considered as an independent experiment and different Thermal Pest Control (TPC) conditions were studied. Experimental design was randomized with a total of two treatment (TPC group *versus* control group) performed in quadruplicate (n=4) in every experimental year. All TPC treatments were applied in partnership with wineries and standardized as a part of industrial projects to reduce the use of additives during crop management.

Thus, experiment I was performed in a vineyard from Santana do Livramento (30°53´27´´S, 55°31´58´´W at 208 meters, Rio Grande do Sul, Brazil) in 2011/2012, being harvested in the beginning of 2012. Equipment (Thermal Pest Control, Lazo TPC do Brasil Ltda) used for heat application (TPC) was tow by a tractor in a constant speed of 2 km/h. Air at a temperature of 140 °C was applied by a distance of 20 cm from grapes espaliers. TPC applications were used in a total of four applications from flowering to fruit set. Experiment II was performed in the same local and conditions than in the first. However, an additional TPC application was performed in the vineyard summarizing a total of five, only in the fruit set. Since, grapes were harvested in beginning of 2013, crop year date is better represented as 2012/2013.

A third experiment (Experiment III) was performed in the city of Dom Pedrito (30°58´54´´S, 54°40´39´´W at 230 meters, Rio Grande do Sul, Brazil) during 2013 to 2014. At this time, equipment was towed in a speed of 4 km/h. Air was heated at 130 °C and applied

by a distance of 20 cm from espaliers. There was 19 TPC application dates equally delivered from flowering to harvesting.

For all experiments grapes were harvested in industrial maturity (average results of 22.1 to 24.0 ºBrix of total soluble solids, pH values of 3.00-3.86, and 0.65-0.75 g tartaric acid 100 $mL^{-1}$ of total acidity).

**Wine sample preparation**

Winemaking was performed in micro scale in the Laboratory of Fermentation and Beverage technology that belongs to the Núcleo Integrado de Desenvolvimento em Análises Laboratoriais (NIDAL) from Universidade Federal de Santa Maria (UFSM). Processing technology was performed as previously described Pszczólkowski and Lecco (2011) with some modifications. Must was placed in micro fermenters (2 liters) and added of sulphite, pectolytic enzymes (pectinase, Lafase® He Grand Cru and Lafase® fruit, Laffort® Company, Bordeaux, France), yeast (Zymaflore® FX10, Laffort®), and probiotic additives (vitamins and minerals, Superstart® and Bioactiv®, respectively, Laffort®). Malolatic fermentation was monitored by paper chromatography as preconized by (Daudt 1971). After malolactic fermentation, wines were filtered and bottled to be storage for 12 months of aging.

**Identification of anthocyanins in wine samples by HPLC-PDA-$MS^n$ analysis**

Wine samples were previously purified by solid phase extraction in C-18 cartridges (Stracta®, 6cc, 500 mg of octadecylsilyl resin, Phenomenex, Torrance, EUA) to obtain a separated fraction of anthocyanins free of other polyphenols, sugars, and organic acids as preconized by (Rodriguez-Saona and Wrolstad 2001) with some modifications already reported (Bochi et al. 2015). Previously to injection, dried fraction of anthocyanins was dissolved in acidic water (0.35% v/v of formic acid), made up to a known volume (2 mL) and filtered (0.22µm, PTFE syringe filters, 25 mm, Simple pure, Allcrom, SP, Brazil).

Separation was performed in a reverse phase column (C18, 2.6µm, 100 mm x 4.6 mm, Phenomenex, Torrance, USA) thermostated at 38˚C. The HPLC-PDA equipment was a Prominence 20 A (Shimadzu, Japan) equipped with degasser (DGU20A5 prominence, Shimadzu, Japan), column oven (CTO-20A prominence, Shimadzu, Japan) and coupled to a PDA detector (SPDM-20A prominence, Shimadzu, Japan). Gradient elution for separation was performed using two mobile phases named as (A) an acidic solution (3% v/v of formic acid, 85%, Merck®) in ultrapure water (Milli-Q Gradient System, Millipore Corporation, Massachusetts, EUA) and (B) pure acetonitrile (HPLC grade, Merck, Darmstadt, Germany). Column initial condition was 10 % of B and 90% of A. During first 18 minutes a B concentration was increased until 18%, than B was increased to 50% in 2 minutes (from 18 to 20 minutes) and column was kept for 10 minutes (from 20 to 30 minutes) at an isocratic condition. After elution of the last peak, B concentration was increased to 100% (from 30 to 32 minutes) to clean-up column (from 32 to 37 minutes) and initial conditions (10% of B) were set to be reached in two minutes (from 37 to 40 minutes) and kept for 5 minutes for re-equilibration . Flow rate was set at 0.9 mL $min^{-1}$. DAD signal was recorded from 250-800 nm.

The HPLC system described above was connected to ion trap mass spectrometer (Esquire 6000, Bruker Daltonics, Billerica, MA, USA) equipped with an electrospray interface operating in positive mode. The ESI conditions were as follows: capillary voltage of +4.5kV, nebulizer gas pressure at 30 psi, dry gas at 11 mL $min^{-1}$, and gas temperature at 310 °C. MRM experiments were performed in a full scan range of 200 to 1000 m/z of all fragments formed from 3 major parent ions per second.

**Quantification of anthocyanin in wine samples by HPLC-PDA analysis**

Analysis for quantification of individual anthocyanins was performed in previously dealcoholized (39°C, 10 minutes, Rotavapor® R-300, Buchi, Labortechnik, Germany) wine samples (5 ml). Prior to injection samples were made up to the initial volume (5 ml) using

mobile phase (A) and filtered (0.22µm, PTFE, Phenomenex). The Prominence HPLC Shimadzu 20A and the conditions already described in section 2.3 were used for separation in quantification analysis. A standard curve of malvidin-3-glucoside (Oenin chloride, 97%, Sigma-Aldrich, St. Louis, MO, U.S.A) was developed for quantification and used to determine linearity. Limits of detection (LOD) and quantification (LOQ) were determined as preconized by(ICH 2005)

**Color parameters and monomeric anthocyanin**

Samples were spectrophotometrically (FEMTO, 600 plus) analyzed as described by (Ribéreau-Gayon et al. 2006) using cuvettes of 1mm of optical pathway. Color intensity, Hue value, at 420, 520, and 620 nm were the analyzed parameters. Total monomeric anthocyanins were determined by pH deferential method (Giusti and Wrolstad 2005)

**Sensorial Quantitative Descriptive Analysis (QDA)**

Training and testing sections were performed in the Food Technology and Science department of the Federal University of Santa Maria after project approval by UFSM ethical committee (number 23044813.1.0000.5346). The Quantitative Descriptive Analysis (QDA) procedures were used as previously described by (Stone and Sidel 2004) with some necessary modifications. Judgers were selected and trained as detailed by (Behrens and Silva 2000) using tests for basic taste recognition by triangular discriminative analysis and specific sensorial training sections. Descriptors for aroma were determined as described in the "Standard Terminology of Wine Aroma" published by Noble et al. (1987). Trained panelists must have 90% of accuracy in three test repetitions. The final trained panel was composed of one man and seven women which in additional training section have determined descriptors and standards that should be used to describe 'Tannat' wines (see supplementary material for more information about sensorial descriptors and standards). All sensorial sections were performed in appropriate closed cabins with white tables equipped with openable windows for

samples delivery and superior lights in red, yellow, and tungsten lamps. Sections have a total of two hours long per day, and it was performed twice per week for a whole month, summarizing a total of 8 sections. All attributes were analyzed using a non-structured scale of 9 cm that started with the word “nothing” and “weak” as the minimal score, and ended with the word “strong” for the maximum score. Following this test, judgers have performed the acceptation test using a 7-point verbal hedonic scale which has started in1 for extremely dislike and ended in7 for extremely like.

**Data Statistical Treatment**

Every experimental results (experiments I, II, and III) were submitted to one-way analysis of variance (ANOVA) separately. Post-hoc mean comparison in physicochemical and chromatographic analysis was performed by Tukey test at 5% of probability error using the software Statistica® 9.0 (Statsoft).

Results from tests used during training were evaluated by ANOVA for each panelist to determine its discriminative capacity, accuracy, and agreement level with all panelists. F-test and error probability was used to evaluate sample ($F_{sample}$, p-value) and among repetitions ($F_{repetition}$, p-value) for each tester in every attribute. Only testers with satisfactory description capacity ($p_{sample} < 0.50$), accuracy ($p_{repetition} \geq 0.05$) and agreement with more than 90% all other testers were considered as trained (FARIA; YOUTSUYANAGY, 2002).

**Results and discussion**

**Identification of major ‘Tannat’ wine pigments**

Naturally, during the wine making process some chemical reactions between anthocyanin monomers, polyphenols, and fermentation products could occur giving raise to new wine pigment families as follows: direct and acetaldehyde-mediated products, caftaric

acid and anthocyanin adducts, anthocyanin-flavanol complexes, anthocyanin-anthocyanin condensation products, and pyranoanthocyanins (Moreno-Arribas and Polo 2013). Some of these compounds were found in 'Tannat' sample wines. A tentative identification is proposed for each of the detected compounds as show in Table 1. It was based on information obtained from ultraviolet to visible electromagnetic spectrum, mass spectra fragmentation patters, and elution order. The identification of each one of these wine pigments will be discussed separately as follows.

**Anthocyanin monomers**

In all studied groups of 'Tannat' wine samples it was identified 3-glucoside monomers of Delphinin, Petunidin, Peonidin, and Malvidin (Table 1). Identification of these monomers followed a logical elution order in reverse-phase chromatography and also an expected fragmentation pattern in mass spectrometry. The neutral loss of e 162 m/z possibly correspond a hexose moiety that was identified as glucose due to the abundance of this sugar linked to anthocyanins in nature. These identification results were previously reported for 'Syrah' wine samples with 12 months of aging with same spectral characteristics and elution order in reverse-phase chromatography (Gordillo et al. 2012). The major anthocyanin pigment in all 'Tannat' samples was Malvidin-3-glucoside (peak 12, Figure 1).

Acylated monomeric anthocyanins with acetyl group were detected by a fragment loss of the dehydrated acid mass structure (64 m/z) followed by the same pattern described by for non-acylated anthocyanins in 'Tannat' samples (peak 20, 24 and 25, Table 1). Acylation with p-coumaric acid by a neutral loss of acid dehydrated structure (146 m/z) were also observed in peak 27. Two different p-coumaroyl derivatives were differenced by mass spectrometry and in this peak which were identified as Petunidin-3-(p-coumaroyl)glucoside and Malvidin-3-(p-coumaroyl)glucoside (Table 1).

Usually ‘Tannat’ grape varieties have a greater quantity anthocyanin acylated with acetic acid than with p-coumaric (González-Neves et al. 2004) which is in agreement with our results. As already discussed (Boido et al. 2006), this fact could be attributed by hydrolitic reactions that occur during fermentation process and wine aging, and also, by the lower extractability of p-coumaroyl glucoside derivatives during the winemaking process.

**Pyranoanthocyanins**

Pyranoanthocyanins are produced during fermentation and wine aging as a result of condensation reaction between yeast metabolites and anthocyanins (Moreno-Arribas and Polo 2013). Major pyranoanthocyanins detected in this work were A-type vitisin in all wine samples. High concentration of these compounds was previously detected in different works with ‘Tannat’ wine (Alcalde-Eon et al. 2006; Boido et al. 2006). These compounds are mainly formed due the cycloaddition of pyruvic acid to anthocyanins. Pyruvic acid adducts were observed in peaks 10, 13, 15, 17, 18, 19, 26, and 27. In all of those peaks, adduct presence was detected by aglycon fragments with 68 amu in mass to charge expected values. Thus, A-type vitisin for Petunidin, Peonidin, and Malvidin showed as the aglycon fragments m/z values of 385, 369, and 399, respectively. Losses of hexose and dehydrated organic acids (acetic and p-coumaric) from pyranoanthocyanins structures were similar to that observed for ‘Tannat’ monomeric anthocyanins which possible are precursors of these wine pigments. Except for A-type vitisin of Delphinidin-3-p-coumaroil-glucoside, all pyranoanthocyanins were already reported (Boido et al. 2006; Alcalde-Eon et al. 2014) in Uruguay an ‘Tannat’ wines.

Formed due cycloaddition of acetaldehyde into anthocyanin structures, B-type vitisin were identified as present in peaks 16, 20, and 27 (Table 1). These compounds showed as major fragment loss the malvidin mass to charge ratio plus 24 amu (355 m/z) from acetaldehyde adduct formation. It is relevant to highlight that peaks 20 and 27 are co-elution

of anthocyanin pigments and UV to visible spectra obtained by DAD detection could not be used to reinforce the identification because they do not correspond to absorptions of pure compounds. Nonetheless, for peak 16 no co-elutions were observed by mass spectrometry analysis and PDA is in agreement with an expected behavior for this class of wine pigments with a hypochromic shift of 17 nm in the maximum absorption wavelength (505 nm) in relation to its monomeric anthocyanin precursor (522 nm, peak 12). B-type Vitisin were already reported as showing hypochromic shift in relation to the same anthocyanin without the acetaldehyde adducts (Boido et al. 2006).

**Direct condensation products**

Anthocyanins and other flavonoids in must and wine are electrophilic and nucleophilic substrates for direct condensation reactions yielding anthocyanin-flavanol (A-F) or flavanol-anthocyanin (F-A) condensed structures (Moreno-Arribas and Polo 2013).These compounds were extensively identified in wines from different cultivars (Alcalde-Eon et al. 2014; Willemse et al. 2015; Dipalmo et al. 2016) and also in different 'Tannat' wine samples (Boido et al. 2006; Alcalde-Eon et al. 2006). Results obtained for these work also detected and identified peaks 2, 4, and 5 as direct condensation products due to the characteristic fragmentation pattern of molecules containing catechin linked to glycosylated structures of petunidin, peonidin, and malvidin. This is in agreement with fragmentation pattern observed (Boido et al. 2006) for the same compounds in 'Tannat' wine samples.

Peak 1 was also identified as a direct condensation product of a malvidin-3-glucoside molecule with gallocatechin (molecular ion of 797 m/z) due to a characteristic neutral loss of a deprotonated molecule of gallic acid resulting in a fragment of 467 m/z ($M^+$-168). Moreover, the also detected fragment of 635 m/z is due to a hexose neutral loss from molecular ion ($M^+$-162) confirming that condensed structure was formed in a glycosylated anthocyanin molecule. Elution order is in accordance with an expected behavior for reverse

phase chromatography in with gallocatechin compounds should have a shorter retention time than same anthocyanin direct condensation product with catechin (peak 1 versus peak 5).

Peak 15 was detected as condensation structure of acylated anthocyanins with catechin and identified as malvidin-3-acetylglucosyde-catechin and malvidin-3-p-coumaroylglucosyde-catechin, respectively (Table 1). Besides the characteristic fragmentation pattern, these compounds have exactly 42 amu and 146 amu higher m/z values than that observed for peak 5 (Malvidin-3-glucosyde-catechin), respectively. These masses are the same that can be expected for acetyl and p-coumaroyl structure fragments which is in agreement with proposed identification. Same condensation products were already reported for 'Tannat' wine (Alcalde-Eon et al. 2006; Boido et al. 2006).

**Acetaldehyde-mediated condensation products**

Acetaldehyde-mediated condensation products are formed during must fermentation by starter yeast added in the winemaking process which has been identified in many other studies about wine pigments composition (Boido et al. 2006; Nave et al. 2010; Gordillo et al. 2012; Ivanova-Petropulos et al. 2015). Thus, peaks 21 and 22 were tentatively identified as ethylated malvidin-3-glucoside condensed to catechin and epicatechin molecules, respectively (Table 1). In both cases, molecular ion of 809 m/z was fragmented into 647 m/z due to the neutral loss of as hexose fragment ($M^+$-162), a fragment ion of 519 m/z was formed due catechin loss ($M^+$ -290), and the ionwith an m/z value of 357 was detected as being acetylated aglycon structure (ethyl-malvidin). Distinction between isomers is due to different elution order in which epicatechin should have longer elution times.

In this class of wine pigments, peak 27 showed a co-elution of two ethyl derivate compounds formed from malvidin-3-acetylglucoside and malvidin-3-pcoumaroylglucoside condensed to catechin (Table 1). These compounds showed molecular ions of 851 and 955 m/z that generated the ion 357 m/z in both cases. This mass to charge ratio is expected for

ethylated malvidin aglycon fragment. Furthermore, ion 955 m/z was linkage into 665 m/z fragment ion due to catechin fragment loss ($M^+$ - 290). Same compounds were also observed in previous work with 'Tannat' wine samples (Boido et al. 2006; Ivanova-Petropulos et al. 2015)

**Unknown pigments**

A total of 40 compounds were detected by mass spectrometry analysis in which only 6 of then remained without identification. Some of these peaks (3, 8, and 23, Table 1) showed aglycon fragments (m/z 301, 331, and 331, respectively). However, fragmentation patters and spectral characteristics do not allowed a conclusive identification. The total area of unknown compounds peaks summarize less than 2.18±0.77% of total area and a final tentative identification of almost 97,82±0.77% of the total area in the chromatogram.

**Effect of Thermal Pest Control on 'Tannat' wine pigments**

Experiments were conducted over different years (2011/12, 2012/13, and 2013/14) in different crop regions. Because of it, each experiment has a control group that was managed simultaneously to treated grapes in the closest area as possible. Moreover, data will be statically treated and discussed separately to avoid mistaken conclusions since each experiment is independent and represents the study of TPC in different conditions.

**Experiment I**

Identification analysis of wine anthocyanins produced with grapes managed in 2011/12 (Table 1) do not show any difference between control and treated grapes in relation to synthesis of new anthocyanins. However, ANOVA results revealed a significant treatment effect in the concentration of some pigments that were in higher concentration in 'Tannat' wines produced with treated grapes (Table 2).

Figure 2 has a graphical representation of the total percentage that was changed for each wine pigments classes due to TPC treatment. For this evaluation, levels in the non-

treated samples (control group) were considered as the total initial content that could be observed in 'Tannat' wine at the experimental conditions with TPC as a management technique. Thus wines that received the thermic treatment showed an increase of 49.8% in total monomeric anthocyanin levels, 51.4% in pyranoanthocyanins content, 7.0% in direct-condensation products, and 39.6% in acetaldehyde mediated condensation products (Figure 2A). Thus, heated air used for TPC treatment in 2011/12 experimental conditions contributed to increase final content of all wine pigments in one years aged 'Tannat' wine samples. It possibly is a result of the thermic stress determined by the treatment that produced an elicitor effect in grape changing all anthocyanin pigments in wine produced with this fruits.

It is possible to perceive in quantification results for each detected peaks (Table 2) that there is a significant increase in the levels of some pigments for wines produced with TPC-treated samples. For monomeric anthocyanins, only peak 12 assigned as malvidin-3-glucoside was increased in wine of treated grapes (Table 1). It is in accordance with results obtained in the total quantification of monomeric anthocyanins by pH-differential method (Table 3) in which results were also higher in wine from treated samples ($p \leq 0.05$).

Pyranoanthocyanins in peaks 10 and 13 named as pyruvic acid condensation products (A-type Vitisins) of Petunidin-3-glucoside and Peonidin-3-glucoside (Table 1), respectively, were also detected in higher concentration in wine samples produced with thermic treated grapes (Table 2). Higher levels of this wine pigments possibly have occurred due to the higher concentration of precursor anthocyanins present in grapes after TPC treatment. As reported (Downey et al. 2006), warm climates during crop management are strongly linked to synthesis stimulation of acylated forms of anthocyanins. Thus, the thermic effect in anthocyanins due to warm climates could be similar to that observed after TPC treatment in 'Tannat' wines.

Even using reverse phase columns recognized by its high resolution obtained with smaller core-shell particle sizes (2.6 µm) than the conventional totally porous particles used in

HPLC-reverse-phase chromatography columns (5 µm), the chromatographic method was not able to efficiently separate some pigments resulting in some co-elutions (peaks 4, 15, 20, 24, 26, and 27) detected by mass spectrometry. From all, just in peaks 15, 26, and 27 it were observed increased levels due to treatment. Independently of the individual separation of these compounds, it is possible to conclude that TPC vine treatment resulted in higher levels of anthocyanins and its condensations products in wine.

Additionally, the wine color seems to be altered by increased levels of these pigments in wines that were produced with 2011/12 TPC treated samples (Table 3). Since, the wine produced with TPC treated samples showed higher absorbance in all analyzed wavelength charactering a wine with greater color intensity and quality.

As reported (Parpinello et al. 2009) consumers color preference for Italian Novello red wines evaluated in tasting sessions showed that high colored wines were associated with high quality ratings. However, for (Marquez et al. 2014) showed that small changes in color intensity among young and aged wines do not alter acceptability for specialized testers. It is in agreement with sensorial QDA results (Figure 3A) which has shown that 'Tannat' wines from TPC treated grapes do not have higher acceptation than wines from control even with a greater amount of anthocyanins and derivate pigments (Figure 2A). However, TPC treated grape have resulted in wines with significant reduction in visual translucent and gustatory woodsy attributes and higher scores for gustatory body perception ($p \leq 0.05$, Figure 3A). Wines were aged in bottle and gustatory perception of woodsy taste is majorly linked to reserve wines aged in wood barrels. Thus, the reduction of this descriptor perception cannot be easily linked to our treatment, but it could be a consequence of the greater amount of monomeric anthocyanins in wines from TPC treated grapes than in control (Figure 2 A). Moreover, these monomeric anthocyanins are usually enrolled in formation of condensation products,

vinylflavonol and vinylcatechol adducts formed in wines during aging resulting and possible linked to reserve 'Tannat' wines (Boido et al., 2006).

Significant reduction of translucent descriptor in wines from TPC treated grape group could be easily linked to the greater amount of wine pigments in these samples than in control. Wine body gustatory perception is usually linked to tannins since with anthocyanins this compounds seems to have a smaller contribution. Thus, TPC could also have effect on other polyphenols composition, as condensed tannins. In summary, at the conditions used in the experiment I, TPC in grapes seems to be able of not only increase anthocyanins in 'Tannat' wine, but also alter sensorial quality.

**Experiment II**

The second experiment was performed in crop years of 2012/13 of the same 'Tannat' grape vineyard used in experiment I. However, climate conditions. Identification and results of individual anthocyanin pigments quantification in wines produced during experiment II are shown in Tables 1 and 2. A total of 24 peaks were detected, some of them showed a reduction in wines of treated samples but without significance by the statistic treatment of data (Table 2).

The summary of total compounds quantified by colorimetric assay for total monomeric anthocyanins by pH differential method (Table 3) showed a lower concentration of these compounds in wines from treated grapes than wines from control. These differences were not detected using HPLC-PDA method, possible because the pH differential method is better applied for quantification of monomers being less sensitive for condensation products formed during wine aging. Thus, since no changes were observed in none of detected compounds and neither in the sum of all quantified anthocyanins and derivate compounds, it seems that conditions used for TPC in grapes had no effect on wine anthocyanins and derivate

pigments. It could be a result of weather conditions during the experiment II with a higher rain incidence, smaller differences between maximum and minimal temperatures, and smaller average of sun exposure hours during the 15 days before harvest (see supplementary material, Figure 2B) resulting in a more humid and unfavorable climate for anthocyanin synthesis. Finally, it is interesting to highlight that in the weather and TPC conditions used in experiment II no color changes were observed between treated group *versus* control samples (Table 3). Moreover, the sensorial analysis by a trained panel have not perceived any significant difference between wines from treated and untreated samples which is in agreement with HPLC results and instrumental color analysis.

**Experiment III**

As already detailed, this experiment was performed in different regions than for experiment I and II. This place has also distinct climate conditions (seem supplementary material, Figure 2C). Moreover, TPC was applied from flowering to harvesting in a total of 19 applications in younger vineyards than in experiments I and II. Rainfall incidence in these region (802 mm) was similar to the precipitation observed in experiment II (771 mm), but higher than for experiment I (455 mm). However, TPC treatment was applied from flowering to harvesting summarizing a total that is at least 4 times greater in number of applications than in previous experiments. Thus, it is expected that the increase in TPC application number should minimize the effect of rain in plant answer to the thermic stress induced by treatment. In agreement with this hypothesis, it could be observed (Figure 2B) that the wine produced with treated grapes showed an increase of 30.6% in monomeric anthocyanin content and 11.5% in direct condensation products. However, for pigments formed by reaction of anthocyanin with fermentation products there is a reduction of 16.7% in pyranoanthocyanin

content and 26.2% in acetaldehyde-mediated condensation products (Figure 2B) for one year aged wines.

For the monomeric anthocyanin pigments in wine it could be observed in peaks 11, 12, and 25 an increase in quantified levels for wines produced with treated samples. This increase in monomeric anthocyanins was also observed in colorimetric measurements of these pigments by pH differential method (Table 3). For acetaldehyde-mediated condensation products, peaks 21 (malvidin-3-glucoside-8-ethyl-catechin) and 22 (malvidin-3-glucoside-8-ethyl-epicatechin) a significant decrease was observed in wines from treated samples. For this family of compounds, the total level of this class of wine pigments was reduced in wine due TPC treatment in vineyard. These compounds are a result of reaction between fermentation products and anthocyanin from must. Since direct-condensation products also showed an increase in relation to wines from control samples (Figure 2B) it could be assumed that substrate for wine pigment formation were used to form complexes with flavonols in one years aged wine. Thus, less substrate for reaction with fermentation products and alcohol degradation, as acetaldehyde, will be available at this time of wine aging.

Increased levels of anthocyanins and derivate pigments (Figure 2B) do not have resulted in significant differences in color by instrumental analysis (Table 3) neither in sensorial attributes evaluated by a trained panel. Thus, in experiment II (Figure 2A) greater increments in all pigments were observed than in experiment III (Figure 2B) which could be the reason by which trained panel has only perceived differences between TPC group and control in the first experiment.

Control wines from grapes of experiment III have shown increased levels of total pigments (Table 2 and 3) in comparison with grapes of previous experiments which could be a result of different microclimates and soil since this experiment was performed in a different region than experiment I and II.

## Conclusions

Since it was observed that climate conditions could alter treatment results (experiment I *versus* II), a complete screening of TPC application number and conditions for different weathers should be done in future studies to understand clearly this interaction and stablish optimized conditions.

Thermal Pest Control applied to grapes in vineyard is reported as a promising management technique to reduce fungus contamination. Moreover, as observed for the first time in this work, it is also able to alter anthocyanin levels in 'Tannat' wines with one year old aged. However, the effect seems to be dependent of weather conditions. Thus, treatment (number of applications) should be optimized for conditions in which TPC is able to have a positive effect in wine anthocyanin concentration without alter other important quality parameters of grape and vine development.

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## Tables

**Table 1 Tentative identification of anthocyanins and derived anthocyanins pigments from different vintages of 'Tannat' wines subjected to Thermal Pest Control (TPC) treatments**

| Peak[1] | RT (min) | $M^+$ | MS[2] | $\lambda_{max}$ (nm) | Pigment family (Tentative Identification) | Experiment I | | Experiment II | | Experiment III | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Control | TPC I | Control | TPC II | Control | TPC III |
| | | | | | **Anthocyanins** | | | | | | |
| 4 | 2.41 | 465 | 303 | 286, 526 | Dp-3-glc | * | * | * | * | * | * |
| 9 | 4.31 | 479 | 317 | 287, 518 | Pt-3-glc | nd | nd | * | * | * | * |
| 11 | 6.43 | 463 | 301 | 281, 518 | Pn-3-glc | nd | nd | nd | nd | * | * |
| 12 | 7.64 | 493 | 331 | 277, 522 | Mv-3-glc | * | * | * | * | * | * |
| 20 | 14.19 | 521 | 317 | 279, 522 | pt-3-acetylglc | nd | nd | * | * | * | * |
| 24 | 17.33 | 505 | 301 | 279, 527 | pn-3-acetylglc | nd | nd | nd | nd | * | * |
| 25 | 18.67 | 535 | 331 | 278, 519 | Mv-3-acetylglc | * | * | * | * | * | * |
| 27 | 19.23 | 625 | 317 | 278, 515 | Pt-3-(p-coumaroyl) glc | * | * | * | * | * | * |
| 27 | 22.82 | 639 | 331 | 279, 522 | Mv-3-(p-coumaroyl)glc | * | * | * | * | * | * |
| | | | | | **Pyranoanthocyanins** | | | | | | |
| 10 | 5.52 | 547 | 385 | 278, 510 | A type vitisin of Pt-3-glc | * | * | * | * | * | * |
| 13 | 8.35 | 531 | 369 | 278, 510 | A type vitisin of Pn-3-glc | * | * | * | * | * | * |
| 15 | 9.72 | 561 | 399 | 273, 508 | A type vitisin of mv-3-glc | * | * | * | * | * | * |
| 16 | 11.12 | 517 | 355 | 277, 505 | B type vitisin of mv-3-glc | * | * | * | * | * | * |
| 17 | 11.43 | 573 | 369 | 275, 513 | Acetone derivative of Mv-3-acetylglc | nd | nd | * | * | nd | nd |
| 18 | 12.12 | 603 | 399 | 277, 514 | A type vitisin of Mv-3-acetylglc | * | * | * | * | * | * |
| 19 | 13.08 | 589 | 385 | 277, 512 | A type vitisin of Pt-3-acetylglc | * | * | * | * | nd | nd |
| 20 | 14.19 | 559 | 355 | 279, 522 | B type vitisin of Mv-3-acetylglc | nd | nd | * | * | * | * |
| 26 | 19.23 | 677 | 369 | 278, 515 | A type vitisin of Dp-3-(p-coumaroyl)glc | * | * | * | * | * | * |
| 26 | 19.23 | 707 | 399 | 278, 515 | A type vitisin of Mv-3-(p-coumaroyl)glc | * | * | * | * | * | * |
| 27 | 22.82 | 663 | 355 | 279, 522 | B type vitisin of Mv-3-(p-coumaroyl)glc | * | * | * | * | * | * |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 27 | 22.82 | 805 | 643. 491 | 279, 522 | Mv-3-glc-4-vinyl-C | * | * | * | * | * | * |
| 27 | 22.82 | 847 | 685. 643. 491 | 279, 522 | Mv-3-acetylglc-4-vinyl-C | * | * | * | * | * | * |
| | | | | **Direct condensation products** | | | | | | | |
| 1 | 1.44 | 797 | 635. 467 | 271, 531 | Mv-3-glc-GC | * | * | * | * | * | * |
| 2 | 1.80 | 767 | 605 | 279, 530 | Pt-3-glc-C | nd | nd | * | * | * | * |
| 4 | 2.41 | 751 | 589. 437 | 286, 526 | Pn-3-glc-C | * | * | * | * | * | * |
| 5 | 2.64 | 781 | 619. 467 | 278, 517 | Mv-3-glc-C | * | * | * | * | * | * |
| 15 | 9.72 | 823 | 619. 467. 373 | 273, 508 | Mv-3-acetylglc-C | * | * | * | * | * | * |
| 24 | 17.33 | 927 | 619. 467. 373 | 279, 527 | Mv-3-(p-coumaroyl)glc-C | nd | nd | nd | nd | * | * |
| | | | | **Acetaldehyde-mediated condensation products** | | | | | | | |
| 21 | 14.89 | 809 | 357. 519. 647 | 279, 531 | Mv-3-glc-8-ethyl-C | nd | nd | nd | nd | * | * |
| 22 | 16.02 | 809 | 357. 519. 647 | 277, 522 | Mv-3-glc-8-ethyl-EC | * | * | nd | nd | * | * |
| 27 | 22.82 | 851 | 357 | 279, 522 | Mv-3-acetylglc-8-ethyl-EC | * | * | * | * | * | * |
| 27 | 22.82 | 955 | 357. 665 | 279, 522 | Mv-3-(p-coumaroyl)glc-8-ethyl-EC | * | * | * | * | * | * |
| | | | | **Unknown** | | | | | | | |
| 3 | 2.20 | 579 | 427. 301 | 303, 528 | Unknown | * | * | * | * | * | * |
| 6 | 3.17 | 511 | 493 | 288, 528 | Unknown | * | * | * | * | nd | nd |
| 7 | 3.63 | 645 | 517 | 302, 531 | Unknown | * | * | * | * | * | * |
| 8 | 3.97 | 511 | 493. 331 | 297, 543 | Unknown | * | * | * | * | * | * |
| 14 | 8.83 | 657 | 495 | 280, 535 | Unknown | * | * | * | * | * | * |
| 23 | 16.76 | 671 | 509. 331 | 277, 513 | Unknown | * | * | * | * | * | * |

[1]RT: retention time (min); $M^+$: positive charged molecular íon; $MS^2$:fragmentation of $M^+$. Control identification results were from samples without TPC treatment. Abbreviations: Dp-delphinidin. Cy-cyanidin. Pt-petunidin. Pn-peonidin. Mv-malvidin. C-catechin. EC-epicatechin. glc-glucoside. *Detected; nd – not detected. Experiment I performed during season of 2011/12 with 4 TPC applications; Experiment II from season 2012/13 and 5 TPC applications; Experiment III from season 2013/14 with 19 applications.

**Table 2 Quantification of individual anthocyanins in different vintages of 'Tannat' red wine before and after Thermal Pest Control (TPC) treatments**

| Peak number[1] | Experiment I | | Experiment II | | Experiment III | |
|---|---|---|---|---|---|---|
| | Control | TPC I | Control | TPC II | Control | TPC III |
| 1 | 3.99±0.66[a] | 4.10±0.78[a] | 6.52±0.23[a] | 5.01±1.00[a] | 9.12±0.00[b] | 10.26±0.15[a] |
| 2 | nd | nd | 0.08±0.05[a] | 0.18±0.08[a] | 0.83±0.00[a] | 0.86±0.05[a] |
| 3 | 0.04±0.01[a] | 0.08±0.02[a] | 0.12±0.03[a] | 0.13±0.03[a] | 0.39±0.04[b] | 0.62±0.03[a] |
| 4 | 0.20±0.09[a] | 0.29±0.02[a] | 0.18±0.09[a] | 0.18±0.09[a] | 11.15±2.38[b] | 15.17±0.50[a] |
| 5 | 0.38±0.11[a] | 0.60±0.15[a] | 0.93±0.28[a] | 0.72±0.23[a] | 0.69±0.12[b] | 0.90±0.02[a] |
| 6 | 0.06±0.00[a] | 0.07±0.02[a] | 0.13±0.05[a] | 0.12±0.04[a] | nd | nd |
| 7 | 0.11±0.00[a] | 0.14±0.14[a] | 0.32±0.16[a] | 0.10±0.04[a] | 1.25±0.10[a] | 1.01±0.10[b] |
| 8 | 0.47±0.18[a] | 0.66±0.23[a] | 0.63±0.16[a] | 0.72±0.44[a] | 0.69±0.00[b] | 0.99±0.05[a] |
| 9 | nd | nd | 0.23±0.09[a] | 0.25±0.20[a] | 11.04±2.72[a] | 14.18±0.17[a] |
| 10 | 0.38±0.09[b] | 0.78±0.17[a] | 1.35±0.44[a] | 0.99±0.33[a] | 0.45±0.15[a] | 0.50±0.03[a] |
| 11 | nd | nd | nd | nd | 2.50±0.66[b] | 3.82±0.03[a] |
| 12 | 0.85±0.03[b] | 2.02±0.30[a] | 1.32±0.27[a] | 1.93±1.09[a] | 52.27±12.28[b] | 74.54±1.29[a] |
| 13 | 0.15±0.01[b] | 0.30±0.04[a] | 0.85±0.28[a] | 0.61±0.22[a] | 0.22±0.03[a] | 0.27±0.00[a] |
| 14 | 0.13±0.07[a] | 0.20±0.11[a] | 0.15±0.02[a] | 0.25±0.08[a] | 0.95±0.12[a] | 0.93±0.04[a] |
| 15 | 2.95±0.73[b] | 5.57±0.84[a] | 8.28±2.85[a] | 6.12±2.50[a] | 4.89±1.04[a] | 5.38±0.11[a] |
| 16 | 0.13±0.04[b] | 0.31±0.01[a] | 0.12±0.03[a] | 0.15±0.07[a] | 0.77±0.00[a] | 0.30±0.01[b] |
| 17 | nd | nd | 0.18±0.05[a] | 0.14±0.02[a] | nd | nd |
| 18 | 0.50±0.15[a] | 1.18±0.15[a] | 1.72±0.44[a] | 1.26±0.60[a] | 1.15±0.21[a] | 1.15±0.05[a] |
| 19 | 0.26±0.09[a] | 0.35±0.00[a] | 0.19±0.04[a] | 0.19±0.13[a] | nd | nd |
| 20 | nd | nd | 0.16±0.02[a] | 0.15±0.04[a] | 1.25±0.23[b] | 1.94±0.09[a] |
| 21 | nd | nd | nd | nd | 1.02±0.02[a] | 0.51±0.05[b] |
| 22 | 0.32±0.05[b] | 0.53±0.01[a] | nd | nd | 2.09±0.10[a] | 0.96±0.07[b] |
| 23 | 1.84±0.80[a] | 1.85±0.55[a] | 0.98±0.21[a] | 0.96±0.68[a] | 1.79±0.21[a] | 0.88±0.05[b] |
| 24 | nd | nd | nd | nd | 0.93±0.13[b] | 1.19±0.01[a] |
| 25 | 0.50±0.21[a] | 0.67±0.16[a] | 0.46±0.02[a] | 0.38±0.32[a] | 6.70±1.76[b] | 11.92±0.39[a] |
| 26 | 0.21±0.02[b] | 0.42±0.06[a] | 0.98±0.18[a] | 0.63±0.43[a] | 1.15±0.28[a] | 0.97±0.03[a] |
| 27 | 52.71±1.11[b] | 96.60±0.18[a] | 130.59±14.29[a] | 101.32±69.69[a] | 174.81±28.46 [a] | 100.27±6.01[b] |
| **Total** | 66.30±3.12[b] | 116.82±2.83 [a] | 156.58±10.53[a] | 122.59±77.59[a] | 279.11±50.94[a] | 239.37±6.62[a] |

[1]Results are mean ± standard deviation of three independent micro wine making processes (n=3). Means that the average is statistically different than control for each experiment ($p<0.05$). Concentration in malvidin-3-glucoside (mg $L^{-1}$). Control results were from samples without TPC treatment.

**Table 3 Color evaluation by colorimetric analysis from different vintages of 'Tannat' wines before and after Thermal Pest Control (TPC) treatments**

| Parameters[1] | Experiment I | | Experiment II | | Experiment III | |
|---|---|---|---|---|---|---|
| | Control | TPC I | Control | TPC II | Control | TPC III |
| $A_{420nm}$ | 0.40±0.02[b] | 0.59±0.02[a] | 0.56±0.02[a] | 0.57±0.01[a] | 0.39±0.01[a] | 0.38±0.02[a] |
| $A_{520nm}$ | 0.55±0.05[b] | 0.84±0.05[a] | 0.72±0.01[a] | 0.73±0.02[a] | 0.72±0.01[a] | 0.70±0.02[a] |
| $A_{620nm}$ | 0.11±0.01[b] | 0.17±0.01[a] | 0.13±0.00[a] | 0.14±0.01[a] | 0.10±0.00[a] | 0.09±0.01[a] |
| CI | 1.07±0.06[b] | 1.61±0.08[a] | 1.42±0.03[a] | 1.45±0.04[a] | 1.21±0.65[a] | 1.18±0.04[a] |
| Hue | 0.73±0.08[a] | 0.70±0.02[a] | 0.77±0.03[a] | 0.78±0.02[a] | 0.53±0.00[a] | 0.54±0.02[a] |
| MA | 38.9±1.94[b] | 77.5±2.07[a] | 63.3±1.60[a] | 48.5±4.34[b] | 175.9±1.69[b] | 241.9±11.88[a] |

[1]A absorbance; CI color intensity (units of absorbance); MA monomeric anthocyanin (mg $L^{-1}$). Means that average is statistically different than control for each season ($p<0.05$). Control was from samples without TPC treatment.

**Figure captions**

**Figure 1** Chromatographic separation of anthocyanins and related red wine pigments detected in one year old 'Tannat' wine samples obtained vineyards with different Thermal Pest Control management treatments.

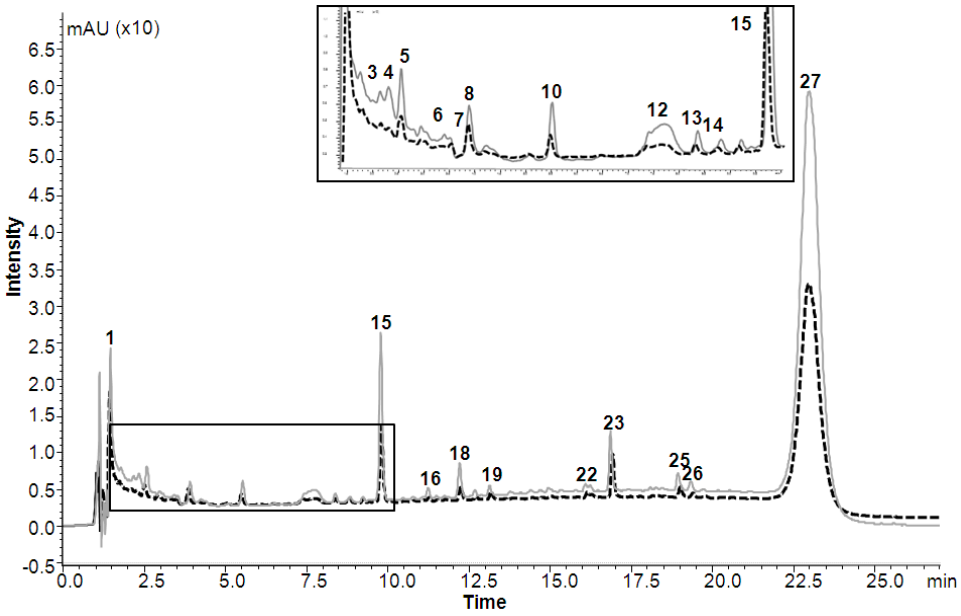
(a) is from Experiment I performed during season of 2011/12 with 4 TPC applications; (b) is from Experiment II from season 2012/13 and 5 TPC applications; (c) is from Experiment III from season 2013/14 with 19 applications. Continuous line corresponds to the wine obtained from treated grapes and the dashed line is from wine produced with control grapes.

**Figure 2** Relative increment of main pigments classes in TPC (Thermal Pest Control) treated samples of one year old 'Tannat' wine *versus* control.

Results are from different vintages corresponding to independent experiments. (a) is from Experiment I performed during season of 2011/12 with 4 TPC applications; (b) is from Experiment III from season 2013/14 with 19 applications. Experiment I were performed in a plain grasslands region at 2011/12. Experiment III was developed in a sierra grassland region during 2013/14 crop management period.

**Figure 3** Sensory descriptive analysis in one year old 'Tannat' wine from TPC: wines produced with *Thermal Pest Control* samples Control: wines produced with untreated samples. V – visual; O – olfactory; G – gustatory. *Significance differences by Tukey test ($p \leq 0.05$).

**(a)**




**(b)**

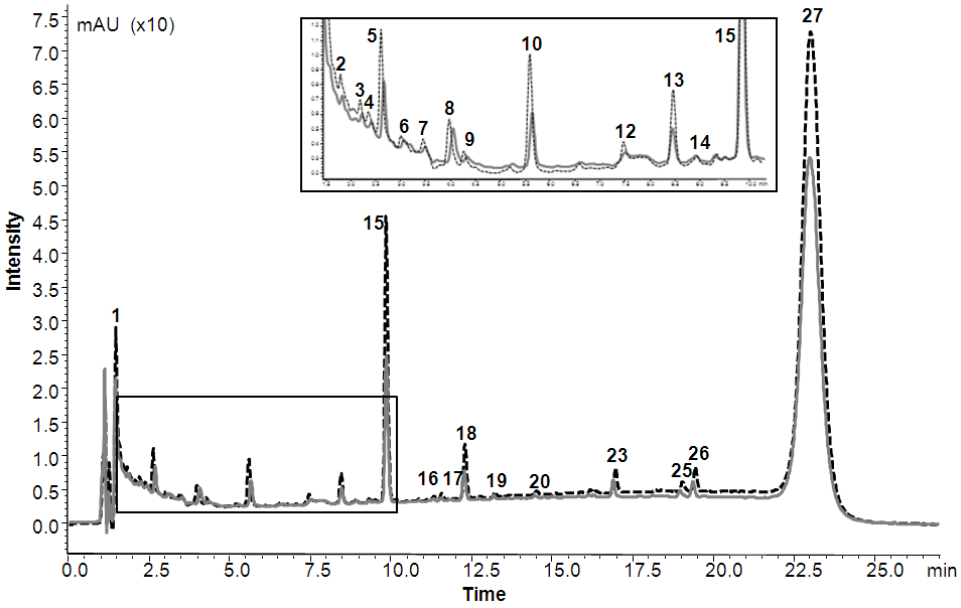



Figure 1 (a) and (b). Treptow *et al.*

**(c)**

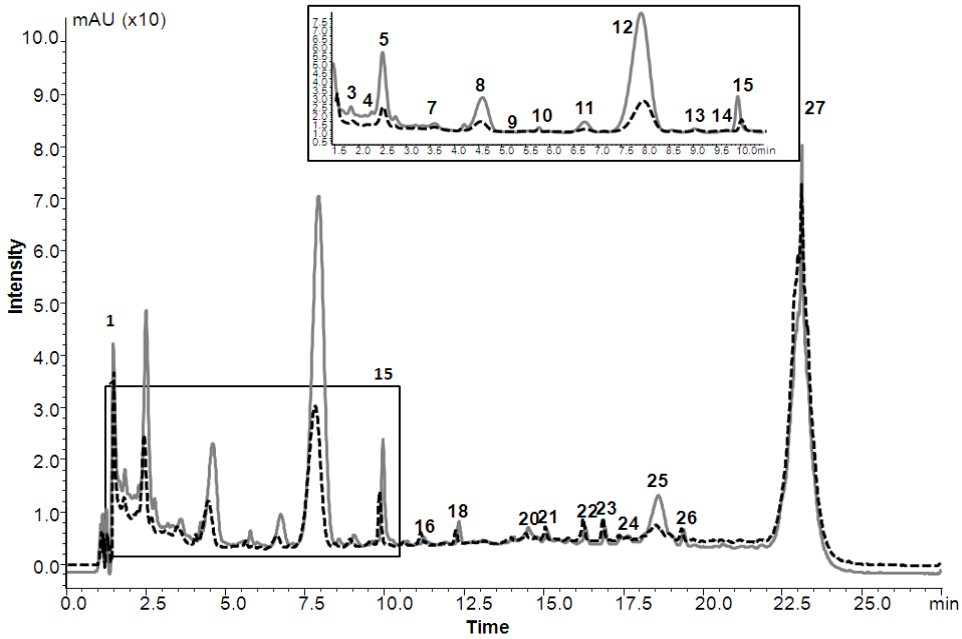
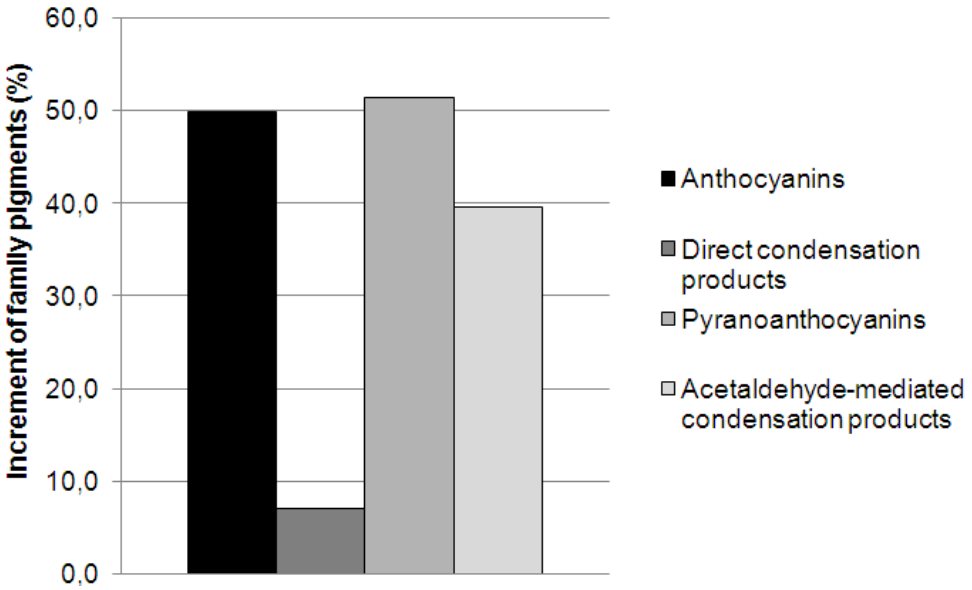



Figure 1 (c). Treptow *et al.*

**(a)**




**(b)**

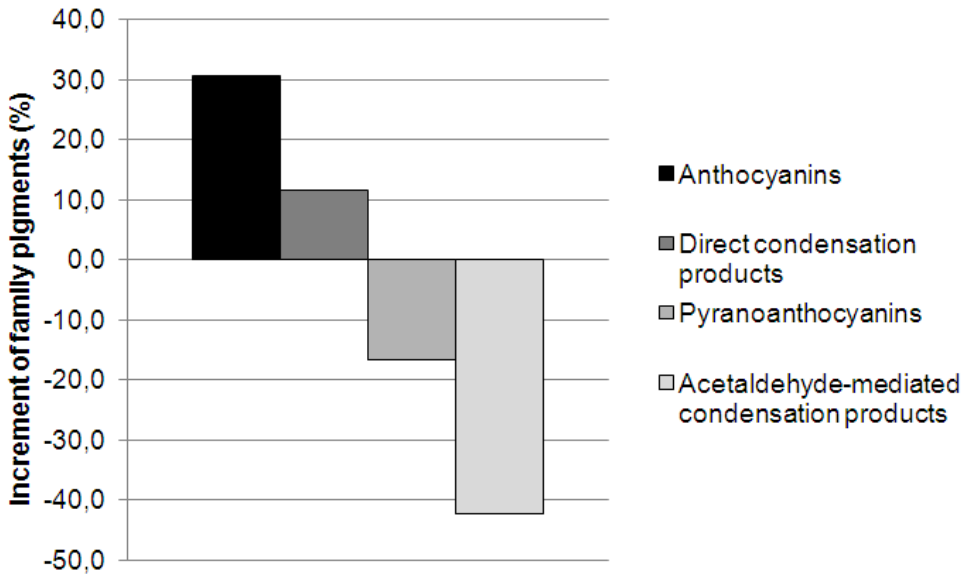



Figure 2 (a) and (b). Treptow *et al.*

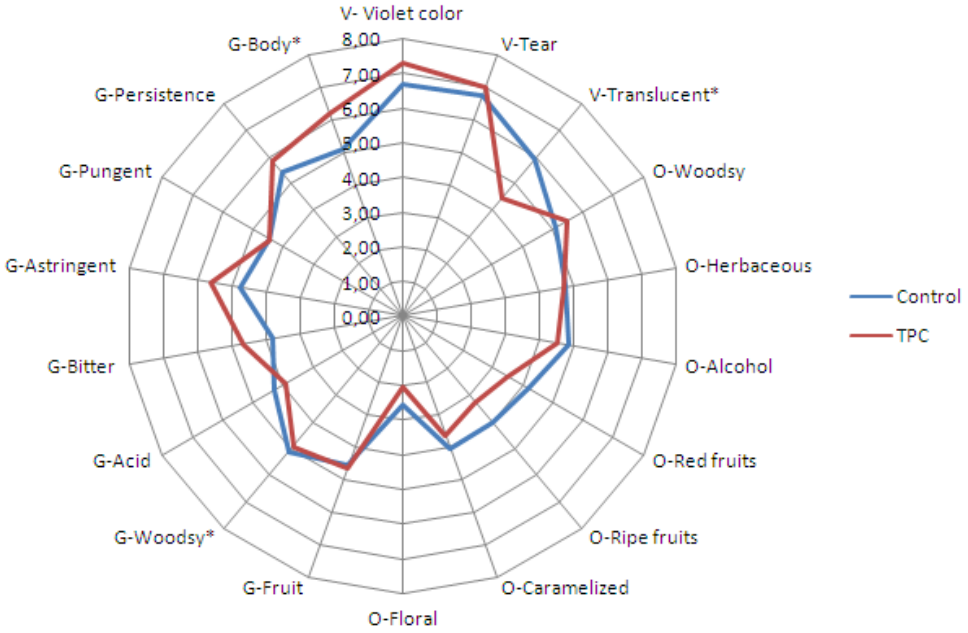



Figure 3. Treptow *et al.*

# Thermal Pest Control in 'Tannat' grapes: effect on anthocyanins, sensorial and color of one year old wines

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## SUPPLEMENTARY MATERIAL

**Table 1** Terms descriptors and references used in the evaluation of samples of 'Tannat' wine.

| Descriptor | Reference |
|---|---|
| | VISUAL |
| Violet color | Weak: 50 mL of 'Tannat' wine in 50 mL of water |
| | Strong: 100 mL of 'Tannat' wine |
| Viscosity | Little: 10 mL of brandy (40%) in 90 mL of water |
| | Very: 100 mL of brandy (40%) |
| Translucent | Little: 50 mL of grape juice in 50 mL of water |
| | Very: 100 mL of grape juice |
| | OLFATORY |
| Woodsy | Nothing: 10 mL of 'Tannat' wine |
| | Strong: 10 mL of reserve 'Tannat' wine/10g smoked bacon/One cigarette tobacco |
| Herbaceous | Nothing: 10 mL of water |
| | Strong: 10 g of sliced fresh pepper/10g of olive |
| Alcohol | Weak: 10 mL of 5% v/v ethanol in water |
| | Strong: 10 mL of 20% v/v ethanol in water |
| Red fruits | Nothing: 10 mL of water |
| | Strong: 10g of plum/strawberry/raspberry/blueberry |
| Ripe fruits | Nothing: 10 mL of water |
| | Strong: 10 g of raisin/ 10g of ripe plum |
| Caramelized | Nothing: 10 mL of water |
| | Strong: 3 drops of 0.25% of vanillin solution/ 10g of brown sugar |
| Floral | Nothing: 10 mL of water |
| | Strong: 10 mL of water with 1 drop of rose aroma |
| | GUSTATORY |
| Fruit | Nothing: 10 mL of 'Tannat' wine |
| | Strong: 10 mL of 'Tannat' wine with 10 g of plum/strawberry/blueberry |
| Woodsy | Nothing: 10 mL of 'Tannat' wine |
| | Strong: 10 mL of reserve 'Tannat' wine |
| Acid | Weak: 10 mL of red wine with 0.02% w/v of citric acid |
| | Strong: 10 mL of red wine with 0.9% w/v of citric acid |
| Bitter | Weak: 10 mL of red wine with 0.01% w/v of caffeine |
| | Strong: 10 mL of red wine with 0.1% w/v of caffeine |
| Astringent | Weak: 10mL of red wine |
| | Strong: 10 mL of red wine with the addition of 1 ml of grape seed infusion |
| Pungent | Weak:10 mL of red wine |
| | Strong: 10 mL of red wine with 2 sliced ginger |
| Persistence and Body | Weak:10 mL of 'Tannat' wine |
| | Strong: 10 mL of reserve 'Tannat' wine |

**Table 2** Initial total soluble solids (TSS) in 'Tannat' grapes at each crop management year

| Parameters[1] | Experiment I | | Experiment II | | Experiment III | |
|---|---|---|---|---|---|---|
| | Control | TPC I | Control | TPC II | Control | TPC III |
| TSS | 23.2±0.2[b] | 24.3±0.1[a] | 22.1±0.1[a] | 20.1±0.1[b] | 19.5±0.2[b] | 20.2±0.3[a] |

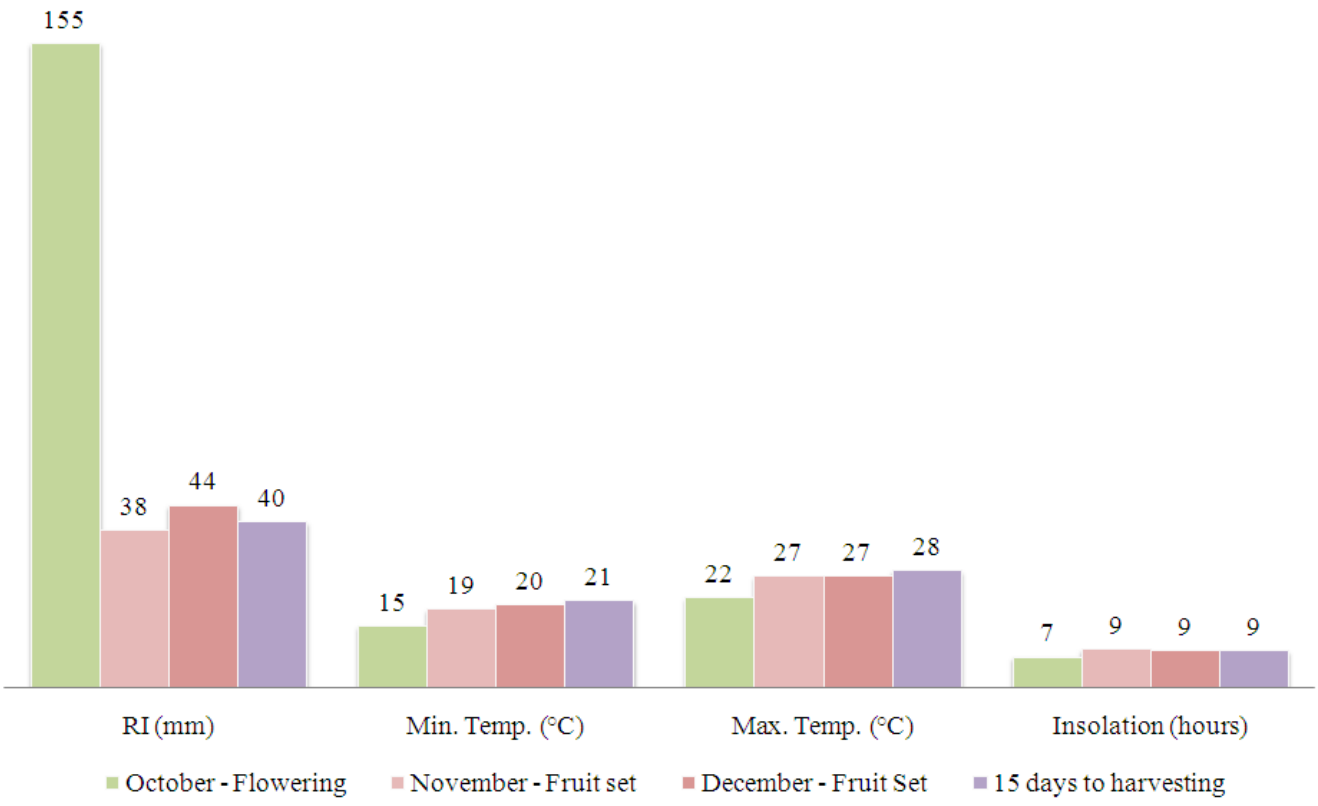
[1]TSS (total soluble solid - °brix). Means that average is statistically different than control for each season ($p<0.05$).

**(a)**

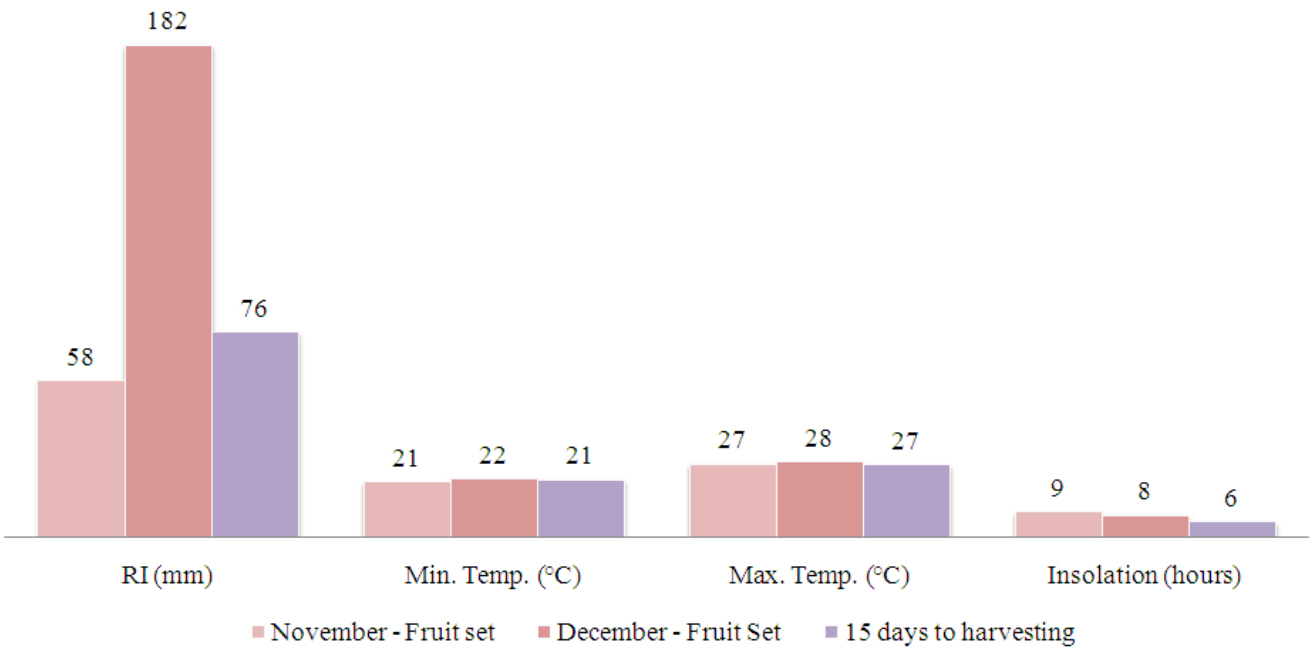
**(b)** **(c)**


**Figure 1** Thermal Pest Control equipment used for grape treatment. A) Lateral overview of the equipment being towed by a tractor. B) Back view of the equipment. C) Control monitor for Thermal Pest Control treatment conditions.

**(a)**


155
38
44
40
15
19
20
21
22
27
27
28
7
9
9
9
RI (mm)
Min. Temp. (°C)
Max. Temp. (°C)
Insolation (hours)
October - Flowering
November - Fruit set
December - Fruit Set
15 days to harvesting

**(b)**


182
58
76
21
22
21
27
28
27
9
8
6
RI (mm)
Min. Temp. (°C)
Max. Temp. (°C)
Insolation (hours)
November - Fruit set
December - Fruit Set
15 days to harvesting

**(c)**

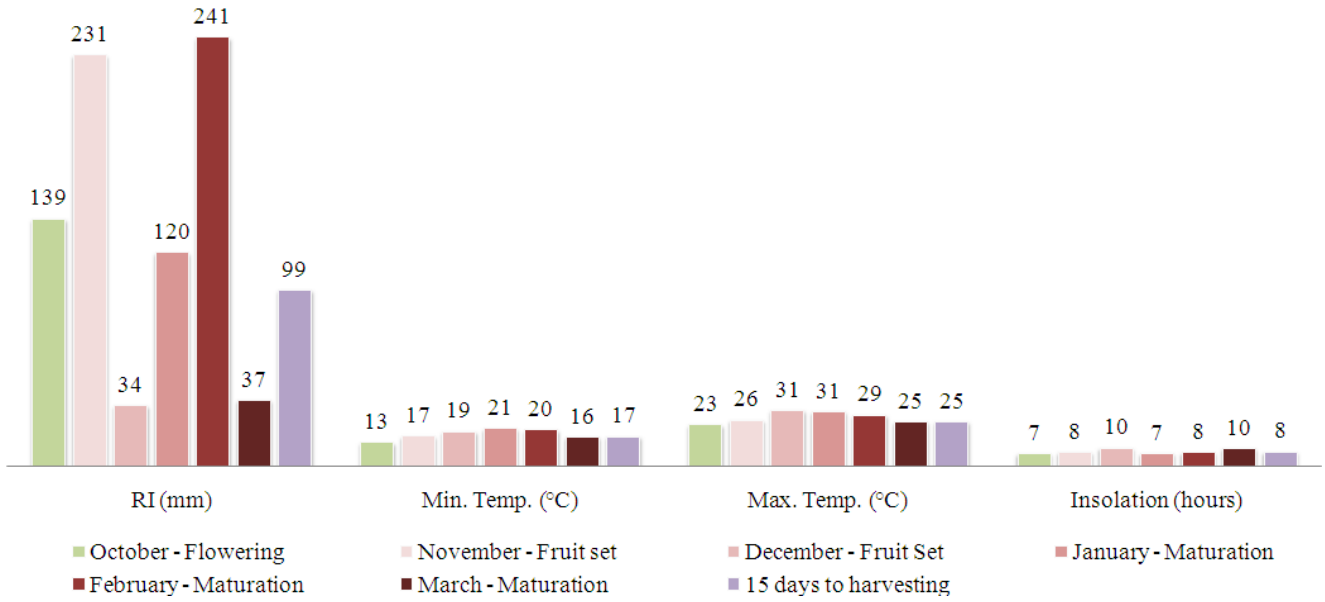



**Figure 2** Climate conditions during *Thermal Pest Control* treatment. (a) is from Experiment I performed during season of 2011/12 with 4 TPC applications (from flowering to fruit set); (b) is from Experiment II performed during season of 2012/13 with 5 TPC applications (only in fruit set) and (c) is from Experiment III performed during season of 2013/14 with 19 TPC applications (from flowering to maturation). Abbreviature: rainfall index (RI), minimum temperature (Mín. Temp), maximum temperature (Máx. Temp). All data was obtained from INMET available at http://www.inmet.gov.br acessed in setember of 2016.

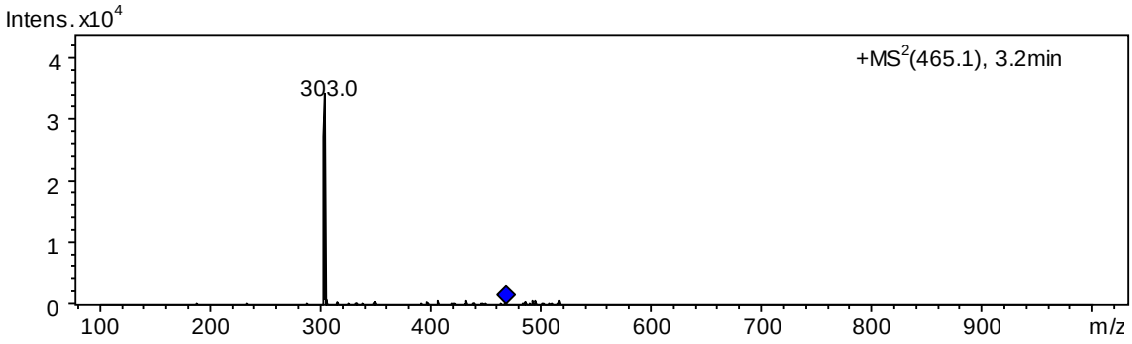



**Figure 2:** Fragmentation ($MS^2$) mass spectra of Delphinidin-3-glucoside.

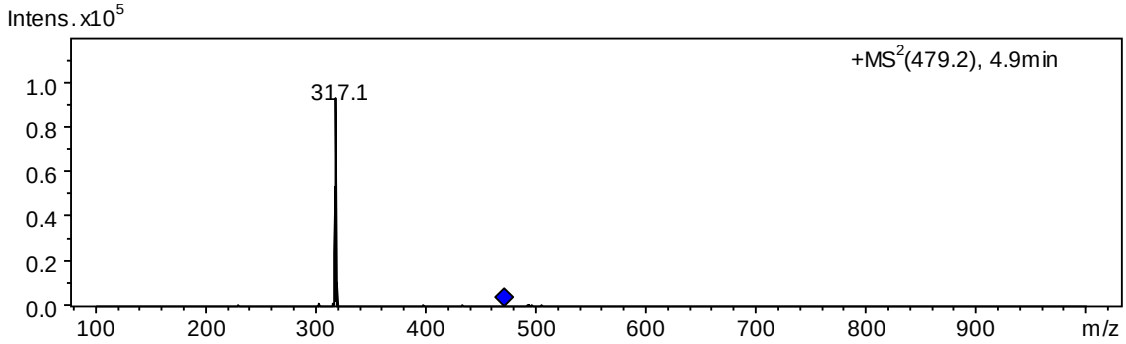



**Figure 3:** Fragmentation ($MS^2$) mass spectra of Petunidin-3-glucoside.

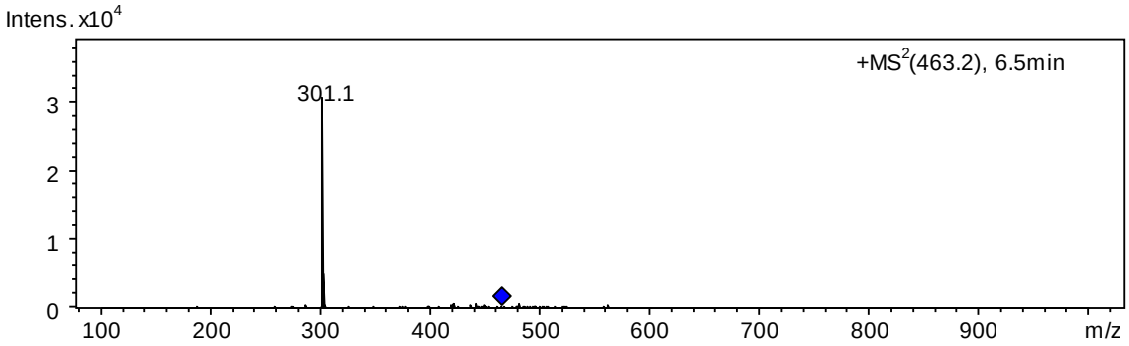



**Figure 4:** Fragmentation ($MS^2$) mass spectra of Peonidin-3-glucoside.

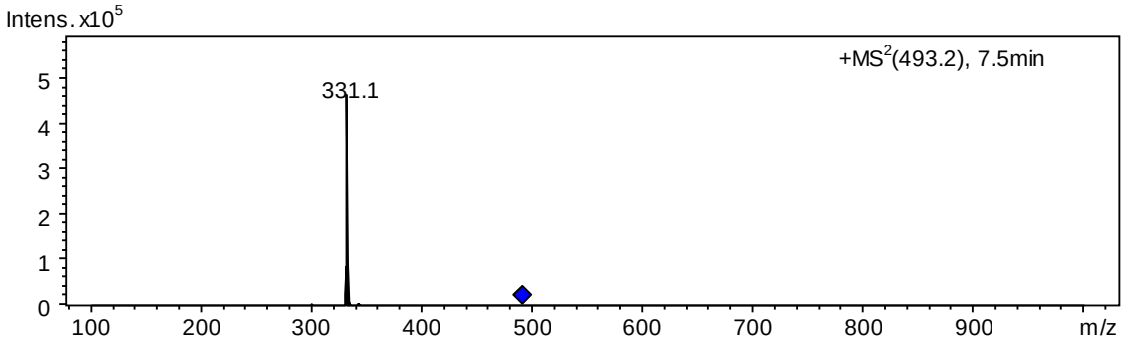



**Figure 5:** Fragmentation ($MS^2$) mass spectra of Malvidin-3-glucoside.

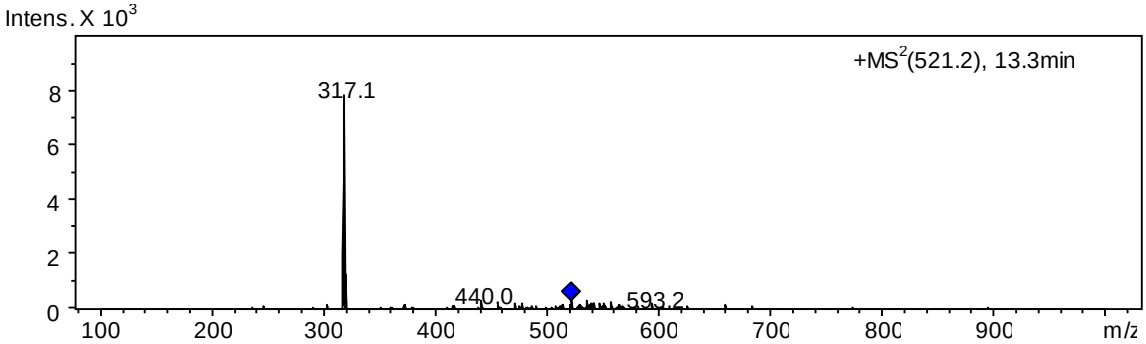



**Figure 6:** Fragmentation ($MS^2$) mass spectra of Petunidin-3-acetylglucoside.

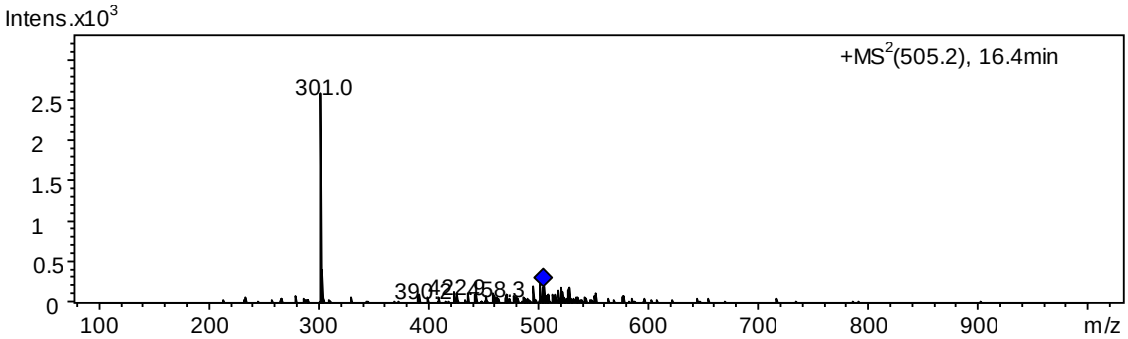



**Figure 7:** Fragmentation ($MS^2$) mass spectra of Peonidin-3-acetylglucoside.

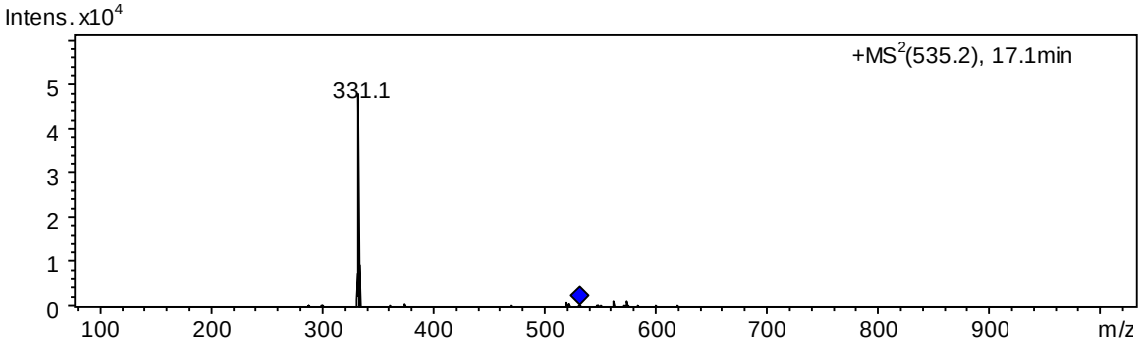



**Figure 8:** Fragmentation ($MS^2$) mass spectra of Malvidin-3-acetylglucoside.

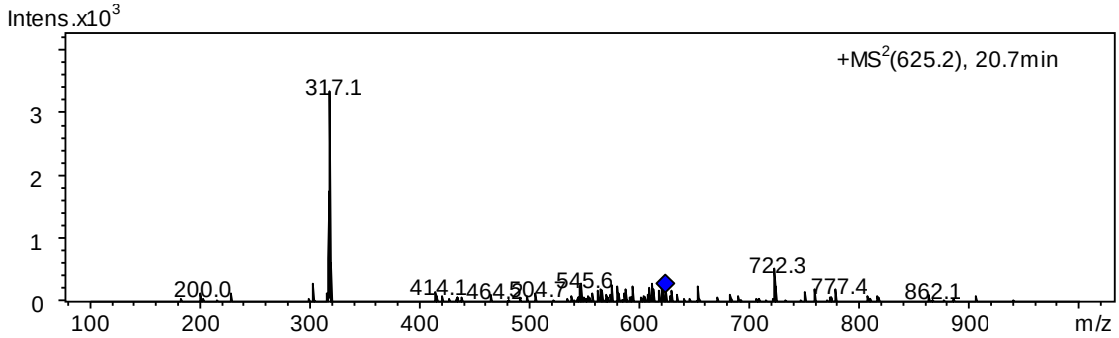



**Figure 9:** Fragmentation ($MS^2$) mass spectra of Petunidin-3-(p-coumaroyl)glucoside.

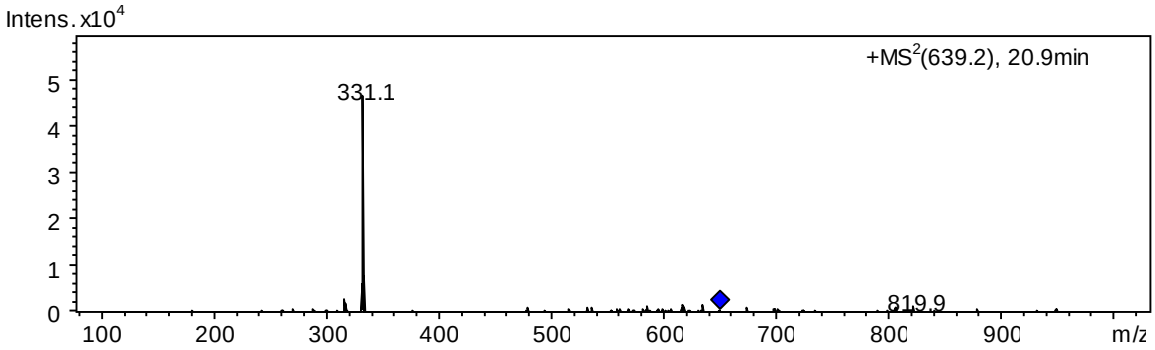



**Figure 10:** Fragmentation ($MS^2$) mass spectra of Malvidin-3-(p-coumaroyl)glucoside.

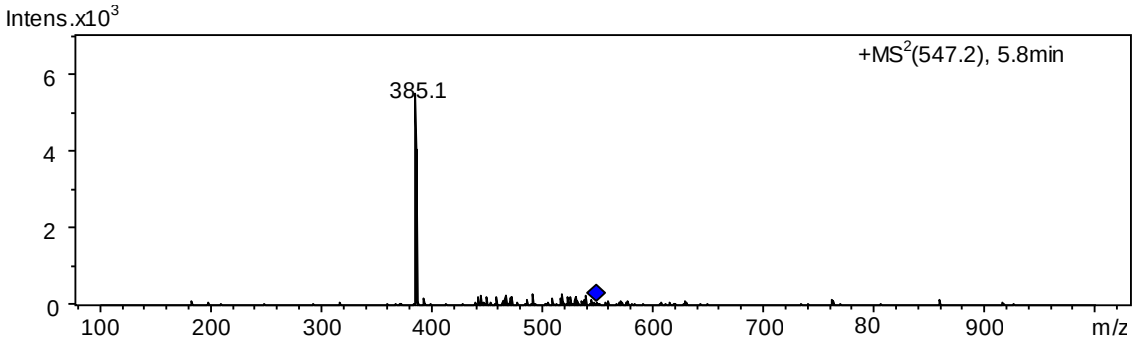



**Figure 11:** Fragmentation ($MS^2$) mass spectra of Petunidin-3-glucoside-pyruvic acid.

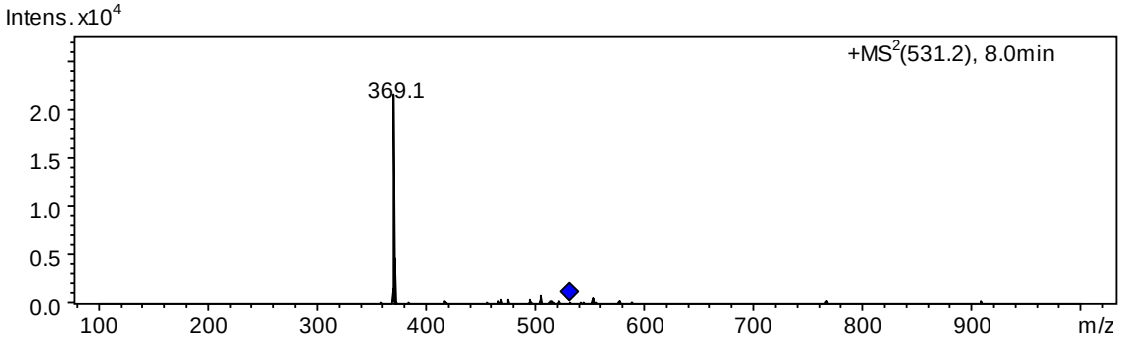



**Figure 12:** Fragmentation ($MS^2$) mass spectra of Peonidin-3-glucoside-pyruvic acid.

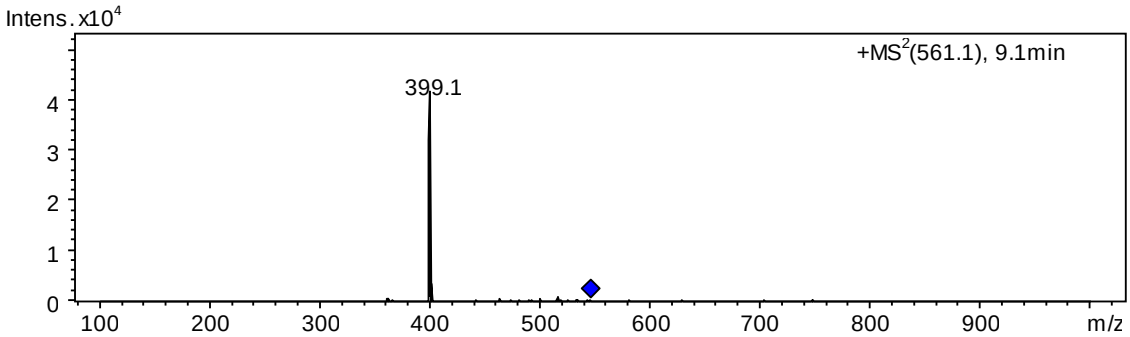



**Figure 13:** Fragmentation ($MS^2$) mass spectra of Malvidin-3-glucoside-pyruvic acid.

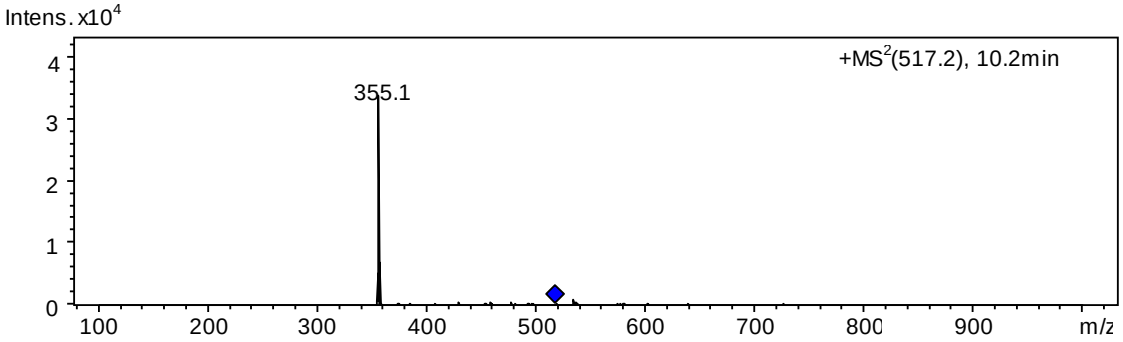



**Figure 14:** Fragmentation ($MS^2$) mass spectra of Malvidin-3-glucoside-acetaldehyde.

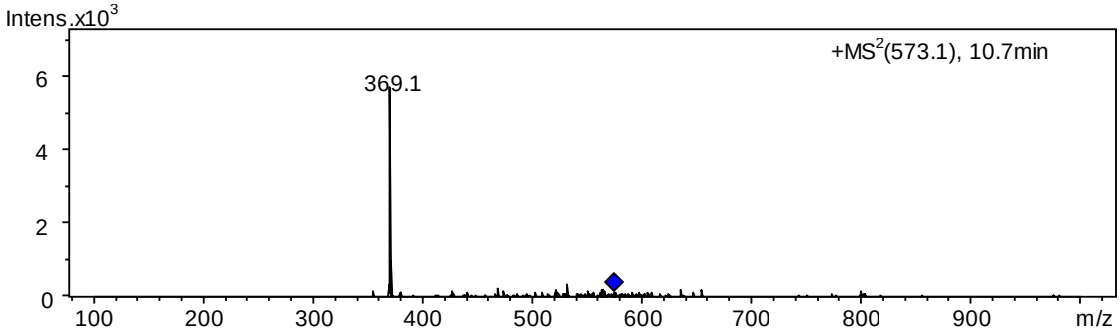



**Figure 15:** Fragmentation ($MS^2$) mass spectra of Malvidin-3-glucoside-Acetone.

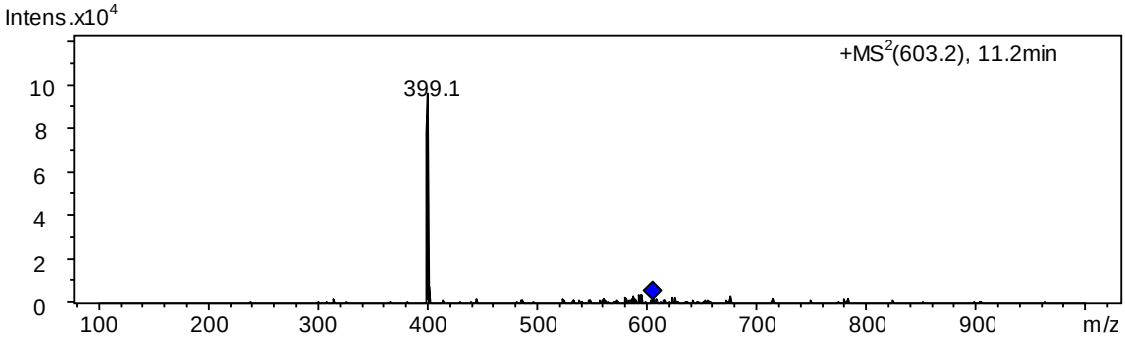



**Figure 16:** Fragmentation ($MS^2$) mass spectra of Malvidin-3-acetylglucoside-pyruvic acid.

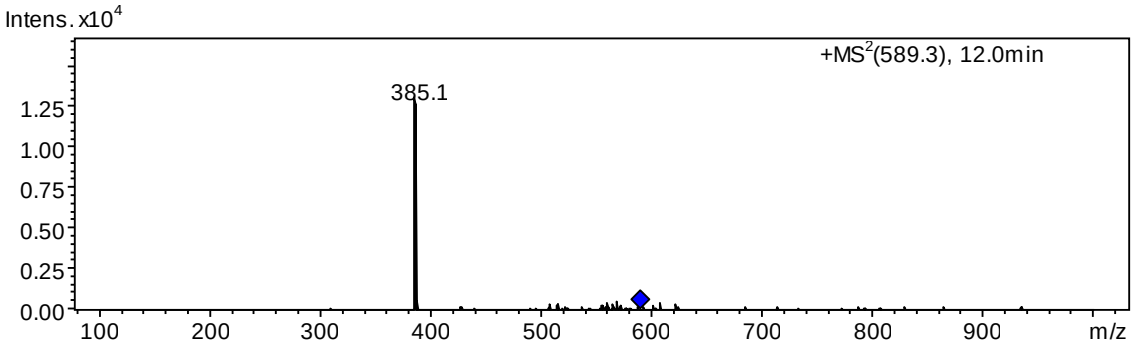



**Figure 17:** Fragmentation ($MS^2$) mass spectra of Petunidin-3-acetylglucoside-pyruvic acid.

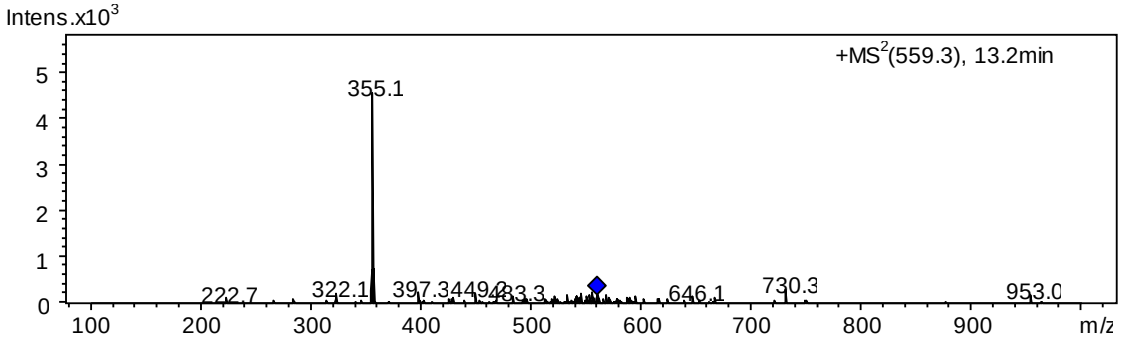



**Figure 18:** Fragmentation ($MS^2$) mass spectra of Malvidin-3-acetylglucoside-acetaldehyde.

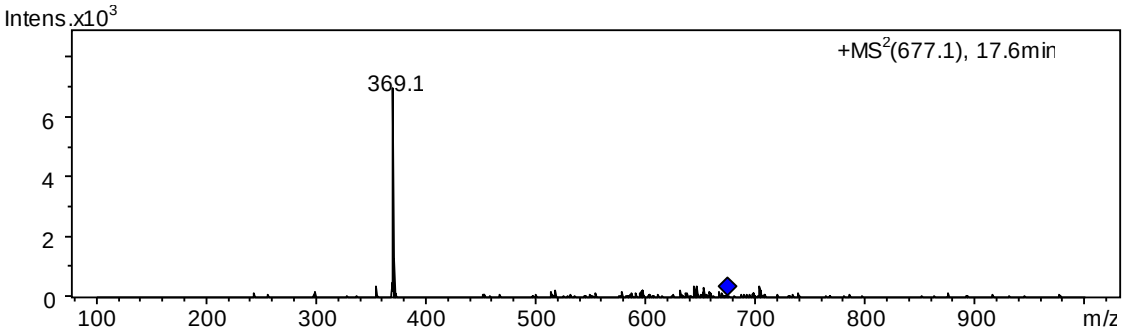



**Figure 19:** Fragmentation ($MS^2$) mass spectra of Delphinidin-3-(p-coumaroyl)glucoside-pyruvic acid.

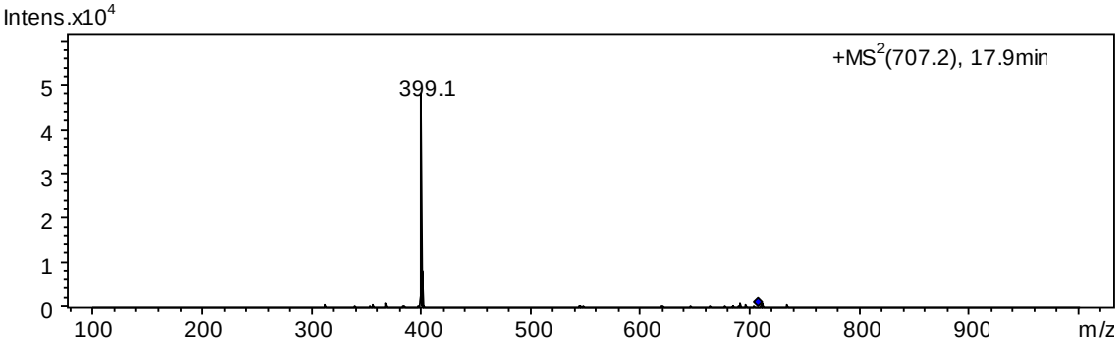



**Figure 20:** Fragmentation ($MS^2$) mass spectra of Malvidin-3-(p-coumaroyl)glucoside-pyruvic acid.

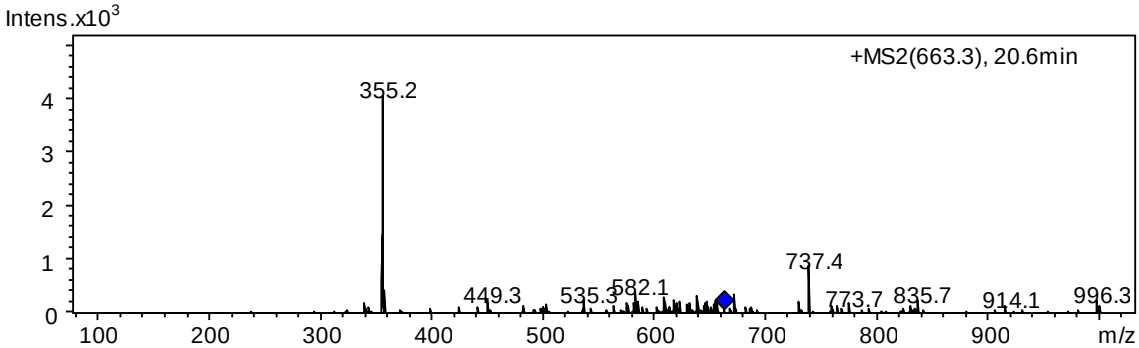



**Figure 21:** Fragmentation ($MS^2$) mass spectra of Malvidin-3-(p-coumaroyl)glucoside-acetaldehyde.

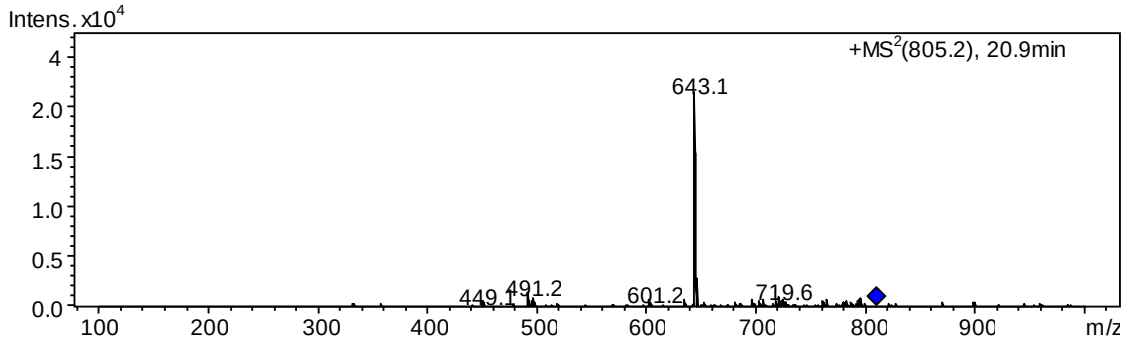



**Figure 22:** Fragmentation ($MS^2$) mass spectra of Malvidin-3-glucoside-4-vinyl-catechin

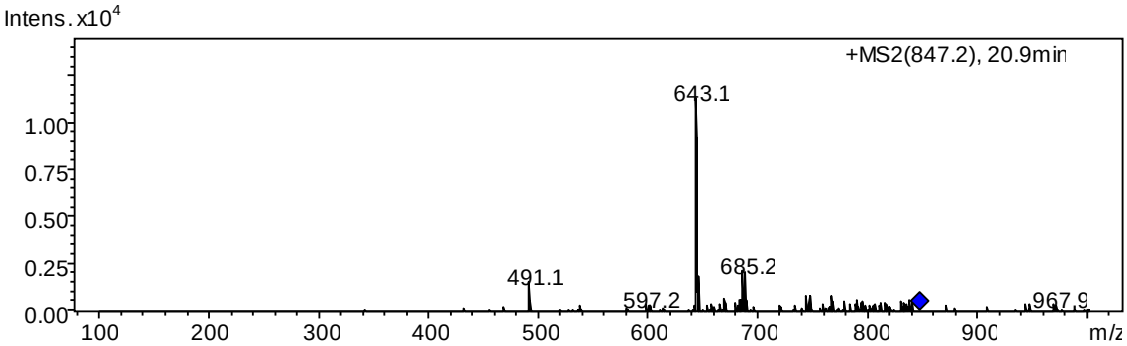



**Figure 23:** Fragmentation ($MS^2$) mass spectra of Malvidin-3-acetylglucoside-4-vinyl-catechin

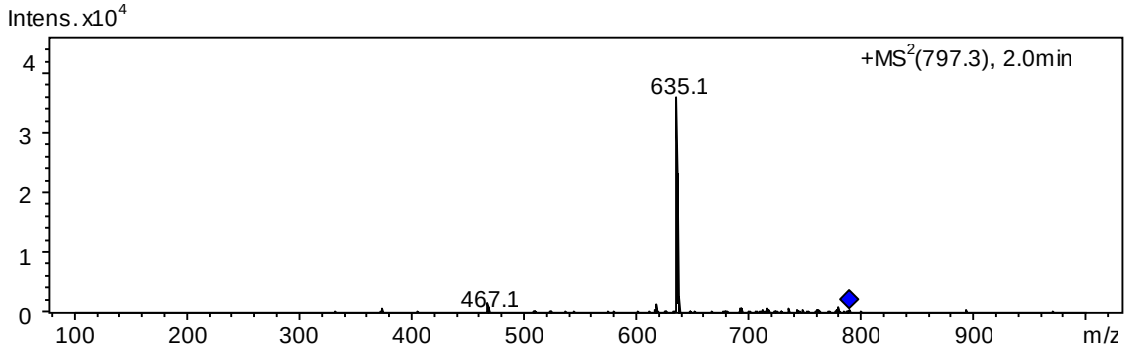



**Figure 24:** Fragmentation ($MS^2$) mass spectra of Mv-3-glc-gallocatechin direct condensation product.

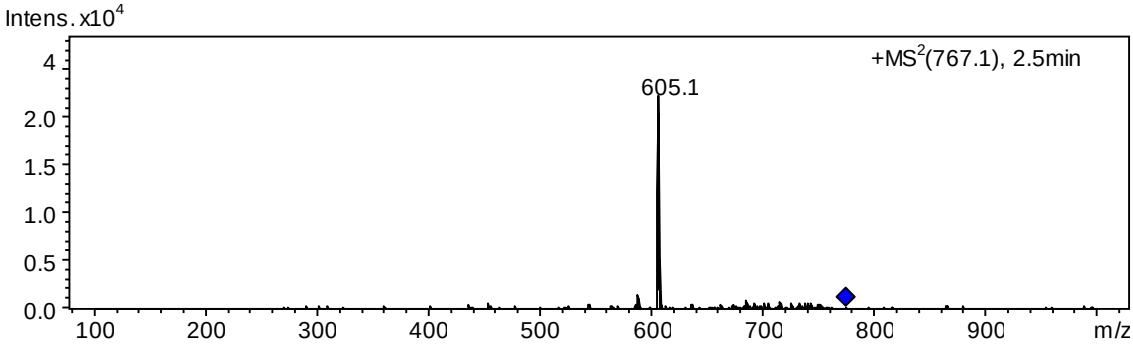



**Figure25:** Fragmentation ($MS^2$) mass spectra of Pt-3-glucoside-catechin direct condensation product.

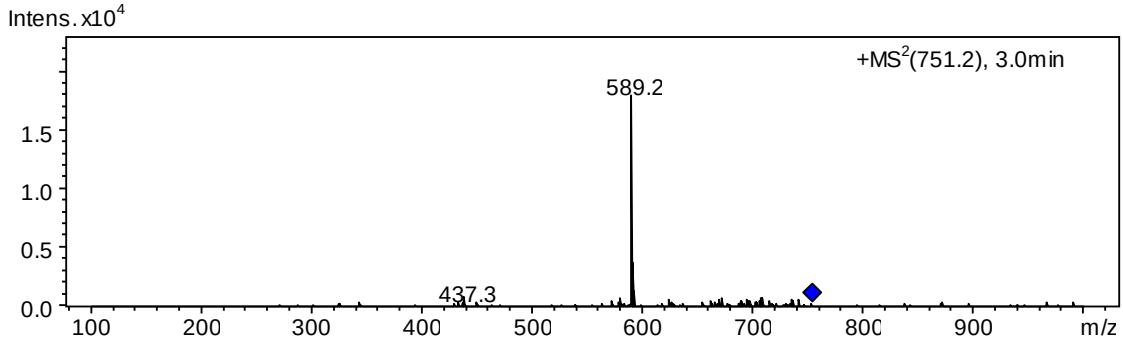



**Figure 26:** Fragmentation ($MS^2$) mass spectra of Pn-3-glucoside-catechin direct condensation product.

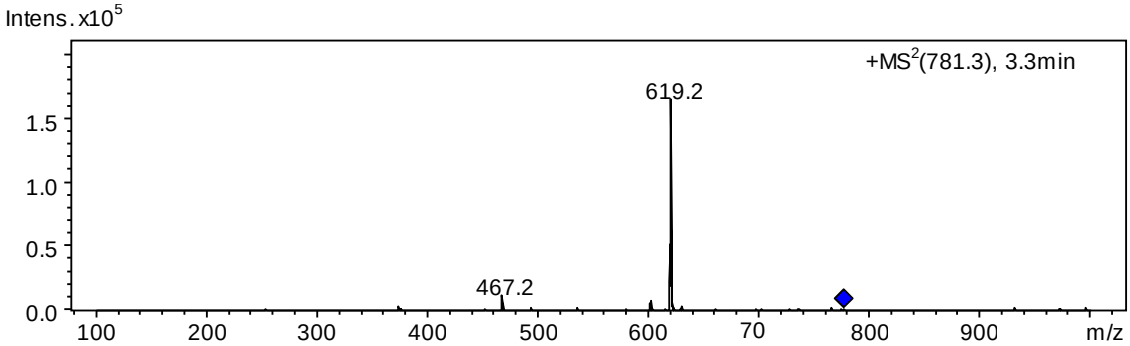



**Figure 27:** Fragmentation ($MS^2$) mass spectra of Mv-3-glucoside-catechindirect condensation product.

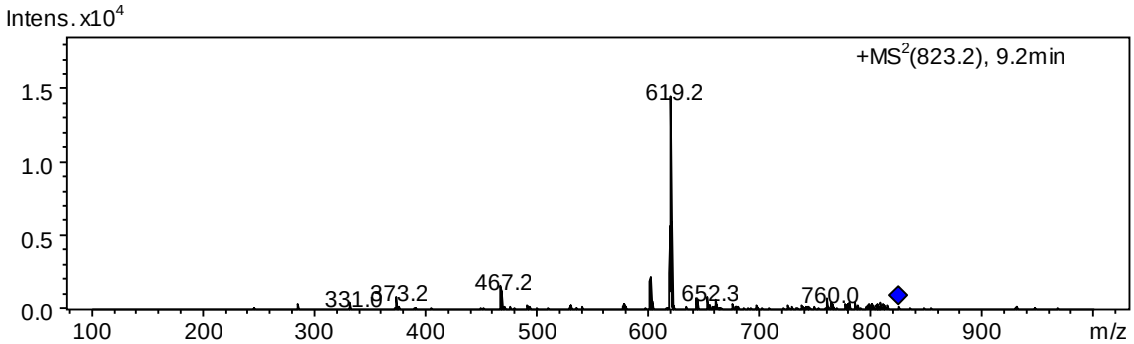



**Figure 28:** Fragmentation ($MS^2$) mass spectra of Malvidin-3-acetylglucoside-catechin direct condensation product.

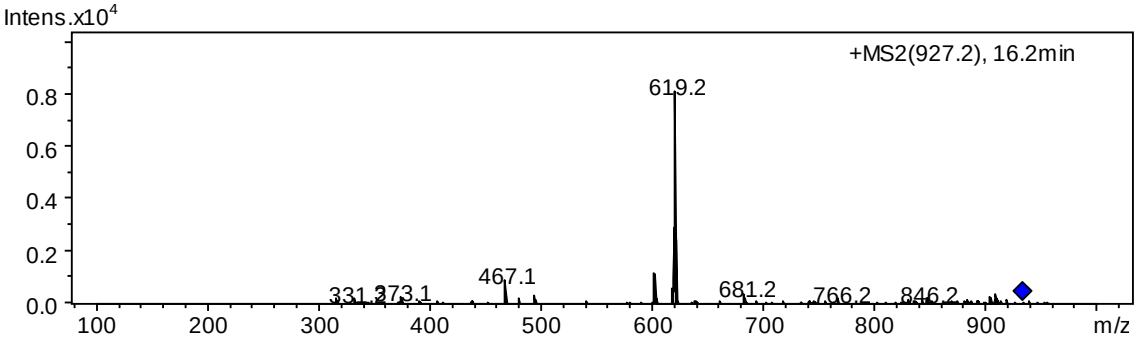


**Figure 29:** Fragmentation ($MS^2$) mass spectra of Malvidin-3-(p-coumaroyl)glucoside-catechin direct condensation product.

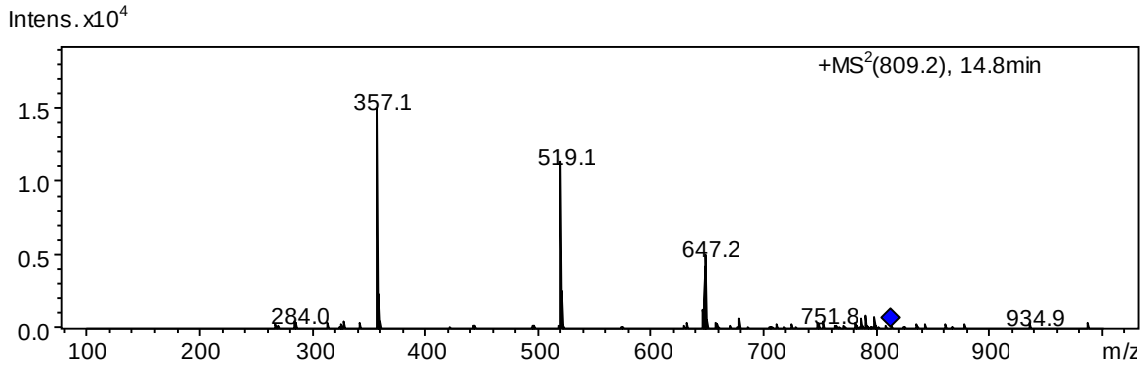



**Figure 30:** Fragmentation ($MS^2$) mass spectra of Malvidin-3-glucoside-8-ethyl-catechin

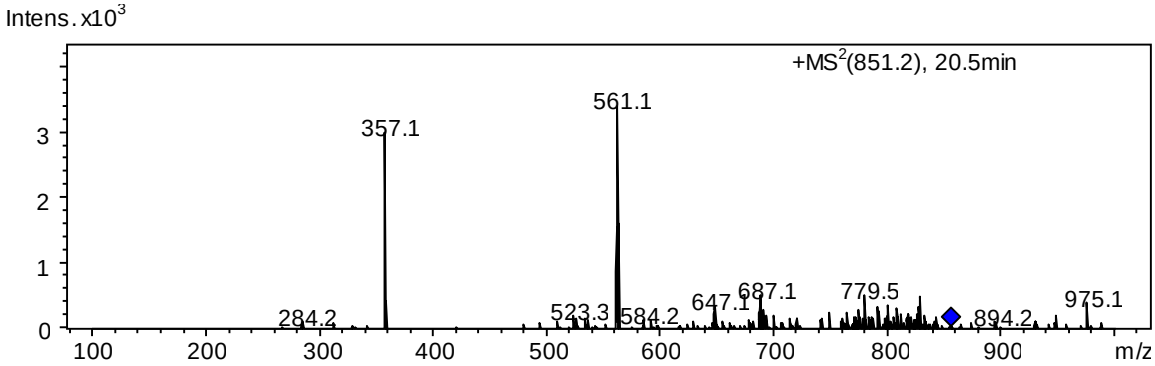



**Figure 31:** Fragmentation ($MS^2$) mass spectra of Malvidin-3-acetylglucoside-8-ethyl-epicatechin

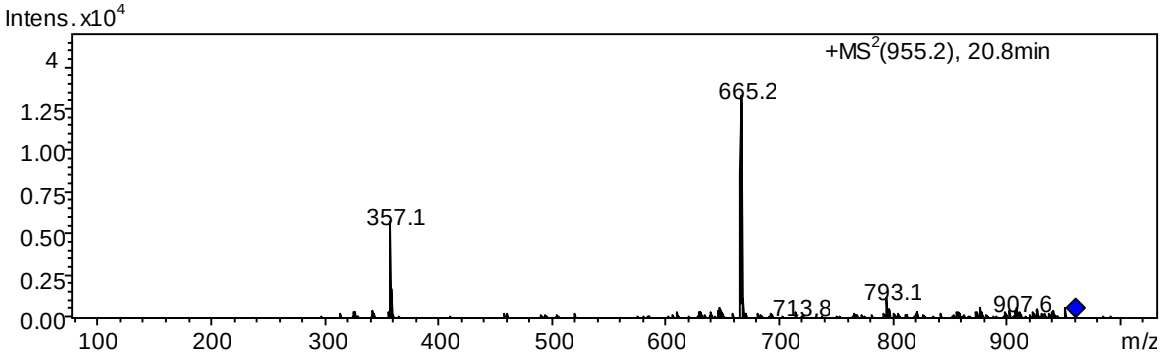



**Figure 32:** Fragmentation ($MS^2$) mass spectra of Malvidin-3-(p-coumaroyl)glucoside-8-ethyl-epicatechin

# 4 ARTIGO 3 - Thermoculture on 'cabernet sauvignon' grapes: effects on anthocyanin composition and sensorial attributes of wine

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**ABSTRACT**

Thermoculture is an alternative technology implemented in the vineyard to control fungus diseases development. However, it could also act as an elicitor due plant thermic stress and, thus, modulating secondary metabolism. This work has aimed to determine the role of themoculture in 'Cabernet sauvignon' grapes on wine sensory properties and its anthocyanin profile. The vineyard was divided in two blocks for control and TPC treatment each. a total of 21 applications was used from flowering to harvesting. A micro scale wine making process and wines were stored for 12 months previously to analysis. Wines were analyzed for physicochemical analysis, identification and quantification by high performance liquid chromatographic and sensory descriptive analysis. Thermic treatment of 'Cabernet sauvignon' grapes in the vineyard has increased 4.5% of the total content of anthocyanins monomers, 29.4% of the pyranoanthocyanins, and 29.5% of the direct condensation products formed in wine making and aging. Panel of judges have significantly perceived differences in attributes of visual (tear, red color) and gustatory (woodsy flavor) perceptions. Even that TPC in grapes was not able to alter wines acceptance, it has significantly affected the sensorial quality of wine. Thermoculture is an innovative technology for crop research and it could be a promising tool to increase anthocyanins and 'Cabernet Sauvignon' wine quality.

**Key-Words:** Treatment thermal, *Vitis vinifera*, malvidin-3-glucoside, sensory descriptive.

## 1.INTRODUCTION

Brazilian viticulture has as one of its major production region two distinct regions in the state of Rio Grande do Sul located in south of this country. Situated in the parallel 30°S in the east of this state there is a plain grassland territory with great production of *Vitis vinifera* grapes for wine production. This region has two distinct annual growing cycles (hibernal rest and crop growing) (PROTAS; CAMARGO; MELO, 2014), a climate with high temperatures in the summer (35 ºC) and a cold winter with high air humid, frost, and rain indices, in a lesser extinction some snowing short-periods (VINHOS DA CAMPANHA, 2016).

Wet climates and increased rain incidence (BATTILANI et al, 2003; BELLÍ et al., 2005) are the major reasons by which fungus contamination is developed in vineyards (WILCOX, 2015) compromising grape integrity and hygienic safety. Reduced rain incidence during grape crop management was already linked to higher wine quality (GRIFONI et al., 2006).

'Cabernet Sauvignon' is a French grape variety (SWEET, 2008; GALET, 1998), that, nowadays, in Brazil it has vigorous and median production. However, *Vitis vinifera* and its hybrid cultivars, as 'Cabernet Sauvignon' have more liability than American cultivars (TRIGIANO; WINDHAM; WINDHAM, 2010). which can reduce productivity or premature plant death (CAMARGO, 2003). Thus, thermoculture or Thermal Pest Control (TPC) technology was implemented in some vineyards to control fungus diseases development. A previous report about preliminary studies using temperatures of 110 ºC in heated air by TPC equipment has shown as able to control fungus diseases and decrease insects (BARRA, 2006). Furthermore, there are some evidences that this technology could also act on plant secondary metabolism in answer to a thermic stress, and thus, acting as an abiotic elicitor (FISCHER, 2012). Phenolic compounds, as anthocyanins, are compounds that have its synthesis stimulated during plant stressing situations (TREUTER, 2010), and it could be at increased levels in fruits previously treated by thermic treatment during crop management.

Secondary metabolites are enrolled in plant surveillance and answer to stressing conditions including abiotic stressors, as temperatures above ideal conditions for development (HUMMEL et al., 2004, JOCHUM et al., 2007). Anthocyanins are major pigments in grape and grape products that are formed in metabolic routes of this metabolism. These compounds could have increased levels due the many environmental stressors, including increased solar UV radiation and temperatures (RAMAKRISHNA; RAVISHANKAR, 2011).

In literature, anthocyanins and the pigments formed during wine making and aging (pyranoanthocyanins, direct condensation products, and acetaldehyde mediate products) are

majorly linked to some important sensorial properties of wine, as color (HE et al., 2012a; HE et al., 2012b) and some mouth perceptions (VIDAL et al., 2004).

Determination of the anthocyanin profile in 'Cabernet Sauvignon' wines using appropriate and high selective and sensible techniques, as high performance liquid chromatographic (HPLC-PDA-MS/MS) are vastly reported in literature (ALAÑÓN et al., 2016, AVIZCURI, et al., 2016, BLANCO-VEGA et al., 2014). Nonetheless, no previous studies about the effect of thermoculture practices on wine anthocyanin profile and sensorial properties were found.

Finally, this work has aimed to determine the effect of Thermoculture using TPC techniques in 'Cabernet Sauvignon' on the wine anthocyanins and sensorial properties. Total levels of other important class of polyphenols, the condensed tannins, and total measurements of this secondary metabolites will be also evaluated.

## 2. MATERIALS AND METHODS

### 2.1 Thermal Pest Control

This experiment was conducted in the city of Dom Pedrito, Rio Grande do Sul, Brasil (30°58´54´´S, 54°40´39´´W e altitude de 230 m). The vineyard was divided in two blocks for control and TPC treatment each. Experimental design was randomized with two treatment groups and four repetitions each.

TPC equipment was towed by a tractor which moves at a speed of 4 km $h^{-1}$ releasing the heated air (130 ºC) at a distance of 20 cm from espaliers. A total of 19 applications was used from flowering to harvesting. At the commercial maturity degree grapes were harvested (Control - SST: 20.2 ºBrix, pH: 3.94, TA: 0.56 g $100mL^{-1}$; TPC - SST: 20.2 ºBrix, pH: 3.98, TA: 0.56 g $100mL^{-1}$).

### 2.2 Wine making practices

A micro scale wine making processing technology was as preconized by Pszczókwski & Lecco (2011) with some modifications. Must was addition of sulfur dioxide, enzymes pectinase (Lafase® He Grand Cru and Lafase® fruit, Laffort®). Next, yeast was inoculated (Zymaflore® FX10, Laffort®) with an activating agent (Superstart®, Laffort®). After 24 hours of fermentation, another activating agent was used (Bioactiv®, Laffort®). Temperature of fermentation was kept at 25 °C and malolactic fermentation was controlled by paper chromatography as recommendations from Daudt (1971). After this fermentation malolática

sulfur dioxide was add wine was transfused and bottled. Wines were stored for 12 months previously to analysis.

### 2.3 Identification by HPLC-PDA-MS$^n$ and quantification by HPLC-PDA analysis

Wine samples were previously purified in solid phase extraction cartridge (SPE-C18) using Rodriguez-saona & Wrolstad (2001) with modifications by Bochi et al. (2015) from identification and was performed in previously dealcoholized (5 ml, 39°C for 10 minutes; Rotavapor® R-300, Buchi, Labortechnik, Germany) and filtered (0.22µm, PTFE, Phenomenex) wine samples from quantification.

Identification equipment performed in HPLC-PDA-MS$^n$ described above was connected to ion trap mass spectrometer (Esquire 6000, Bruker Daltonics, Billerica, MA, USA) equipped with an electrospray interface operating in positive mode. The ESI conditions were as follows: capillary voltage of +4.5kV, nebulizer gas pressure at 30 psi, dry gas at 11 mL min$^{-1}$, and gas temperature at 310 °C. MRM experiments were performed in a full scan range of 200 to 1000 m/z of all fragments formed from 3 major parent ions per second. And quantification performed in HPLC-PDA equipment was a Prominence 20 A (Shimadzu, Japan) equipped with degasser (DGU20A5 prominence, Shimadzu, Japan), column oven (CTO-20A prominence, Shimadzu, Japan) and coupled to a PDA detector (SPDM-20A prominence, Shimadzu, Japan)

Identification and quantification was using a reverse phase column (C18, 2.6 µm, 100 mm x 4.6 mm, Phenomenex, Torrance, USA) thermostated at 38˚C and gradient elution for separation was performed using two mobile phases named as (A) an acidic solution (3% v/v of formic acid, 85%, Merck®) in ultrapure water (Milli-Q Gradient System, Millipore Corporation, Massachusetts, EUA) and (B) pure acetonitrile (HPLC grade, Merck, Darmstadt, Germany). Thus, elution gradient has as initial condition 10% of mobile phase B (pure HPLC grade acetonitrile, Merck, Darmstadt, Germany) and 90% of A (3% v/v formic acid ultra-pure water solution, 85%, Merck®). During the first 20 minutes, gradient has increased to 25% of B. After 20 minutes, gradient was increased to 80% of B in 5 minutes, kept in this condition for 2 minutes to elute highly retained compounds from injected ‘Cabernet sauvignon wines’. After column cleanup, the initial conditions were re-established kept for 5 minutes for column re-equilibration. All eluted compounds were monitored from 250-800nm. Using standard curve malvidin-3-glucoside (Oenin chloride, 97%, Sigma-Aldrich, St. Louis, MO, U.S.A) analyzed in relation to linearity and limits of detection (LoD) and quantification (LoQ) using recommendation from ICH validation guide (2005).

### 2.4 Physicochemical and color analysis

Wines were analyzed for ethanol concentration using an equipment of Gibertini, procyanidins, total acidity by OIV (2010), color intensity (IC), and all other color parameters by the methods from Ribéreau-Gayon et al (2006). Monomeric anthocyanins (MA) was determined using the method described by Giusti & Wrolstad (2005) and total polyphenols by Singleton & Rossi (1965).

### 2.5 Quantitative Descriptive Analysis (QDA)

'Cabernet sauvignon' wines were analyzed in the laboratory for sensorial analyses from Food Science and Technology department of UFSM using QDA procedures described by Stone & Sidel (2004) with necessary modifications. During this time, discrimination tests were performed to remember basic gustatory perceptions, a test sensorial memory, attribute formation and description, and a final group discussion for consensus among the panelists and scale presentation. Moreover, at every sensorial training section standards for sensorial reference were presented (Table 1) and kept available for trained panelists during evaluation.

Sensorial training sections were performed in appropriate sensorial closed cabins (white tables equipped with openable windows for samples delivery and superior red, yellow, and tungsten lamps) in a total of two hours per day for two weeks. Thus, female and male panelists with 25 to 59 years old were selected in the university public summarizing a total of 8 in which seven of them were women and one man. This work was approved by UFSM ethical committee (project number 23044813.1.0000.5346).

Eighteen attributes (Visual – color, translucent, tear; Olfatory – woodsy, herbaceous, alcohol, red fruits, ripe fruits, caramelized and floral; and Gustatory – fruit, woodsy, acid, bitter, astringent, pungent, persistence, body) were selected as best descriptors for 'Cabernet sauvignon' wines. This attributes were evaluated using a non-structured scale of 9 cm that started with the word "nothing" as the minimal score, followed by "weak" and "little" as two medium scores with increasing intensity, and in the another extremity of the line, the word "strong" for the maximum score. Following this test, judgers have performed the acceptation test using a 7-point verbal hedonic scale (1= extremely dislike e 7= extremely like).

### 2.6 Statistical treatment of data

All analytical data was represented as the average of an experiment performed in triplicate. Data was submitted to factorial analysis of variance (ANOVA) and Tukey test at

5% of error probability was used for mean comparisons. The software Statistica® 9.0 was used for statistical treatment of data.

## 3. RESULTS AND DISCUSSION

### 3.1. Physicochemical and color analysis

All physicochemical wine quality parameters were in agreement with local and international regulation (BRASIL, 1988; OFFICIAL JOURNAL OF THE EUROPEAN UNION, 2006). Furthermore, except for alcohol concentration (Table 2), there are no significant differences between 'Cabernet sauvignon' wines produced in this work from control and TPC treated samples.

Wine color analysis (Table 2) has shown a treatment effect in parameters related to the overall color intensity (CI), hue angle, and the absorbance at 520 nm which is high related to the anthocyanin levels and its related pigments composition. However, no significant differences were observed in the total levels of monomeric anthocyanins. It possible indicates that colorimetric method is not enough sensitive to detect oscillation on these compounds that could justify differences in color. Moreover, the difference ($p \leq 0.05$) in hue values due to TPC treatment possibly indicates that anthocyanin composition could also be affected. Thus, a high selective and sensitive analytical method by HPLC-PDA-MS/MS was required and used to determine the treatment effects in each anthocyanin and in the pigment profile as a whole. Results from total polyphenolic assay and proanthocyanidin quantification in wines were not affected by TPC in grapes.

### 3.2. Identification and quantification of Cabernet sauvignon wine pigments

A total of 24 different peaks were detected by the HPLC-PDA method in which the combined information of elution order, ultraviolet to visible (UV-Vis) spectra, and mass spectrometry fragmentation patterns allowed the identification of more than 30 different anthocyanins and related pigments in 'Cabernet sauvignon' wines (Table 3, Figure 1). No new compounds in wines were detected as result of TPC treatment on grapes. However, concentration of some of these pigments were increased due treatment. Identification of each of these peaks will be discussed in separate followed by comparison of data from quantification assay of wines produced with treated and untreated samples.

#### 3.2.1 Anthocyanins monomers

‘Cabernet Sauvignon’ wines have shown 13 different monomers of anthocyanin (peaks 2b, 5-8, 10a, 14, 15a, 16a, 17, 19, 21 and 22a, Table 3, Figure 1). In this class of anthocyanin, only one compounds was a diglucosylated anthocyanin (Malvidin-3,5-diglucoside, 5.70 minutes) identified by two neutral losses of 162 m/z and with a shorter elution time than the monoglucoside form (Malvidin-3-glucoside, 7.66 minutes). The presence of only one diglucosylated anthocyanin in smaller concentration than all other compounds (0.02 mg - control and 0.05 mg - TPC of Mv-3-glc equivalents/L wine) is in agreement with the literature that recognizes wines from *Vitis vinifera* species as having reduced quantities of these compounds (LAMBERT et al., 2011, MANNS; MANSFIELD, 2012) and it was also reported in ‘Cabernet sauvignon’ grapes (PICARIELLO et al., 2012, XING et al., 2015) and wines (HE et al., 2012, PEDASTSAAR et al., 2014). Moreover, TPC has not significantly altered the concentration of this compound.

Four monoglucosylated anthocyanins were found in samples of ‘Cabernet sauvignon’ wines (peaks 2b, 5, 7 and 8, Table 3, Figure 1) identified by a single neutral loss of 162 m/z from the molecular ion and detection of the aglycon ion (303, 317, 301, and 331 for delphinidin, petunidin, peonidin, and malvidin, respectively. Elution order was determined by substitution pattern in the aglycon with shorter times for high hidroxylated (delphinidin) than for methoxylated (malvidin) compounds. Only peonidin-3-glucoside had its levels increased in wine by TPC treatment in the vineyard.

Accordingly to the expected sequence in the elution order for anthocyanin, in ‘Cabernet sauvignon’ wines five acetyl derivated compounds (peaks 10a, 14, 15a, 16a, and 17, Table 3, Figure 1) were detected with longer retention times than non-acylated compounds and with the characteristic fragmentation pattern in mass spectrometry. P-coumaroyl derivatives were also found in these samples linked to peonidin and malvidin, this last anthocyanin in two isomeric forms determined by same fragmentation patters but in two different retention times. In literature, it is already stablished that *cis* isomers have a lesser intense retention in C-18 columns than *trans* structures (BOIDO et al., 2006, ALCALDE-EON et al., 2006). In the present study, p-coumaroyl derivatives were less abundant than acetyl derivatives. This observed profile behavior is in agreement with previous literature data for ‘Cabernet sauvignon’ wines (BURNS et al., 2002, AROZARENA et al. 2002, NUÑEZ et al. 2004).

TPC had significantly increased only two of the p-coumaroyl derivatives, the malvidin-3-*cis*-(p-coumaroyl)glucoside and the peonidin-3-(p-coumaroyl)glucoside. For acetyl derivatives, treatment could have affected delphinin-3-acetylglucoside and petunidin-3-

acetylglucoside. However, it could not be affirmed because in these PDA-detected peaks (10a/10b and 15a/15b) there are co-elutions with pyranoanthocyanins. For peak 10a/10b (delphinidin-3-acetylglucoside plus A type visitin of Mv-3-glucoside), it could be assumed that peak is majorly composed of the pyranoanthocyanin due to the characteristic UV-visible spectra which was with the maximum absorbance at 510 nm, a 15 nm hypsochromic shift from the precursor anthocyanin (malvidin-3-glucoside, 525 nm).

For this class of anthocyanins pigments that were derivate from grape, all compounds detected in 'Cabernet sauvignon' wines were previously reported for different wines obtained from the same grape variety (HE et al., 2012, PEDASTSAAR et al., 2014; BURNS et al., 2002, AROZARENA et al. 2002, NUÑEZ et al. 2004).

### 3.2.2 Pyranoanthocyanins

Eleven pyranoanthocyanins were detected in wine samples (peaks 3, 4, 10b, 11, 12a, 13b, 15b, 18, 22b, 23, and 24) in which only two of them were formed by cycloaddition acetaldehyde into anthocyanin structure (B type vitisins). Identification of this compounds were based detection of characteristic fragments with m/z values that were equal to the mass to charge ration of the aglycon plus 68 amu and 24 amu for A type and B type vitisins, respectively. Among all these compounds, at least three of them seems to be altered by TPC treatment in the vineyard since it were detected in higher amounts and as pure compounds in peaks with no co-elutions. These anthocyanin derivate pigments are formed due cycloaddition of fermentation products (pyruvic acid and acetaldehyde) during winemaking and aging, and it were already detected in other reports in literature for 'Cabernet sauvignon' wines (CEREZO et al., 2010, AGUIRRE et al., 2011).

### 3.2.3 Direct condensation products

Wine samples are recognized as having great amount of other polyphenols than not only anthocyanins. Thus, by electrophilic attack flavonols, as catechin and gallocatechin, can easily form adducts with anthocyanins. These compounds were already detected in a different range of wines samples (ALCALDE-EON et al., 2006; GORDILLO et al., 2012). In the present work, a total of seven direct condensation products were identified in 'Cabernet sauvignon' wines in both experimental groups (control and TPC treated grape). However, only one of them (peak 1, Table 3) were significantly found in higher concentration in wine produced with grapes from a TPC treated vineyard.

Thermal Pest Control has increased 4.5% the total content of anthocyanins monomers, 29.4% the pyranoanthocyanins and 29.5% the direct condensation products. The increase in anthocyanins monomers was not detected by the colorimetric measurement by pH differential method (Table 2) possibly by the smaller sensitivity of this method in comparison with HPLC-PDA-MS/MS techniques. Moreover, the greatest increases in pigments were in derivate-anthocyanin pigments (pyranoanthocyanins and direct condensation products) which are not properly measured in this colorimetric assay. The anthocyanin increments due to TPC detected by HPLC is in agreement with color results (Table 2) that were significantly affected in intensity and in absorbance at 520 nm.

TPC wine has presented greater scores values for visual tear and gustatory woodsy perception than wine produced with untreated grapes ($p \leq 0.05$, Figure 2).

**1.3 Sensorial analysis**

Visual tear is related to wine viscosity and, thus; any substance that could alter this property. Ethanol (Table 2) in 'Cabernet sauvignon' wines produced with TPC treated grapes had more than 3% lower content than the wine manufactured with grapes from control groups. The relationship between wine tear intensity and its ethanol is still unclear in literature. Moreover, other compounds could also change wine viscosity and impact in sensorial perception as the concentration of condensed tannins that by colorimetric measurements showed a small tendency to be in greater levels in the wine produced with TPC treated grapes (p-value of 0.11). Another evidence that could support the hypothesis that TPC is altering tannin content and composition is the increased levels of direct condensation products of anthocyanins with proanthocynidin monomers (catechin and epicatechin) in wines from grape TPC group (29.5%).

During wine making and aging no wood materials or barrels were used. However, the woodsy gustatory descriptor was detected in all samples, inclusive, with higher scores in wines produced with TPC treated samples ($p \leq 0.05$). Previous literature has already stablished that wood flavor is a combination of wood, toasted coffee, Tabaco, and phenolics (ARENHART, 2015). Phenolics were also linked to aroma of wood in wines (NOBLE, 1987). Thus, taking into account that control samples also were detected as having this flavor, for the grape variety used in this work, a polyphenol could have some contribution to the overall perception of wood. In agreement with the possible relationship of polyphenols with wood flavor in wine, TPC grape treatment had significantly affect wine pigments that are majorly formed during aging (pyranoanthocyanins and direct condensations products) which could

also be enrolled in this woodsy taste by increase of phenolic gustative perception. Previous literature has already reported that anthocyanins could have some relationship with gustatory sensorial wine descriptors, as wine mouth fullness (VIDAL et al., 2004).

At 90% of confidence level, 'Cabernet Sauvignon' wines were affected in sensorial descriptor for olfactory red fruits aroma and visual red color intensity ($p \leq 0.10$, Figure 2). Increased perception red color in 'Cabernet Sauvignon' wines by TPC grape treatment is in accordance with results obtained instrumental measurement and characterization of color (Table 2) which has shown greater absorbance at 520 nm and color intensity ($p \leq 0.05$). Moreover, it is acceptable that instrumental analysis should be more sensitive for measurement of differences between groups than an experiment with human perception for discrimination. Individual quantification of anthocyanins and wine anthocyanin derivate pigments (Table 3, Figure 1) was also increased in wines from treated samples which also corroborates for greater perception of visual red wine.

Red fruits aroma was already observed in 'Cabernet Sauvignon' wines (CHAPMAN ET AL. 2004; BINDON et al. 2014). In the present work, it was also perceived. However, treatment was not able to decrease this sensorial characteristic ($p \leq 0.05$), and just has shown a tendency to reduce this perception ($p \leq 0.10$).

Therefore, some sensorial descriptors in wine were affected by application TPC in grape vineyard, but; it was not able to alter wine's acceptability which was not altered in wines from grape TPC group. Wines were scored with values of 6.18±0.65 and 5.87±1.18 for wines from control and TPC grape groups, respectively. These values do not differ statistically ($p \leq 0.05$), and both of them correspond to the same verbal qualification of 'moderately like'.

## 4. CONCLUSIONS

Thermic treatment of 'Cabernet sauvignon' grapes in the vineyard has increased the concentration of anthocyanins and, in a greater extension, the derivate pigments formed in wine making and aging. These results suggest that TPC could stimulate secondary metabolism increasing levels of these polyphenols. It should be highlighted that significant differences in anthocyanins were just perceived in HPLC analysis and not by colorimetric measurement. Thus, other classes (proanthocyanidins and other non-anthocyanin polyphenols) that do not differed by simple measurements (colorimetric/spectrophotometric assays) could have its behavior in wine due TPC application in grapes better tracked if highly selective and sensible techniques are applied.

Even that TPC in grapes was not able to alter wines acceptance, it has significantly affected the sensorial quality of wine, majorly in attributes of visual (perception of tear, red color) and gustatory (woodsy flavor) perceptions.

Thermoculture is an innovative technology for crop research and it could be a promising tool to increase anthocyanins and 'Cabernet Sauvignon' wine quality. Future with the same specie but across different regions and crop years should be done to determine soil and climate interactions with TPC effect. Moreover, HPLC-PDA-$MS^n$ techniques should be used to determine with greater sensitivity the effect of TPC in tannins and other polyphenolic classes that are relevant for wine quality.

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**Table 1** Terms descriptors and references used in the evaluation of samples of 'Cabernet Sauvignon' wine[a]

| Descriptor | Reference |
|---|---|
| | VISUAL |
| Red rubi Color | Weak: 50 mL of 'Cabernet Sauvignon' wine in 50 mL of water |
| | Strong: 100 mL of 'Cabernet Sauvignon' wine in 15 drops of dye |
| Viscosity | Little: 10 mL of brandy (40%) in 90 mL water |
| | Very: 100 mL of brandy (40%) |
| Translucent | Little: 50 mL of grape juice in 50 mL of water |
| | Very: 100 mL of grape juice |
| | OLFATORY |
| Woodsy | Nothing: 10 mL of 'Cabernet Sauvignon' wine |
| | Strong: 10 mL of reserve 'Cabernet Sauvignon' wine/One cigarette tobacco |
| Herbaceous | Nothing: 10 mL of water |
| | Strong: 10 g of sliced fresh pepper/10g of olive |
| Alcohol | Weak: 10 mL of 5% v/v ethanol in water |
| | Strong: 10 mL of 20% v/v ethanol in water |
| Red fruits | Nothing: 10 mL of water |
| | Strong: 10g of plum/strawberry/raspberry/blueberry |
| Ripe fruits | Nothing: 10 mL of water |
| | Strong: 10 g of raisin/ 10g of ripe plum/ |
| Caramelized | Nothing: 10 mL of water |
| | Strong: 3 drops of 0.25% of vanillin solution/ 10g of brown sugar |
| Floral | Nothing: 10 mL of water |
| | Strong: 10 mL of water with 1 drop of rose aroma |
| | GUSTATORY |
| Fruit | Nothing: 10 mL of 'Cabernet Sauvignon' wine |
| | Strong: 10 mL of 'Cabernet Sauvignon' wine with 10 g of plum/strawberry/blueberry |
| Woodsy | Nothing: 10 mL of 'Cabernet Sauvignon' wine |
| | Strong: 10 mL of reserve 'Cabernet Sauvignon' wine |
| Acid | Weak: 10 mL of red wine with 0.02% w/v of citric acid |
| | Strong: 10 mL of red wine with 0.9% w/v of citric acid |
| Bitter | Weak: 10 mL of red wine with 0.01% w/v of caffeine |
| | Strong: 10 mL of red wine with 0.1% w/v of caffeine |
| Astringent | Weak: 10mL of red wine |
| | Strong: 10 mL of red wine with the addition of 1 ml of grape seed infusion |
| Pungent | Weak:10 mL of red wine |
| | Strong: 10 mL of red wine with 2 sliced ginger |
| Persistence and Body | Weak:10 mL of 'Cabernet Sauvignon' wine |
| | Strong: 10 mL of reserve 'Cabernet Sauvignon'wine |

Table 2 – Physicochemical behavior and color of wines produced from grapes treated with TPC *

| Parameters | Experiment | |
|---|---|---|
| | Control | TPC |
| TA | 107.5±2.9 a | 110.0±11.5 a |
| alcohol | 11.8±0.23 a | 11.4±0.00 b |
| PROC | 1.32±0.06 a | 1.40±0.03 a |
| $A_{420nm}$ | 0.19±0.01 a | 0.20±0.00 a |
| $A_{520nm}$ | 0.19±0.00 b | 0.24±0.00 a |
| $A_{620nm}$ | 0.03±0.00 a | 0.04±0.00 a |
| CI | 0.42±0.01 b | 0.49±0.00 a |
| Hue | 1.03±0.09 a | 0.85±0.00 b |
| MA | 135.02±16.9 a | 144.60±3.11 a |
| TP | 1353.2±8.07 a | 1359.4±25.22 a |

*Means that the average is statistically different than control for each experiment ($p<0.05$).

Abbreviations: TA-titulable acid (meq $L^{-1}$), PRO-procyanidin (g $L^{-1}$), A-absorbance, CI- color intensity (absorbance), MA-monomeric anthocyanin (mg $L^{-1}$), TP-total polyphenol (mg $L^{-1}$).

Table 3 – Tentative identification and quantification of individual anthocyanins and derived anthocyanins pigments in 'Cabernet Sauvignon' wines subjected to Thermal Pest Control (TPC) treatments[a]

| Peak | RT[a] (min) | $M^+$ | $MS^2$ (Product ions) | $\lambda_{max}$ (nm) | Pigment family | Compound | Experiment | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Control | TPC |
| 1 | 1.45 | 797 | 635, 467 | 527 | Direct condensation product | Mv-3-glc-GC | 2.01±0.10 b | 2.72±0.06 a |
| 2a | 2.56 | 751 | 619 | 524 | Direct condensation product | Pn-3-glc-C | | |
| 2b | 2.57 | 465 | 303 | 524 | Anthocyanin | Dp-3-glc | 2.76±0.57 a | 2.88±0.07 a |
| 2c | 2.58 | 781 | 619, 373 | 524 | Direct condensation product | Mv-3-glc-C | | |
| 3 | 3.63 | 533 | 371 | 517 | Pyranoanthocyanin | A type vitisin of Dp-3-glc | 0.19±0.02 b | 0.22±0.00 a |
| 4 | 4.20 | 573 | 371 | 527 | Pyranoanthocyanin | A type vitisin of Dp-3-acetylglc | 0.17±0.04 a | 0.24±0.00 a |
| 5 | 4.74 | 479 | 317 | 525 | Anthocyanin | Pt-3-glc | 2.56±0.25 a | 2.70±0.03 a |
| 6 | 5.70 | 655 | 331 | 527 | Anthocyanin | Mv-3,5-diglc | 0.02±0.02 a | 0.05±0.00 a |
| 7 | 6.70 | 463 | 301 | 516 | Anthocyanin | Pn-3-glc | 1.98±0.11 b | 2.90±0.01 a |
| 8 | 7.66 | 493 | 331 | 525 | Anthocyanin | Mv-3-glc | 56.8±12.9 a | 61.7±0.14 a |
| 9 | 8.96 | 823 | 619, 467, 373 | 527 | Direct condensation product | Mv-3-acetylglc-C | 0.19±0.06 a | 0.13±0.00 a |
| 10a | 9.16 | 507 | 303 | 510 | Anthocyanin | Dp-3-acetylglc | 1.75±0.33 b | 2.50±0.09 a |
| 10b | 9.17 | 561 | 399 | 510 | Pyranoanthocyanin | A type vitisin of Mv-3-glc | | |
| 11 | 9.34 | 547 | 385 | 485 | Pyranoanthocyanin | A-type vitisin of Pt-3-glc | 0.04±0.01 b | 0.08±0.00 a |
| 12a | 9.86 | 517 | 355 | 488 | Pyranoanthocyanin | B type vitisin of Mv-3-glc | 0.19±0.02 a | 0.19±0.01 a |
| 12b | 9.87 | 943 | 635 | 488 | Direct condensation product | Adduct gallocatechin-mv-3-O-glc | | |
| 13a | 10.85 | 801 | 331 | 514 | Direct condensation product | Mv-3-trans-cmglc-5-glc | 0.93±0.17 b | 1.31±0.00 a |
| 13b | 10.86 | 603 | 399 | 514 | Pyranoanthocyanin | A type vitisin of Mv-3-acetylglc | | |
| 14 | 11.23 | 491 | 287 | 527 | Anthocyanin | Cy-3-acetylglc | 0.08±0.00 a | 0.08±0.00 a |
| 15a | 12.21 | 521 | 317 | 527 | Anthocyanin | Pt-3-acetylglc | 0.81±0.10 a | 0.61±0.00 b |
| 15b | 12.22 | 559 | 355 | 527 | Pyranoanthocyanin | B type vitisin of Mv-3-acetylglucoside | | |
| 16a | 14.17 | 505 | 301 | 528 | Anthocyanin | Pn-3-acetylglc | 1.94±0.31 a | 1.98±0.00 a |
| 16b | 14.18 | 927 | 619, 467 | 528 | Direct condensation product | Mv-3-(p-coumaroyl)glc-C | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 17 | 14.79 | 535 | 331 | 527 | Anthocyanin | Mv-3-acetylglc | 24.0±5.02 a | 22.0±0.04 a |
| 18 | 15.43 | 707 | 399 | 515 | Pyranoanthocyanin | A type vitisin of Mv-3-(p-coumaroyl)glc | 0.19±0.02 b | 0.29±0.00 a |
| 19 | 17.76 | 639 | 331 | 530 | Anthocyanin | Mv-3-cis-(p-coumaroyl)glc | 0.40±0.00 b | 0.42±0.00 a |
| 20a | 18.25 | 847 | 685 | 458 | Other compounds | Unknown | 0.10±0.00 b | 0.14±0.00 a |
| 20b | 18.26 | 817 | 655 | 458 | Other compounds | Unknown | | |
| 21 | 19.22 | 609 | 301 | 520 | Anthocyanin | Pn-3-(p-coumaroyl)glc (cis) | 0.55±0.01 a | 0.45±0.00 b |
| 22a | 19.56 | 639 | 331 | 527 | Anthocyanin | Mv-3-trans-(p-coumaroyl)glc | 4.50±0.77 a | 3.76±0.02 a |
| 22b | 19.57 | 625 | 463 | 527 | Pyranoanthocyanin | Mv-3-glc-4-vinylcatecol | | |
| 23 | 21.32 | 609 | 447 | 503 | Pyranoanthocyanin | Mv-3-glc-4-vinylphenol | 0.21±0.03 a | 0.23±0.00 a |
| 24 | 23.33 | 651 | 447 | 505 | Pyranoanthocyanin | Mv-3-acetylglc-4-vinylphenol | 0.05±0.01 a | 0.04±0.00 a |

[a]RT: retention time (min); M+: positive charged molecular íon; $MS^2$:fragmentation of M+. Abbreviations: Dp-delphinidin, Cy-cyanidin, Pt-petunidin. Pn-peonidin. Mv-malvidin. C-catechin. GC-gallocatechin. glc-glucoside. Means that the average is statistically different than control for each experiment ($p<0.05$). Concentration in malvidin-3-glucoside (mg $L^{-1}$)

1

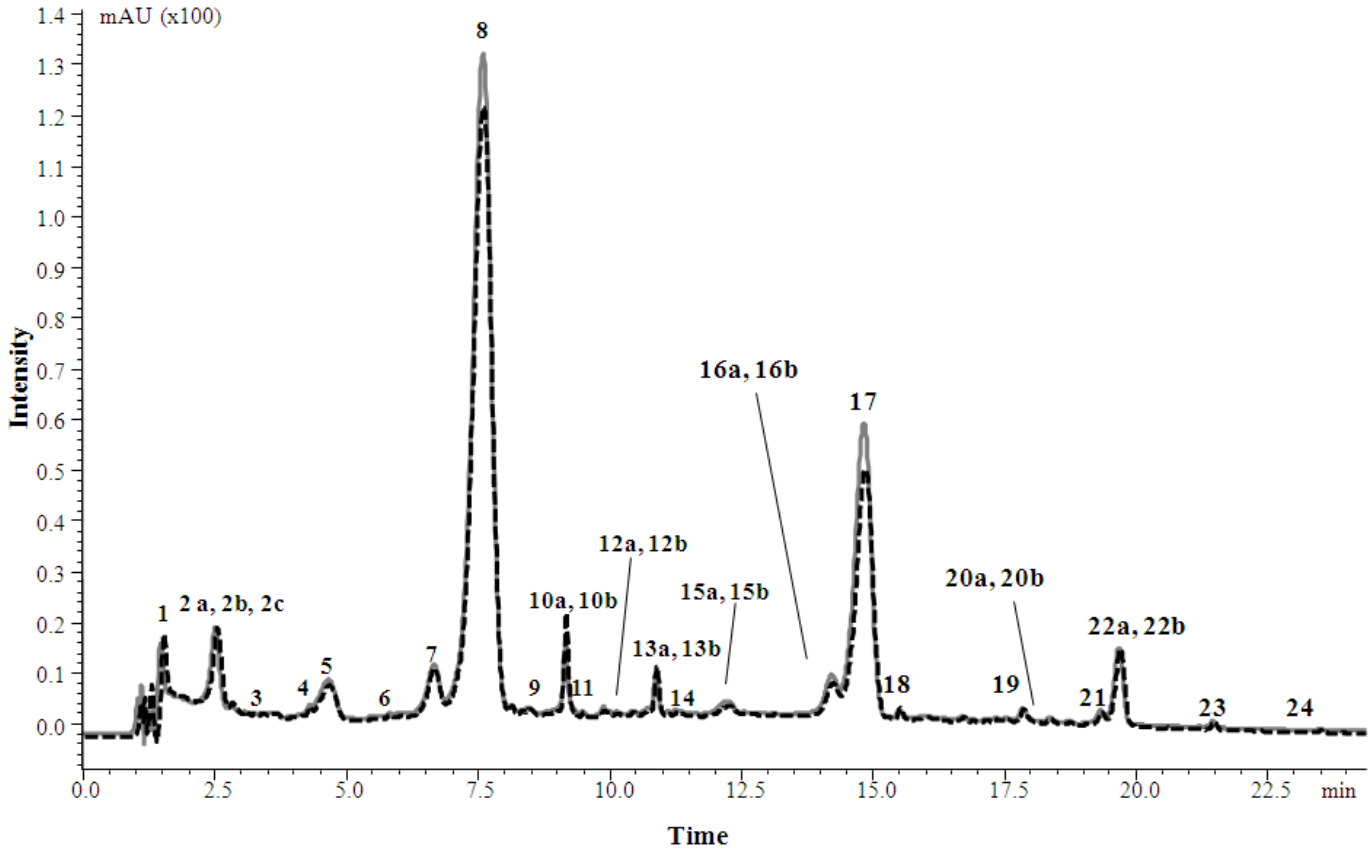


Figure 1 - Chromatographic separation of anthocyanins and related red wine pigments detected in 'Cabernet Sauvignon' wine samples obtained vineyards with Thermal Pest Control management treatments. Continuous line corresponds to the wine obtained from treated grapes and the dashed line is from wine produced with control grapes

2

1

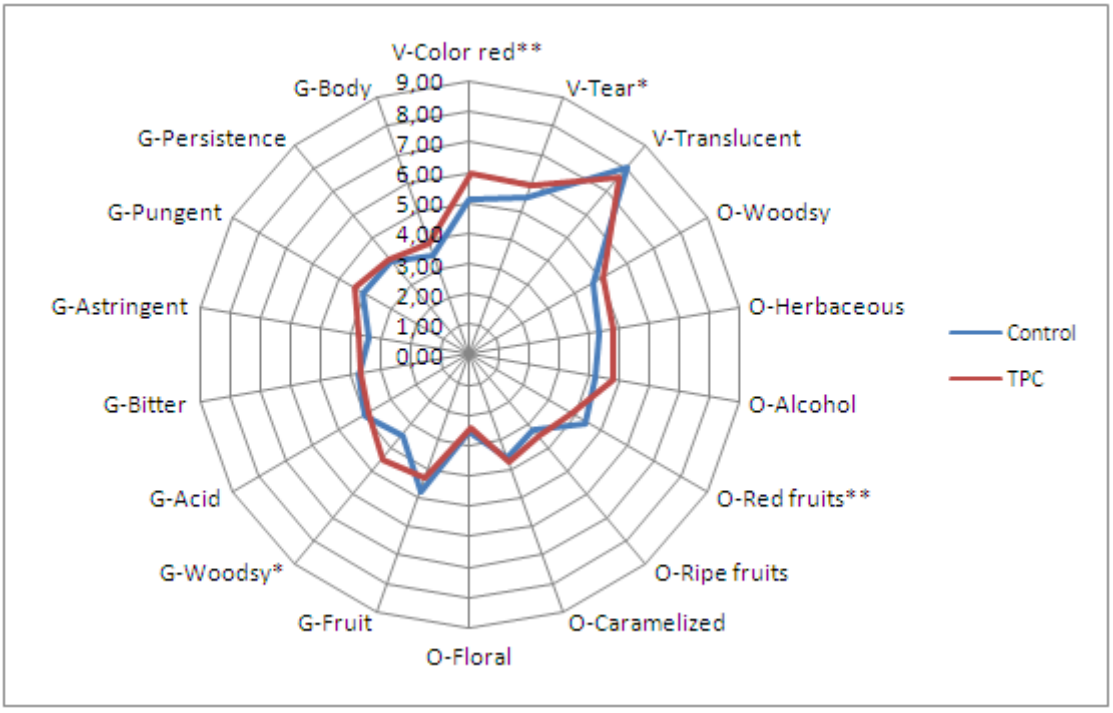


Figure 2 – Effect of Thermal Pest Control in Cabernet Sauvignon wines. V – visual; O – olfatory; G – gustatory. *Significance level of the ANOVA ($p \leq 0.05$) and **($p \leq 0.10$).

2

# 5 DISCUSSÃO

As pesquisas sobre a ação do tratamento térmico (*Thermal Pest Control* - TPC) ou *thermoculture* em videiras são amplas e independentes. Na literatura internacional a investigação do tratamento não é observada no mundo científico, estudos isolados sobre o efeito do tratamento TPC referem um aumento nos polifenóis e antocianinas totais em vinho 'Cabernet Franc' (GRAPEGROWING AND WINEMAKER, 2015).

No Brasil, também são poucas as pesquisas encontradas sobre o efeito do TPC em videiras. São observados estudos sobre o controle de doenças fúngicas, (CAVALCANTI et al. 2014; MONTEIRO et al. 2014) e efeitos físico-químico e fisiológico (DOMINGUES, 2013). No entanto, devido à escassez de estudos sobre os efeitos deste elicitor abiótico em videiras, o presente trabalho objetivou avaliar os efeitos da tecnologia sobre uvas de cultivares Tannat e Cabernet Sauvignon, além do impacto na composição antociânica nos vinhos.

Desta forma, baseado na proposta de que o tratamento térmico pode influenciar no metabolismo secundário das videiras (FISCHER, 2012), e alterar a composição físico-química do vinho (FISCHER; DAWSON, 2014; LOPES et al., 2014; GRAPEGROWING AND WINEMAKER, 2015).

O primeiro artigo foi dividido em dois experimentos, visto que, os mesmos são independentes devido às diferentes cultivares. Desta forma, teve como objetivo determinar o efeito do tratamento térmico sobre a maturação fenólica das uvas 'Tannat' e 'Cabernet Sauvignon' e composição fenólica dos vinhos. Com as técnicas analíticas utilizadas nesse artigo, foi possível observar pequenas alterações na maturação industrial na uva 'Tannat' (Artigo 1 – Tabela 1). Especialmente a redução de acidez que refletiu na maior relação de sólidos solúveis totais e acidez total no tratamento com TPC. Essa relação sugere que a uva apresenta maior maturação quando tratada termicamente. Quanto a maturação fenólica, foi possível observar alterações em ambas as cultivares. Na uva 'Tannat' ocorreu uma perda no índice de compostos fenólicos totais sem interferir na extratibilidade das antocianinas assim como nos taninos. Entretanto a uva 'Cabernet Sauvignon' foi estimulada com o tratamento térmico, aumentando o índice de polifenóis totais assim como os taninos das sementes. No entanto não foi possível observar tais diferenças no vinho com as técnicas espectrofotométricas, com exceção da intensidade de cor aumentada com o uso do tratamento térmico.

Em vista disso, com o intuito de investigar a composição antociânica do vinho 'Tannat', o segundo artigo teve como objetivo determinar o impacto sobre a qualidade do

vinho em relação à cor e composição antocianina devido à aplicação de TPC no manejo da cultura de uva ‘Tannat’ nas safras 2011/12, 2012/13, 2013/14. Na primeira safra as 4 aplicações de TPC estimularam a síntese de antocianinas monoméricas e derivados (Artigo 2 – Figura 2A), no entanto, na safra subsequente devido ao alto índice pluviométrico não foi observado efeito elicitor. Já na safra 2013/14, a mesma do primeiro artigo as 21 aplicações resultaram em um aumento de 30,6% nas antocianinas monoméricas, e 11,5% em produtos diretos de condensação direta, no entanto, houve uma redução de 16,7% das piranoantocianinas e 26,2% dos produtos de condensação mediados pelo acetaldeído (Artigo 2 - Figura 2B), referente às estruturas formadas durante a fermentação e envelhecimento. Segundo MORENO-ARRIBAS e POLO (2009), naturalmente durante o processo de vinificação ocorrem algumas reações entre antocianinas monoméricas, polifenóis e produtos de fermentação que podem promover a formação de novas famílias de pigmentos, tais como os produtos de condensação direta.

Da mesma forma como foi investigado nos vinhos de ‘Tannat’, o terceiro artigo, também procurou elucidar o perfil das antocianinas assim como as propriedades sensoriais dos vinhos ‘Cabernet Sauvignon’ sob o efeito do uso de *Thermoculture* nos vinhedos. Os resultados obtidos demonstram que houve um incremento de 4,5% no conteúdo de antocianinas monoméricas, 29,4% nas piranoantocianinas e 29,5% nos produtos de condensação direta (Artigo 3 – Tabela 2). Esse aumento de antocianinas corresponde ao aumento de intensidade de cor que também foi observado pelos provadores ($p \leq 0,10$). Outros atributos como a lágrima e o sabor amadeirado se destacaram ($p \leq 0,05$) com o tratamento térmico (Artigo 3 – Figura 2). Segundo os painelistas, as lágrimas foram mais intensas, apesar do menor teor alcoólico nos vinhos elaborados com uvas TPC. Essa informação demonstra que apesar da suposta relação entre as lágrimas e o teor alcoólico nesse caso não são correlacionáveis. Portanto, outro fator favorece a produção de lágrimas nos tratamentos com TPC, alterando a viscosidade e consequentemente são necessários mais estudos para elucidar este efeito físico-químico.

O sabor amadeirado foi detectado em todas as amostras de vinho sobressaindo-se no tratamento térmico, e que remetem ao tostado, café, tabaco ou fenólicos, que não apenas a madeira. Ressalta-se que os vinhos dos tratamentos deste terceiro artigo não foram envelhecidos em contato com carvalho. No entanto, a aceitabilidade dos vinhos foi igual para ambos os tratamentos, sendo classificados como “gostei moderadamente”.

# 6 CONCLUSÃO

O tratamento térmico TPC nas condições desse estudo:

- Influencia a maturação industrial da uva ‘Tannat’, reduzindo índice de polifenóis totais, sem alterar a extratibilidade das antocianinas. Não teve efeito na maturação industrial da uva ‘Cabernet Sauvignon’, mas estimulou índice de polifenóis totais e taninos nas sementes.
- Têm pouca influência sobre os parâmetros físico-químicos dos vinhos, exceto a intensidade de cor;
- Aumenta os compostos antociânicos e derivados do processo fermentativo em vinhos ‘Tannat’ e ‘Cabernet Sauvignon’;
- Apresenta uma influência que não é constante, em algumas situações atua e em outras não teve efeito.
- Considera que as reações dos compostos fenólicos podem ser melhor estudada por técnicas altamente seletivas e sensíveis, como cromatografia líquida de alta eficiência acoplada à espectrometria de massas;
- Favorece o aumento na intensidade dos atributos sensoriais: lágrima, cor vermelha e sabor amadeirado em vinhos ‘Cabernet Sauvignon’;
- Não altera a aceitabilidade dos vinhos ‘Cabernet Sauvignon’

As considerações finais sobre o uso do *Thermal Pest Control* como uma tecnologia para melhorar a qualidade do vinho sugere que o TPC estimula o metabolismo secundário das uvas estudadas, visto que, nos vinhos ocorre um aumento do teor dos compostos antociânicos e derivados em ambas as cultivares. Consequentemente, alguns atributos sensoriais foram melhorados com o uso do TPC em vinhos ‘Cabernet Sauvignon’.

No entanto, são necessários mais estudos para reduzir os custos de produção, avaliar o comportamento em outras cultivares, além do solo e clima de outras regiões vitivinícolas. Pesquisas futuras podem auxiliar na comprovação do estímulo elicitor fundamentado na ação enzimática e sua expressão gênica, para elucidar os efeitos deste tratamento em outras classes de compostos fenólicos voltados à saúde humana.

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## APÊNDICE A – TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Título do estudo: Qualidade de uvas e vinhos obtidos de vinhedos submetidos ao controle térmico
Pesquisadora responsável: Cláudia Kaehler Sautter
Instituição/ Departamento: Universidade Federal de Santa Maria, Centro de Ciências Rurais, Departamento de Tecnologia e Ciência dos Alimentos
Telefone para contato: (55) 3220-8254
Local da coleta de dados: Universidade Federal de Santa Maria, Centro de Ciências Rurais, Departamento de Tecnologia e Ciência dos Alimentos, Laboratório de Análise Sensorial.
**Prezado(a) Senhor(a):**
Você está sendo convidado(a) a provar um produto com teor alcoólico e em seguida, responder às perguntas deste questionário de forma totalmente voluntária.
Antes de concordar em participar desta pesquisa e responder este questionário, é muito importante que você compreenda as informações e instruções contidas neste documento. Os pesquisadores deverão responder todas as suas dúvidas antes que você tenha decidido participar. Você tem o direito de desistir de participar da pesquisa a qualquer momento, sem nenhuma penalidade e sem perder os benefícios aos quais tenha direito.
Objetivo do estudo: Avaliar os efeitos das aplicações pré-colheita do tratamento térmico em uvas, e sua influência nas características sensoriais e organolépticas dos vinhos 'Tannat' produzidos a partir deste tratamento
**Procedimentos**: Sua participação nesta pesquisa consistirá apenas em provar as amostras de vinho e o preenchimento deste questionário de acordo com as suas opiniões sobre este produto.
**Benefícios:** Esta pesquisa trará maior conhecimento sobre o tema abordado, sem benefício direto para você.
**Riscos:** A realização desta degustação pode oferecer riscos a indivíduos que possuem intolerância ou hipersensibilidade a substância comumente presentes no vinho, podendo ocasionar quadros alérgicos, bem como os relacionados ao consumo de bebida alcoólica, como tontura, dor de cabeça, mal estar geral, diminuição dos reflexos e coordenação motora.
**Sigilo:** As informações fornecidas por você terão sua privacidade garantida pelos pesquisadores responsáveis. Os sujeitos da pesquisa não serão identificados em nenhum momento, mesmo quando os resultados desta pesquisa forem divulgados em qualquer forma.
Ciente e de acordo com o que foi anteriormente exposto, estou de acordo em participar desta pesquisa, assinando este consentimento em duas vias, ficando com a posse de uma delas.

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Participante

Santa Maria, ....., de maio de 2014
Declaro que obtive de forma apropriada e voluntária o Consentimento Livre e Esclarecido deste sujeito de pesquisa ou representante legal para a participação neste estudo.

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Pesquisador responsável