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NÍVEL DOUTORADO

Avaliação de Parâmetros Enológicos, Sensoriais e Biológicos em Vinhos Espumantes: efeito de agentes moduladores

CLÁUDIA ALBERICI STEFENON

Caxias do Sul

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"Tese apresentada ao Programa de Pós-graduação em Biotecnologia da Universidade de Caxias do Sul, visando a obtenção de grau em Doutor em Biotecnologia"

Orientadora: Profa. Dra. Regina Vanderlinde

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"...filho é um ser que nos emprestaram
para um curso intensivo de
como amar alguém além de nós mesmos,
de como mudar nossos piores
defeitos para darmos os melhores exemplos e
para aprendermos a ter coragem..."
José Saramago

A **Deus**, fonte inesgotável!
Aos meus dois anjos emprestados, **Anderson** e **Caio**, por tornarem os meus dias plenos de fé e de amor, energias indispensáveis para trilhar os passos da vida!
Aos meus pais, **Antonio** (*in memorian*) e **Lourdes** por abençoarem todos os meus vôos!
A minha irmã **Luciana**, espelho de minha alma!
Ao **Diego** e à pequena **Juliana**, por existirem em minha vida!
Ao meu esposo **Ivo**, prova de que o amor é atemporal!

A Sabedoria construiu a sua casa, talhando suas sete colunas. Abateu seus animais, preparou o vinho e pôs a mesa. Enviou seus criados para anunciar nos pontos mais altos da cidade. Os ingênuos venham até aqui! Quero falar aos que não têm juízo. Venham comer do meu pão e beber do meu vinho. Deixem de ser ingênuos e vocês viverão. Sigam o caminho da inteligência!

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SUMÁRIO

LISTA DE FIGURAS E QUADRO

LISTA DE ABREVIATURAS

VE	*Vinhos Espumantes*
PIQs	*Padrões de Identidade e Qualidade*
UV (UVA – UVB)	*Ultravioleta (raios A e B)*
CLAE	*Cromatografia Líquida de Alta Eficiência*
OIV	*Organisation Internationale de La Vigne et du Vin*
$DPPH^{\bullet}$	*1,1 Difenil 2-picrilhidrazil*
SOD	*Superóxido Dismutase*
CAT	*Catalase*
DOC	*Denominação de Origem Controlada*
DOCG	*Denominação de Origem Controlada e Garantida*
OPC	*Oligômeros de Procianidinas*
PT	*Polifenóis Totais*
HCT	*Hidroxicinamatos Totais*
FT	*Flavonóides Totais*
AT	*Antocianinas Totais*
PIXE	*Particle-Induced X-Ray Emission*
RL	*Radicais Livres*
ADN	*Ácido Desoxirribonucléico*
IC_{50}	*Índice de Concentração 50%*

RESUMO

A avaliação de parâmetros enológicos, sensoriais e biológicos em vinhos espumantes (VE) *Charmat* (*brut* e *demi-sec*) e *Champenoise* (*brut*) foi realizada em escala experimental e industrial. As amostras foram obtidas e/ou elaboradas através da parceria com sete empresas do setor vitivinícola brasileiro, situadas em Bento Gonçalves e Garibaldi, no Rio Grande do Sul. Os principais objetivos foram: a) determinar os padrões de identidade e qualidade (PIQs) dos VE; b) quantificar os níveis de ácido ascórbico em VE; c) estudar o perfil fenólico dos VE através de espectrofotometria UV e cromatografia líquida de alta eficiência (CLAE); d) avaliar a capacidade antioxidante dos VE em testes *in vitro* e *in vivo*; e) verificar a presença de atividade β-glicosidásica em VE; f) realizar a análise sensorial dos VE, com a participação de um grupo de experts e segundo as normas da OIV (*Organisation Internationale de la Vigne et du Vin*); e g) comparar os dados entre os três grupos de VE analisados. Os resultados obtidos foram: a) os PIQs dos VE brasileiros encontraram-se dentro dos limites legais; b) os níveis de ácido ascórbico foram influenciados principalmente pela variedade utilizada na obtenção do vinho base e num segundo momento, pelo metabolismo da levedura durante as fermentações e o *sur lie*; c) ao analisarmos os compostos fenólicos em classes (polifenóis totais, flavonóides totais e hidroxicinamatos totais e oligômeros de procianidinas), observou-se que as reações de hidrólise e/ou polimerização (tendo como matriz, a constituição do vinho base) foram influenciadas pelo *sur lie*; constatou-se ainda que os VE apresentaram quantidades consideráveis dos ácidos gálico, caféico e ferulico, (+)-catequina. (-)epicatequina, *trans*-resveratrol, *trans*-piceid e tirosol; d) todas as amostras de VE analisadas apresentaram importante atividade antioxidante, tanto *in vitro* (testes $DPPH^{\bullet}$, SOD-like e CAT-like) quanto *in vivo* (testes com *Saccharomyces cerevisiae*); e) foi verificada a existência de atividade β-glicosidásica em VE, a qual permaneceu constante durante o período de *sur lie* estudado; f) os VE brasileiros apresentaram alta qualidade sensorial, equiparada aos elaborados em países tradicionais como França e Itália; além disto, a perlage exerceu ação positiva sobre a atividade antioxidante (CAT-like); e g) houve significativas correlações entre a composição mineral e os polifenóis presentes em vinhos espumantes (*Charmat* e *Champenoise*, *Brut* e *Demi-sec*) com a atividade antioxidante exercida pelos mesmos. Em síntese, os resultados permitem concluir que: 1) é possível elaborar vinhos espumantes capazes de exercer maior atividade antioxidante através das técnicas enológicas utilizadas; 2) é o *sur lie* (tempo de amadurecimento sobre as borras) e não o método de elaboração (*Charmat* ou *Champenoise*) que determina a variação na atividade biológica, principalmente, devido a sua maior influência sobre os teores dos compostos relacionados com a atividade antioxidante observada em vinhos espumantes; e 3) a presença de açúcar pode interferir na capacidade antioxidante, aumentando-a ou diminuído-a, em função da presença de atividade β-glicosidásica em vinhos espumantes, a qual permanece constante durante o período de *sur lie* e, portanto, está relacionada com as reações bioquímicas envolvidas no processo. Este trabalho contribuiu para ampliar o conhecimento sobre as diferenças entre os métodos de elaboração de vinhos espumantes, demonstrando que ambos possuem o mesmo potencial qualitativo frente aos aspectos industriais, comerciais e científicos.

ABSTRACT

The evaluation of enological, sensorial and biological parameters in sparkling wines (SW) *Charmat* (*brut* and *demi-sec*) and *Champenoise* (*brut*) was performed in experimental and industrial samples, provided by seven wineries, located in Bento Gonçalves and Garibaldi, Rio Grande do Sul. The main objectives were: a) Determine the beverage standards to the SW; b) quantify the ascorbic acid levels in SW; c) study the SW phenolic profile by spectrophotometry UV an high performance liquid chromatography (HPLC); d) evaluate the SW antioxidant capacity by *in vitro* and *in vivo* tests; e) verify the presence of β-glucosidase activity in SW; f) perform the sensorial analysis of the SW with the participation of an experts group by the rules of OIV (International Organization of Vine and Wine); and g) compare the data between the three blocks analyzed. The results obtained were: a) the beverage standards were in accordance with the legal limits; b) the ascorbic acid levels were influenced mainly by the grape used on the base wine production, and a second moment, by the yeast metabolism during the fermentations and sur lie; c) for the classes of phenolic compounds (total polyphenols, total flavonoids, total hidroxycinnamates and oligomeric procyanidins) we observed that the reactions of hydrolysis and/or polymerizations (linked with the base wine constitution) were influenced by the sur lie; SW showed important amounts of gallic, caffeic and ferulic, (+)-catechin, (-) epicatechin, *trans*-resveratrol, *trans*-piceid and tyrosol; d) all the samples of SW analyzed shown important antioxidant activity, both in vitro ($DPPH^{\bullet}$, SOD-like and CAT-like tests) as *in vivo* (*Saccharomyces cerevisiae* assay); e) was verified the presence of β-glucosidase activity in SW and its action was constant during the *sur lie* studied; f) Brazilians SW presented a great sensorial quality, at the same level of the French and Italian ones; furthermore, the *perlage* can act positively on the antioxidant activity (CAT-like); and g) we find correlations between the phenolic profile and mineral composition of the SW (*Charmat* and *Champenoise*, *Brut* and *Demi-sec*) with the antioxidant capacity of them. In synthesis, the results conclude that: 1) the production of SW with highest antioxidant capacity through specific enological techniques is possible; 2) is the *sur lie* (ageing on lees) and not the elaboration method (*Charmat* or *Champenoise*) that determines the changes in the biological activity, mainly due to a greater influence on the compounds related with the antioxidant activity observed in SW; and 3) the sugar concentration can change the antioxidant capacity, increasing or decreasing it, is related with the β-glucosidase activity in SW (this stayed unchanged during the sur lie, therefore, is related with the others biochemical reactions of the process). This work contributed to the understanding of the differences between the SW production methods, showing that both have the same qualitative potential aspects when compares the industrial, commercial, or scientific points of view.

APRESENTAÇÃO

Diversos estudos sobre os vinhos vêm sendo realizados. A composição físico-química da uva e sua correlação com os compostos formados durante o processo de fermentação; o papel dos microrganismos presentes no meio; rotas bioquímicas envolvidas nas etapas que vão do amadurecimento do fruto até a obtenção do produto final fazem parte dos primeiros estudos.

Especialmente a partir de 1992, com o evento do Paradoxo Francês, os interesses se voltaram para as possíveis atividades biológicas que o vinho e/ou seus compostos poderiam desempenhar no organismo humano como protetores da saúde ou promotores de um estilo de vida mais saudável. A partir deste pressuposto, explorado em vários campos, como na cardiologia, neurologia e oncologia, por exemplo, as ações que podem ser atribuídas ao vinho variam em forma e proporção, mas estas parecem estar associadas a um mecanismo comum, o de minimizar os danos causados pelo estresse oxidativo. Até poucos anos atrás, o vinho tinto era o protagonista da maioria dos estudos realizados ao redor do mundo e no Brasil este quadro não era diferente. Atualmente, trabalhos com vinhos brancos, sucos de uva, vinoterapia e vinhos espumantes começam a ser divulgados em revistas indexadas. O universo de investigação é amplo e há muito por ser feito. Para se ter uma idéia, na busca por palavras-chaves na ferramenta de pesquisa www.scopus.com, até o momento, o termo "sparkling wine" representa aproximadamente 5% do número de artigos publicados em comparação à expressão "red wine".

Devido a estas questões e ao mercado crescente de vinhos espumantes (produção, consumo interno e exportação), foi estudado, em um primeiro momento,

vinhos espumantes comerciais, elaborados por nove empresas vinícolas da Serra Gaúcha. Variáveis chaves como a atividade antioxidante e o perfil fenólico foram avaliadas e os resultados obtidos foram publicados na revista *Food Chemistry* (2010), anexo I. Pode-se observar que, a exemplo do que ocorre em vinhos tintos, os vinhos espumantes possuem considerável capacidade antioxidante, a qual pode ser modulada pelo efeito sinérgico dos polifenóis e, estes por sua vez, sofrem a influência das diferentes técnicas enológicas utilizadas. Entre estas técnicas, o tempo de amadurecimento sobre as borras (*sur lie*) alterou significativamente os resultados obtidos.

Para a construção da tese de doutoramento, estes dados foram novamente analisados através de uma abordagem com novos elementos, tais como a análise sensorial, a composição mineral e diferentes atividades enzimáticas. Estes resultados foram publicados na revista *Redox Report* (2010) e no livro *Wine: Types, Production and Health* (2012; capítulo 14). Restava ainda uma questão importante: ao elaborarmos os vinhos espumantes através dos métodos *Charmat* e *Champenoise*, mantendo constantes os principais parâmetros e monitorando o tempo de *sur lie*, iriam os resultados se repetir? Desta forma, através de nova parceria com duas das empresas participantes, novos testes foram conduzidos em escala industrial. Formulou-se a hipótese de que o *Sur Lie* seria o principal agente modulador da capacidade antioxidante observada nos vinhos espumantes. Este conjunto de dados foi aceito para publicação na revista *Food Chemistry* (Article in Press, 2014).

1. INTRODUÇÃO

O setor do vinho no Brasil vem crescendo ano a ano, o que por si só aumenta a importância de pesquisas que favoreçam o constante aprimoramento das técnicas envolvidas nos mais diferentes processos que fazem parte desta cadeia produtiva. Entre estes processos, pode-se salientar o constante aprimoramento na elaboração de vinhos (tranquilos e espumantes), o surgimento de indicações de procedência e o desenvolvimento de novas atividades empresariais (vinoterapia). Esta busca constante pela qualidade envolve o setor como um todo e inclui ações que objetivam a consolidação da marca Brasil.

A composição físico-química, as variáveis sensoriais e a presença de atividades biológicas que possam aportar benefícios à saúde humana formam um conjunto intrínseco de fatores que são responsáveis pelo conceito moderno de qualidade em alimentos e bebidas. Em vinhos, o perfil fenólico e a composição mineral desempenham importante papel, tanto do ponto de vista enológico (estabilidade/qualidade geral) quanto biológico (vinho e saúde), pois estes compostos interagem entre si e com o meio e são responsáveis pela qualidade geral, uma vez que estão, direta ou indiretamente, relacionados aos parâmetros visuais, olfativos e gustativos específicos de cada produto.

Estas interações têm relação direta com a origem da uva (procedência), o que torna muito importante realizar uma caracterização regional, passível de comparação com os demais centros vitícolas do mundo. Além do rico universo fenólico, os vinhos possuem diversos componentes inorgânicos, que embora representem uma pequena percentagem da composição final, desempenham um papel importante no processo de

vinificação e, consequentemente, na qualidade final do produto. Além disso, como uma bebida consumida em nível mundial, o vinho torna-se uma importante fonte de macro e oligoelementos que são essenciais para os seres humanos. Dado o fato de que os íons metálicos desempenham um papel importante para o estresse oxidativo nas células, que por sua vez, pode estar relacionado ao envelhecimento, a análise da concentração elementar em vinhos e espumantes é pertinente.

Neste sentido, a viticultura, a enologia e os processos biotecnológicos são os principais responsáveis pelas diferenças analíticas e sensoriais que estes produtos podem apresentar e que estão ligadas a fenômenos de adsorção, sinergismo entre os diferentes grupos fenólicos, processos de óxido-redução, interações entre compostos orgânicos, ação enzimática, entre outros. Diversos autores sugerem que a capacidade autolítica das leveduras modifica sobremaneira a qualidade do produto final, pois, entre outros aspectos, a enzima β-glicosidase presente em leveduras e bactérias envolvidas nos processos fermentativos, são capazes de hidrolisar compostos mono ou diglicosilados como, por exemplo, o *trans*-piceid (liberando sua aglicona, o resveratrol). Portanto, estudos sobre os mecanismos envolvidos nas reações entre grupos fenólicos e moléculas de glicose são de amplo interesse para a indústria vinícola, uma vez que o teor de açúcar encontrado nos diferentes vinhos espumantes (VE), como por exemplo, *demi-sec*, *brut*, entre outros, se constitui em um fator importante para o consumidor final, no momento da compra.

Estas ações conjuntas podem gerar perspectivas na busca pela definição de técnicas enológicas apropriadas para alcançar estas metas e demonstram a importância deste trabalho, o qual teve como objetivos: a) determinar os padrões de identidade e qualidade (PIQs) dos VE; b) quantificar os níveis de ácido ascórbico em VE; c) estudar o perfil fenólico dos VE através de espectrofotometria UV e cromatografia líquida de

alta eficiência (CLAE); d) avaliar a capacidade antioxidante dos VE em testes *in vitro* e *in vivo*; e) verificar a presença de atividade β-glicosidásica em VE; f) realizar a análise sensorial dos VE, com a participação de um grupo de experts e segundo as normas da OIV (*Organisation Internationale de la Vigne et du Vin*); e g) comparar os dados entre os três grupos de VE analisados.

2. REVISÃO BIBLIOGRÁFICA GERAL

2.1 Aspectos Gerais sobre Vitivinicultura Brasileira

A superfície territorial com cultivo da videira no Brasil atinge cerca de 77.000 ha (Figura 1), sendo que no Rio Grande do Sul, sua cadeia produtiva (incluindo vinhos e derivados) compreende mais de 700 vinícolas e é responsável pela geração de milhares de empregos diretos e indiretos (Paiva & Lentz Jr. 2012). O Brasil é o 5º maior produtor de vinhos do hemisfério sul e tem desenvolvido uma capacidade excepcional para a produção de vinhos de qualidade (Jeziorny & Ortega, 2012). Neste contexto, as demais atividades relacionadas ao setor (produção e exportação, suco de uva, enoturismo, enogastronomia, vinoterapia, etc.) vêm aumentando gradativamente.

A elaboração de VE é uma das áreas de maior destaque na Serra Gaúcha, devido ao alto padrão de qualidade que foi conquistado. Este segmento vem ganhando espaço e notoriedade mundial e é responsável por um consumo de mais de sete milhões de litros por ano (Santos, 2009). As exportações se encontram em expansão, alcançando hoje mais de 20 países, entre eles, Estados Unidos, Alemanha e Inglaterra. Este cenário se tornou possível devido ao reconhecimento mundial de que somos uma das melhores regiões no mundo para o cultivo de uvas destinadas a elaboração de VE (De Mello, 2011).

A comercialização dos produtos obtidos a partir dos métodos *Charmat* e *Champenoise* no período entre 2008 e 2012 apresentou um crescimento ao redor de 10% (De Mello, 2012), tendência que tem se mantido constante nos últimos anos (Figura 2). Entre as uvas mais utilizadas para a elaboração de VE, pode-se citar a

Riesling Itálico, a Chardonnay e a Pinot Noir (Figura 3) (Tonietto, 2007). Este vinho é obtido através da segunda fermentação de vinhos convencionais, objetivando a produção natural de CO_2 (tomada de espuma) através dos métodos *Champenoise* (segunda fermentação na própria garrafa) ou *Charmat* (segunda fermentação em grandes recipientes vinários) (Miolo & Miele, 2003).

Figura 1. Principais regiões vitivinícolas do Brasil (Monezzi, 2012).

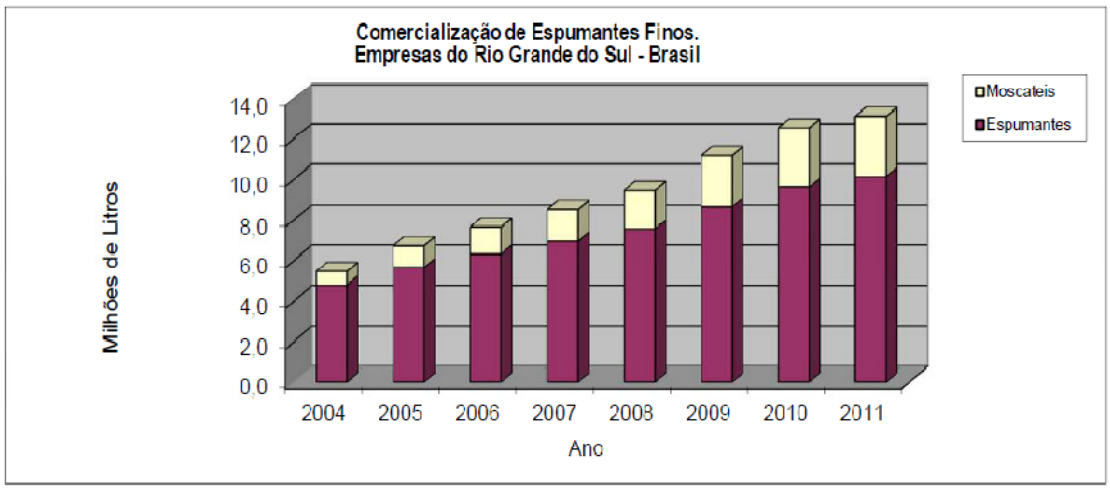

Figura 2. Comparativo da comercialização de vinhos espumantes entre 2004 e 2011 (IBRAVIN, 2011).

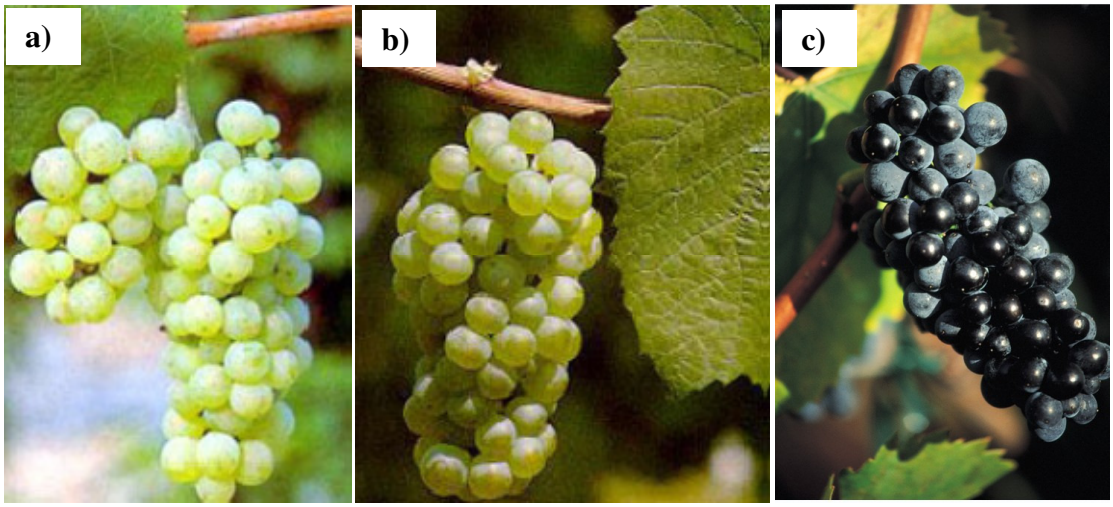

Figura 3. Cachos das uvas Riesling Itálico (a), Chardonnay (b) e Pinot Noir (c) (Simon, 2000).

A Serra Gaúcha também vem se destacando pela aptidão em elaborar vinhos de elevada qualidade. A adequada maturação das uvas devido à melhoria dos vinhedos, bem como os avanços na tecnologia de vinificação vem permitindo aumentar o percentual de vinhos tintos brasileiros "*Premium Quality*" (Zanus & Mandelli, 2004). O vinho, ao contrário de outros produtos agroindustriais, sempre esteve ligado ao conceito de *terroir*, que o remete a um espaço no qual está se desenvolvendo um conhecimento coletivo das interações entre o ambiente físico e biológico e as práticas

enológicas aplicadas, proporcionando características distintas aos produtos originários deste espaço (Mampieri, 2010; OIV, 2010). Neste sentido, é importante salientar que algumas das principais vinícolas situam-se no chamado Vale dos Vinhedos (Figura 4) e que esta foi a primeira região brasileira a ter seus vinhos certificados com Indicação Geográfica de Procedência (IGP) pelo Instituto Nacional de Propriedade Industrial em 2002 e a tornar-se, em 2012, também a primeira Denominação de Origem de vinhos e espumantes do país.

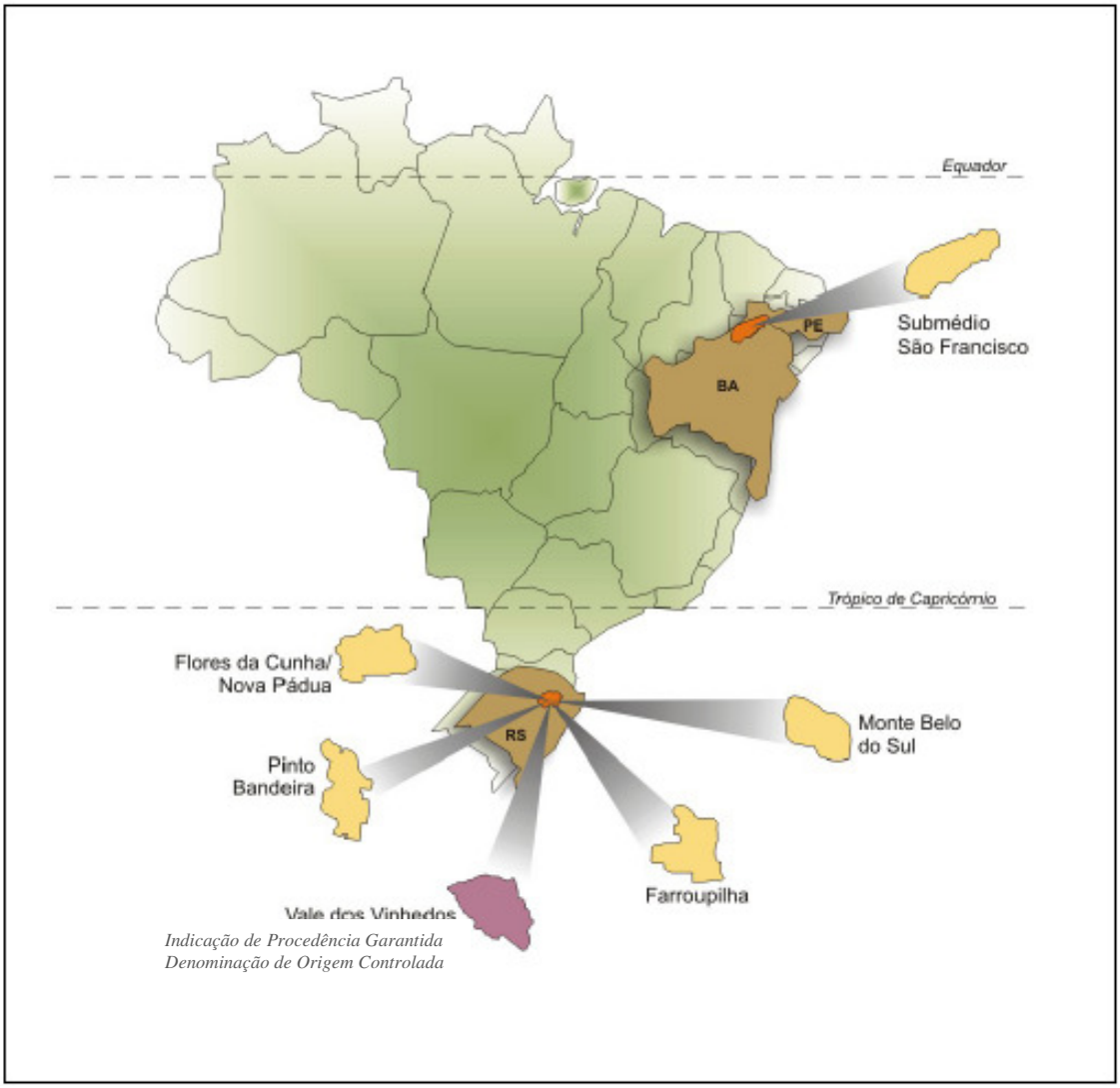

Figura 4. Indicações geográficas e denominação de origem controlada de vinhos finos do Brasil (Adaptado de Tonietto & Zanus, 2007).

A Região Uva e Vinho, no Rio Grande do Sul, compõem-se por 28 municípios, sendo que a atividade turística data do início do século XX, quando os visitantes já buscavam lazer no clima ameno dessa região serrana. Atualmente, os roteiros de enoturismo vêm se consolidando, devido aos atrativos da uva e seu entorno, os quais exercem apelo sobre os aproximadamente 1 milhão de turistas que visitaram a região em 2006 (Valduga *et al*., 2007). O consumidor do vinho é, em geral, exigente, pois de acordo com De Lemos (2005), "o valor turístico está assentado no conjunto de produtos sociais de uma comunidade no espaço e no tempo", isto é, o valor é produto do meio, e como tal, precisa ser preservado. Exatamente por isto, a preocupação com a sustentabilidade, da cadeia produtiva em si e do meio-ambiente, vem se desenvolvendo cada vez mais na vitivinicultura brasileira. Até pouco tempo atrás, os resíduos da indústria vinícola não recebiam destino adequado, ou pelo menos, não eram vistos como subprodutos capazes de gerar renda. Hoje, existem diversas opções de uso para estes resíduos, que vão desde o uso de restos da poda (ou seja, ainda na parte vitícola) como adubo (Ciota, 2007), até o aproveitamento das borras de vinificação para a obtenção de matérias-primas para a indústria farmacêutica e cosmética (Maraschin *et al*., 2002; Franzoni, 2006).

No que diz respeito a este último aspecto abordado, o interesse por bioativos da uva e do vinho vem ganhando destaque em nível mundial, não somente pelos conhecidos aspectos de "*vinho e saúde*" (Covas *et al*., 2010) válidos para um consumo regular, moderado e às refeições, mas também sob o ponto de vista da manutenção da saúde e da beleza do corpo como um todo (Pereira, 2008; Maier *et al*., 2009), através da vinoterapia (Núñez-Sellés, 2005; Neves, 2008). Afinal, inúmeros são os benefícios associados à ingestão de produtos que possuam compostos fenólicos em sua composição, os quais são capazes de agir como queladores de íons metálicos e/ou

seqüestradores de espécies reativas, justificando o potencial antioxidante dos mesmos (Stefenon, 2006). Neste sentido, a uva e seus derivados possuem papel de destaque (Spada, 2003; Franzoni, 2006; Stefenon, 2006; Dani, 2006).

Segundo Maraschin *et al*. (2002), a maioria dos subprodutos vegetais é pobre em nutrientes como proteínas e vitaminas, o que não acontece com os resíduos provenientes da uva (película, semente, engaço, resíduos de vinificação). Assim, surge a perspectiva de utilização destes materiais para fins econômicos, com o adicional de redução dos impactos ambientais gerados pela liberação de grandes volumes de resíduos nos sistemas agrícolas (produção limpa). De fato, produtos a base de sementes de uvas, por exemplo, são utilizados há vários anos em países da Europa e nos Estados Unidos como complemento nutricional, vitamínico, na cosmética (óleo essencial) (Figura 5) e como agentes profiláticos (antioxidantes) (Figura 6).

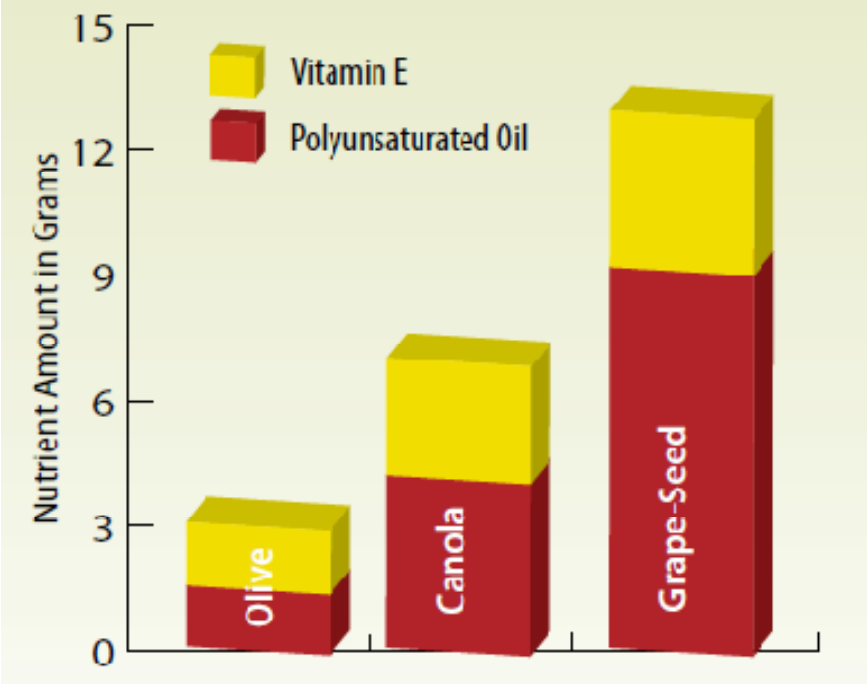

Figura 5. Exemplo de composição nutricional de diferentes óleos vegetais (Leber, 2012).

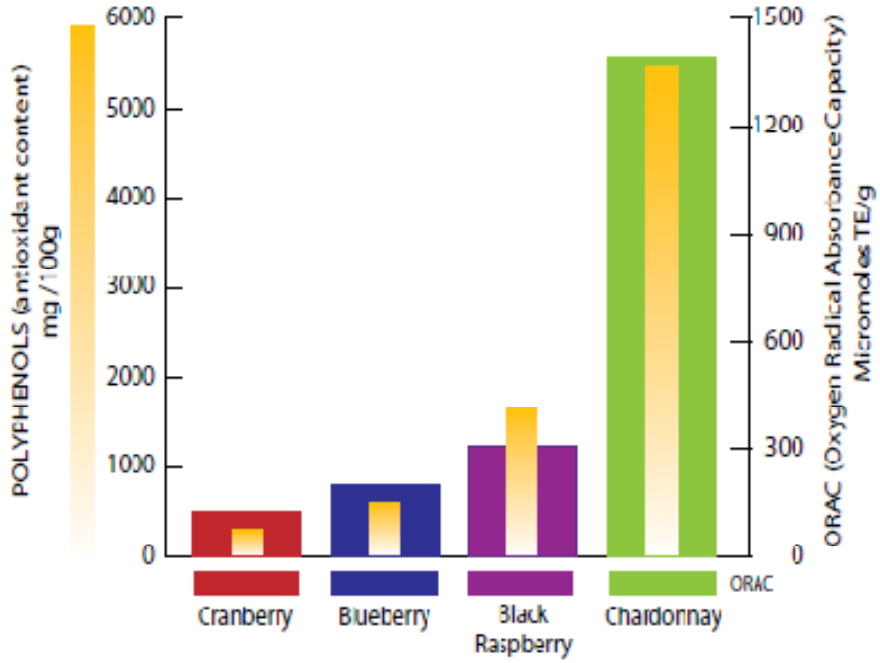

Figura 6. Teor de polifenóis e respectiva atividade antioxidante de sementes de diferentes frutas (Leber, 2012).

No Brasil, as sementes de uva já são utilizadas para a produção de adubos através da adoção de procedimentos adequados de preparo daquela biomassa (Ciota *et al*., 2007). Todavia, a utilização como fonte de compostos de interesse (em especial, polifenóis com reconhecida atividade antioxidante) às indústrias química, de cosméticos e farmacêutica, deve ser levado em consideração, já que os bioativos da uva e do vinho possuem valor agregado e resultam na obtenção de produtos de alta qualidade e competitivos economicamente (Floris *et al*., 2010).

Entre os principais subprodutos da uva e do vinho para a área farmacêutica e cosmética, pode-se citar: a) extrato de semente de uva, rico em lipídios, proteínas, carboidratos e polifenóis (incluindo oligômeros de procianidinas (OPC); b) óleo extraído das sementes de uva, o qual contém os ácidos, palmítico, palmitoléico, esteárico, oléico, linoléico, assim como alfa- linoléicos, icosanóico e docosanóico e OPC; c) leite de uvas, que consiste em uma emulsão contendo o extrato de semente de uva e o óleo de semente de uva de *Vitis vinifera;* e d) extrato de vinho, obtido após a

vinificação, o qual contém ácidos, málico e tartárico, sais minerais e compostos fenólicos (Maier *et al*., 2000; Pereira, 2008).

Durante muito tempo, a vitivinicultura brasileira (concentrada no Rio Grande do Sul, estado que detém 90% da produção nacional) esteve alicerçada na produção de vinhos tranquilos, em especial, tintos. Hoje, devido à importância do elo entre o meio, o produto e o consumidor já discutidos anteriormente, diversos comitês de regulamentação garantem alta qualidade final (Figura 7). Graças à introdução de novas tecnologias tanto nos vinhedos como nas vinícolas (Tonietto & Zanus, 2007), o mercado de outros produtos vem crescendo ano a ano, como é o caso dos VE (De Mello, 2012).

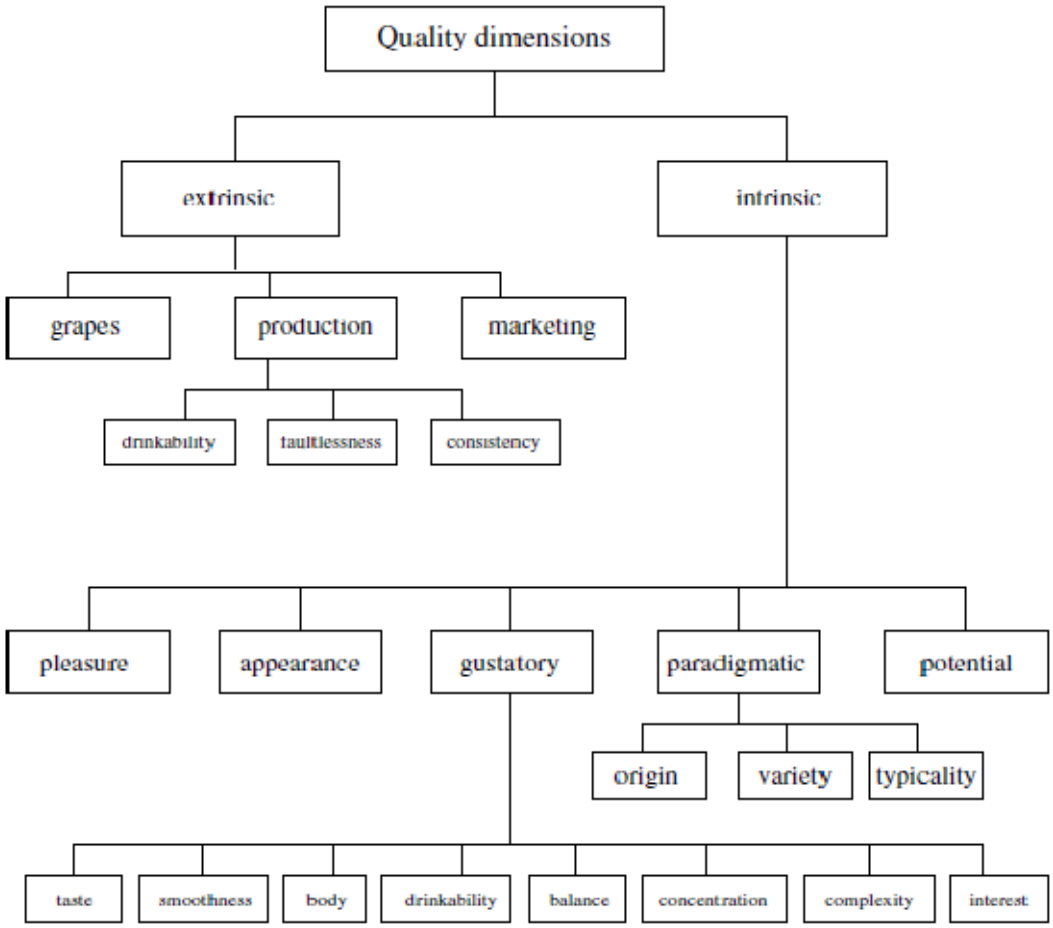

Figura 7. Parâmetros envolvidos no conceito de qualidade dos vinhos (Charters & Pettigrew, 2007).

Entretanto, as pesquisas que resultaram nestas inovações ainda podem ser consideradas escassas quando comparadas às realizadas em outros países produtores, como Espanha, Portugal, França e Austrália, o que reforça a necessidade de promover a caracterização do produto regional em função de sua origem geográfica, avaliando parâmetros físicos, químicos e biológicos, entre os quais o teor de minerais tem alta correlação (Almeida & Vasconcelos, 2003; Thiel *et al*., 2004). O vinho, como todo produto de origem vegetal, possui uma composição variada de elementos minerais que depende de diversos fatores (tipo de solo, clima, cultivar, práticas culturais e enológicas, além do contato do vinho com materiais e equipamentos utilizados nas fases de elaboração, conservação, estabilização e engarrafamento) (Thomas *et al*., 1993; Jaganathan & Dugar, 1998; Rizzon, 2005). É importante salientar, ainda, que alguns dos minerais presentes nos vinhos são indispensáveis para o processo fermentativo, como fonte de nutrientes para as leveduras (fósforo, enxofre, etc.), ao mesmo tempo em que outros podem ser tóxicos, como por exemplo, chumbo e cobre, tanto aos microrganismos como para o ser humano, devendo-se, portanto, estabelecer limites para preservar a saúde do consumidor (Kocsonya *et al*., 2002; Rizzon, 2005). Estes aspectos reforçam a relevância deste estudo (Charters & Pettigrew, 2007).

2.2 Vinhos Espumantes

Os primeiros sinais da elaboração de vinho espumante em *Champagne*, na França, ocorreram no final do século XVII, embora hajam vestígios sobre vinhos nesta região desde a época romana (aproximadamente 50 a.C.). Durante a Idade Média, estes vinhos foram caracterizados como tendo uma efervescência passageira e suave devido à incompleta fermentação do mosto. Surgiram então as primeiras *assemblages* entre uvas brancas e tintas, ou seja, os vinhos que seriam precursores dos base para espumantes

(Méheut & Griffe, 1997; Díaz de Mendívil *et al*., 1999; Buxaderas & Lópes-Tamames, 2012). O início de um processo mais metódico e preciso nasceu com *Dom Pérignon*, em *Hautvillers*. Suas observações foram publicadas no livro "A arte de cuidar das vinhas e dos vinhos de *Champagne*", publicado três anos depois de sua morte por Canon Godinot. Entre elas estão: um conjunto de diretrizes sobre como colher e obter vinhos brancos a partir da uva Pinot Noir (*blanc de noirs*); a forma de poda da videira para produzir uma safra menor; as precauções necessárias para garantir que as uvas não sejam danificadas durante a colheita; a recomendação para que cada uva fosse vinificada de forma independente, entre outras. Nesta época, a primeira parcela de mosto dava origem a um vinho encorpado, porém delicado e leve, chamado *vin de goutte*, enquanto que as próximas parcelas denominavam-se primeiro e segundo *taille* (Díaz de Mendívil *et al*., 1999; Buxaderas & Lópes-Tamames, 2012).

Até o século XVIII, os VE de *Champagne* foram transportados em barris para a Inglaterra, onde eram envasados em garrafas espessas, mais resistentes à pressão exercida pelo dióxido de carbono. De acordo com alguns escritores, em 1640, *Sir Kenelm Digby* criou a primeira fábrica de garrafas em carvão betuminoso, mais resistente do que o vidro feito na França ou em qualquer outro lugar. A cortiça fixada à garrafa, com auxílio de um arame, favoreceu a dispersão do vinho, não só na Inglaterra, mas também para outras aristocracias européias, tornando-o um sinal de personalidade e elegância. Apesar de *Pasteur* somente ter esclarecido a origem da *perlage* no século XIX, a primeira evidência da segunda fermentação nos vinhos de *Champagne* existe a partir da metade do século XVIII, devido à adição de licor de tiragem. Esta era uma prática empírica utilizada para garantir a efervescência desejada, embora a quantidade precisa de açúcar necessária para gerar uma pressão de gás particular, ainda não tivesse sido calculada (Díaz de Mendívil *et al*., 1999; Buxaderas & Lópes-Tamames, 2012).

Também no século XIX, outra importante figura no desenvolvimento do vinho espumante, em especial, os *Champenoise*, entrou em cena. Madame *Clicquot* queria eliminar a nebulosidade *off-white* e inventou os *pupitres* e a arte da *remuage*. As garrafas eram colocadas horizontalmente e a cada 1/8 de volta, ao dia, iam sendo posicionadas em um ângulo cada vez mais vertical, até ficarem perpendiculares ao chão; ocorria então a sedimentação das borras no gargalo da garrafa, que ao serem removidas, tornavam o espumante limpo e com uma coloração amarela brilhante. Muito aconteceu deste período até a elaboração do primeitro vinho espumante no Brasil, há 100 anos. O crescimento deste segmento é resultado da busca constante pela qualidade, que envolve o setor vitivinícola como um todo e inclui ações que objetivam a consolidação da marca Brasil (Olavarrieta, 1995; Buxaderas & Lópes-Tamames, 2012).

Atualmente este vinho é produzido na maioria dos países vitivinícolas, recebendo diferentes denominações em função do local de origem e do método de elaboração. Além dos *Champagnes* franceses (os *Crémants* - elaborados em outras regiões da França e os *Vins Mousseux* - espumantes *Charmat*), outros produtos também merecem destaque (Payne *et al*., 2008). Na Espanha, a Catalunha é a região onde são elaborados os Cavas - Denominação de Origem Controlada (DOC). Este vinho espumante de alta qualidade também é elaborado através do método *Champenoise*, porém com variedades distintas, as autóctones, Macabeo, Xarel.lo, e Parellada (Daban, 2005). Na Alemanha, a Riesling é considerada a melhor variedade para a elaboração de VE (Sket), devido a sua acidez equilibrada, ao aroma frutado e ao sabor agradável (Woller, 2005; Buxaderas & Lópes-Tamames, 2012). Os VE italianos são conhecidos como *Spumanti* e também estão relacionados com os aromas típicos de suas cultivares nativas e às áreas de produção (Zironi & Tat, 2005). Neste sentido, Franciacorta (Denominação de Origem Controlada e Garantida – DOCG) e Trento DOC possuem

importância relevante, além dos elaborados com a variedade Moscato (Asti DOCG) e com a casta Prosecco (da região do Vêneto) (Buxaderas & Lópes-Tamames, 2012). No Brasil adotou-se o termo Vinho Espumante (VE) para atender às normas internacionais no que diz respeito às DOC. De acordo com a Legislação Brasileira (Decreto nº 99.066 de 08/03/1990), os VE podem ser elaborados somente com cultivares *Vitis vinifera*, sendo que as mais apreciadas são Chardonnay, Riesling Itálico e Pinot Noir.

Segundo Boulton (1995), o processo de elaboração de VE inicia com a obtenção do vinho base, fase que contempla etapas distintas daquelas empregadas nos vinhos brancos de mesa, como por exemplo, a exclusão ou não da fermentação malolática, passagem pelo caravalho, etc., para que se obtenha um produto com características diferenciadas. O mosto é obtido através de prensagem suave (prensas pneumáticas), para evitar ao máximo a extração de compostos indesejáveis, como os responsáveis pelo amargor. A clarificação geralmente ocorre por centrifugação ou filtração, a dose de dióxido de enxofre não atinge mais de 50 $mg.L^{-1}$ e o pH costuma ser baixo, por volta de 2,8 a 3,0 (devido ao grau de maturação das uvas). Antes da primeira fermentação, o mosto é resfriado para protegê-lo da oxidação, sendo que o processo em si ocorre em temperaturas entre 18°C a 22°C. A escolha da levedura é baseada em um caráter neutro e na baixa formação de ésteres e derivados do enxofre.

No momento da decisão final sobre o perfil do vinho base espumante, podem ser utilizados somente vinhos brancos (base *blanc de blancs*) ou de mesclas entre cultivares brancas e tintas (base *blanc de noirs*), sendo que a segunda fermentação pode ocorrer através dos métodos *Charmat* (grandes recipientes vinários) ou *Champenoise* (na própria garrafa) (Flanzy, 2003; Ribéreau-Gayon *et al*., 2003). Nos primeiros, é comum encontrarmos variações no teor de açúcar, tais como, até 6,0 $g.L^{-1}$ (extra *brut*), de 6,1 a 15,0 $g.L^{-1}$ (*brut*), de 15,1 a 20,0 $g.L^{-1}$ (seco), de 20,1 a 60,0 $g.L^{-1}$ (d*emi-sec*) e mais de

60,0 $g.L^{-1}$ (doce) (Decreto nº 99.066 de 08/03/1990). Já os espumantes *Champenoise* são normalmente elaborados dentro das classes extra-*brut* a seco.

O método *Charmat* (Figura 8a) é iniciado com a escolha de um *assemblage* (mescla de variedades) ou de um vinho varietal (vinho com no mínimo 75% de uma determinada uva). Para a segunda fermentação faz-se uso do *liqueur de tirage* (parcela de vinho base, açúcar refinado e leveduras em uma proporção necessária para atingir uma pressão de cerca seis (6) atmosferas). A temperatura é controlada (+/- 12ºC) e o período de fermentação pode levar de dias a algumas semanas. A técnica *sur lie* (amadurecimento sobre as borras - autólise das leveduras) possui duração variável e pode ser realizada após o término desta etapa. Previamente ao engarrafamento, utiliza-se *liqueur d'expédition* para determinação dos teores finais de açúcar e conservante (Flanzy, 2003; Miele & Miolo, 2003).

Depois da escolha do vinho base, no método *Champenoise* (Figura 8b) a segunda fermentação ocorre na própria garrafa, o que torna o processo mais lento, em ambientes com temperatura controlada (+/- 12ºC) e dura em média 60 dias. Durante o *sur lie*, o vinho espumante permanece por um período mínimo de 8 meses a uma temperatura que varia de 15 a 18ºC. Em seguida, efetua-se o *remuage* para decantar os sedimentos (aproximadamente 20 dias). O processo é finalizado através do *dégorgement* (descarte dos sedimentos) e adição de *liqueur d'expédition* (Flanzy, 2003; Miele & Miolo, 2003).

Sabe-se que os diferentes vinhos podem apresentar composição variável em função de diversos fatores que fazem parte do *terroir* e da metodologia de elaboração. Durante a obtenção de VE, além da *perlage*, ocorre a formação de características complexas, principalmente, em função do período de amadurecimento (Escudero *et al*., 2000).

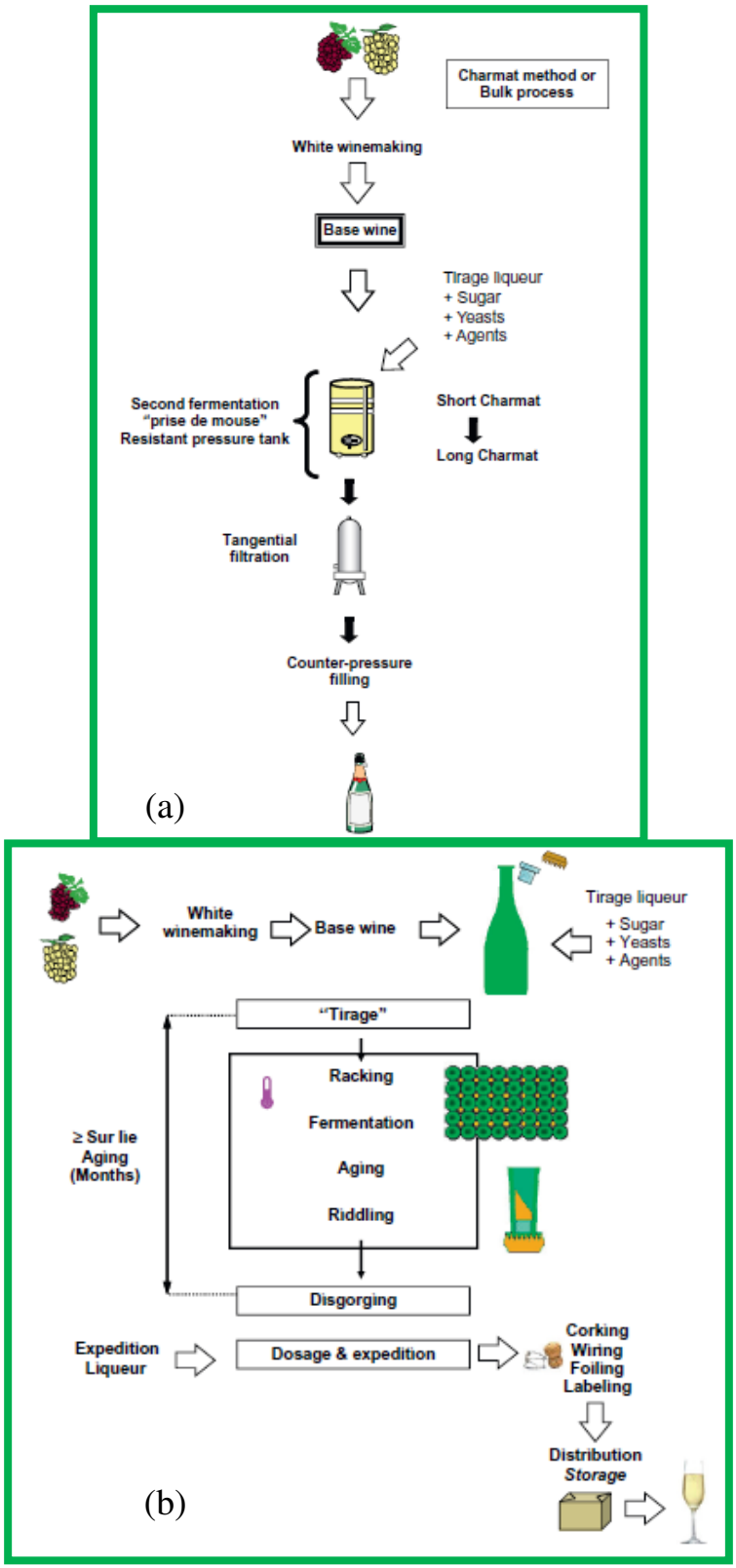

Figura 8. Esquema simplificado do método *Charmat* (a) e *Champenoise* (b) para a elaboração de VE (Buxaderas & Lópes-Tamames, 2012).

Longos períodos de armazenamento na vinícola representam um grande investimento de capital, o que faz com que o controle de qualidade seja rigoroso para a manutenção da estabilidade do produto até o momento do consumo. A estabilidade pode ser definida como ausência de mudanças físico-químicas e sensoriais por certo período de tempo (Bosch-Fusté *et al*., 2009). Portanto, é necessário ampliar conhecimentos sobre as alterações decorridas do período de contato com as leveduras para que se obtenham resultados cada vez melhores.

Esta prática enológica vem ganhando destaque nos últimos anos, sendo inclusive obrigatória em alguns casos, como para *Champagnes* e *Cavas* (Buxaderas & Lópes-Tamames, 2012). Sua duração é condicionada pela forma na qual a segunda fermentação é conduzida e pelo tipo de vinho espumante (Colagrande *et al*., 1994). Assim, as principais razões que a justificam são de natureza sensorial. Vinhos com mais estrutura, corpo e complexidade aromática são alcançados com períodos de *sur lie* maiores (Alexandre & Guilloux-Benatier, 2006). Embora o vinho base, a lise celular e o tempo de amadurecimento sejam considerados os principais fatores que regulam as características sensoriais, ainda existem lacunas significativas em relação a nossa compreensão das interações físico-químicas entre borras e vinho (Moreno-Arribas & Polo, 2009; Pozo-Bayón *et al*., 2009a, b).

As borras do vinho são constituídas, em sua maior parte, por células de levedura de aproximadamente 5mm, cristais de ácido tartárico, restos celulares e agentes de clarificação (Buxaderas & Lópes-Tamames, 2012). Quando o vinho permance em contato com as borras, ocorrem transferências de constituintes, destas para o vinho, impactando em sua estabilidade e nas características sensoriais (Martínez-Rodríguez *et al*., 2001), como por exemplo, na complexidade aromática (Cebollero & Reggiori,

2009), na amplitude gustativa (Charpentier *et al*., 2005) e no maior potencial de envelhecimento (Caridi, 2007; Escot *et al*.,2001).

A autólise das leveduras é representada por uma autodegradação enzimática, inicialmente, de glucanos em manoproteínas, que começa no fim da fermentação alcoólica e está associada com a morte celular (Buxaderas & Lópes-Tamames, 2012). Além disto, as borras contêm uma grande variedade de enzimas de hidrólise, como as proteases (Rowe *et al*., 2010; Tirelli *et al*., 2010). A Protease A é uma endopeptidase responsável pela liberação de 85% do nitrogênio e da maioria dos peptídios (Alexandre *et al*., 2001), sendo que a hidrólise destes varia dependendo da estirpe de levedura (Caridi, 2007), bem como da temperatura e da duração do *sur lie*. Os polissacarídeos de membrana podem atuar também como agentes de absorção, auxiliando na estabilidade físico-química e microbiológica dos VE (Gallardo-Chacón et al., 2010; Andújar-Ortiz *et al*., 2010;. Pérez-Serradilla & Luque de Castro, 2011).

É importante lembrar ainda que a oxidação de polifenóis (por via química e/ou enzimática) é considerada como a principal causa da depreciação qualitativa em VE (Cheynier *et al*., 1993) e, embora a atmosfera em VE seja redutora (devido a pressão em torno de 5 atm de CO_2), o pardeamento em *Cavas* já foi descrito (Ibern-Gómez *et al*., 2000). Neste sentido, a resistência à oxidação associada à presença de leveduras vem sendo alvo de estudos em soluções modelo (Lopez-Toledano *et al*., 2002), vinhos de sobremesa (Barón *et al*., 1997) e vinhos (Bonilla *et al*., 2001). Esta resistência pode estar relacionada à interação entre as borras de *Saccharomyces cerevisiae* e o oxigênio (presente em pequenas doses, quer por homogeneização da massa, quer por adição) durante o *sur lie*; tal consumo de oxigênio é completamente independente de qualquer viabilidade celular (Fornairon *et al*., 1999; Salmon *et al*., 2000; Mazauric & Salmon,

2005), ou ainda, como resultado indireto da absorção da cor pelos compostos formados durante este período de amadurecimento (Lopez-Toledano *et al.,* 2006).

Além disso, diversos compostos do vinho, como por exemplo, os terpenos e polifenóis, são encontrados na forma livre ou conjugados com moléculas de glicose (Romero-Pérez *et al*., 1999; Hernández *et al*., 2003; Sun *et al*., 2006). É importante salientar que compostos voláteis (especialmente terpenos) e não voláteis podem ser hidrolisados pela ação enzimática das β-glicosidases (Sánchez-Torres *et al*., 1996; Hernández *et al*., 2003) já descritas em *S. cerevisiae* há algum tempo (Duerksen & Halvorson, 1959; Delcroix *et al*., 1994). Em relação aos polifenóis, a possível formação de aductos com moléculas de glicose, pode influenciar parâmetros qualitativos extremamente importantes, tais como, cor, amargor, maciez, longevidade, atividade antioxidante, entre outros (Auger *et al*., 2005; Lee *et al*., 2005; Valko *et al*., 2005; Totlani & Peterson, 2006).

Neste sentido, os atributos de um vinho são a razão para o seu consumo; VE são produtos alimentares agradáveis que proporcionam prazer para o consumidor, sendo que a *perlage*, a cor e o aroma são os três principais que devem ser considerados. Especialmente no que diz respeito à *perlage*, os quatro aspectos qualitativos mais relevantes são: espuma inicial (aspecto e quantidade assim que o VE é servido na taça), área de espuma (avalia se a mesma cobre toda a superfície áerea do VE), colarinho de espuma (mede o aparecimento de uma coroa parcial) e tamanho da borbulha (quanto mais fina, melhor) (Obiols *et al*., 1998; Gallart *et al*., 2004) (Figura 9). Embora a capacidade de produção de espuma dependa da composição do VE e, esta seja oriunda das práticas vitícolas e enológicas aplicadas em sua elaboração, o seu comportamento na taça também depende da temperatura, da maneira como a garrafa é aberta, da forma como o líquido é servido, da limpeza e secagem da taça, bem como, do formato da

mesma e da qualidade do cristal, o que exige a padronização do protocolo de serviço, para que se obtenham resultados objetivos e consistentes (Casey, 1995; Obiols *et al*., 1998).

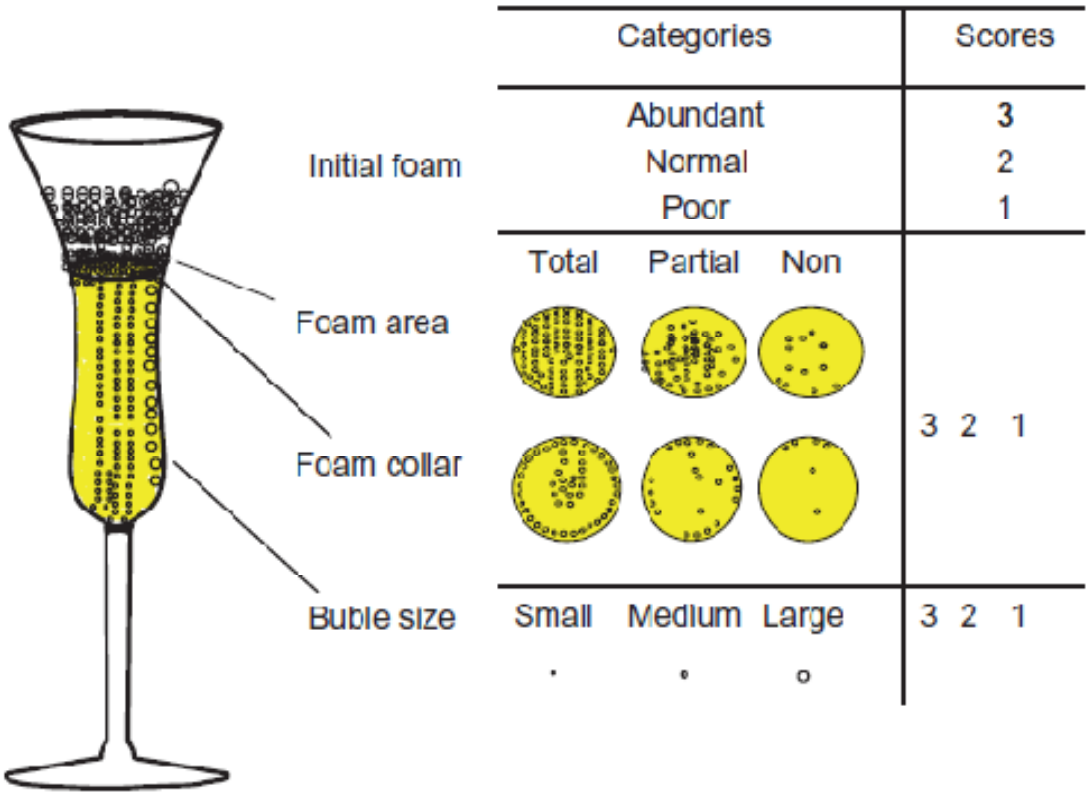

Figura 9. Descritores da perlage (Gallart *et al*., 2004).

A tonalidade de um vinho espumante depende em grande parte das variedades utilizadas na sua produção, sendo que a biossíntese dos compostos responsáveis pela cor ocorre até a *veraison* (mudança de cor) e é reforçada pela exposição do cacho a luz solar direta. É importante lembrar ainda, que em vinhos obtidos através da vinificação em branco, os ácidos hidroxicinâmicos e os flavonóides são os principais grupos relacionados a este parâmetro (Buxaderas & Lópes-Tamames, 2012). As diferenças na tonalidade são principalmente resultantes da oxidação destes compostos, pois esta reação intensifica a cor amarela, a qual pode tornar-se acastanhada em VE envelhecidos (Nagel & Graber, 1988; Cilliers & Singleton, 1990), devido, por exemplo, à

polimerização do ácido cafeico com ortoquinonas (Guyot *et al*., 1996). Poucos estudos têm avaliado a composição fenólica de VE, logo, pesquisas que auxiliem no entendimento destes aspectos são necessárias. Os compostos voláteis dos VE também dependem da constituição do vinho base, mas são influenciados pela segunda fermentação, em função do método utilizado. A influência dos aromas varietais é especialmente importante em VE *Charmat*, pois para os *Champenoise*, o *sur lie* torna-se preponderante (Alexandre & Guilloux-Benatier, 2006). Além disso, processos químicos e bioquímicos ainda não totalmente compreendidos, envolvendo as interações entre levedura e vinho, também estão envolvidos (Stefenon, 2006; Torrens *et al*., 2010a) e as mudanças sensoriais podem afetar claramente a qualidade percebida e, portanto, o valor comercial do vinho em questão. Esta questões demonstram o quanto é essencial que o enólogo trabalhe em prol do equilíbrio, visando a otimização das propriedades sensoriais (Buxaderas & Lópes-Tamames, 2012).

Juntam-se a estes fatores a análise sobre estudos que mostrem possíveis propriedades funcionais de alimentos e bebidas, os quais estão cada vez mais atraindo o interesse não só da comunidade científica, mas também da indústria e consumidores em geral, devido à busca por produtos que proporcionem ao mesmo tempo, maior satisfação e segurança alimentar comprovada (Auger *et al*., 2005). Nesta relação entre "*vinho e saúde*" (sendo que estas duas palavras englobam não somente vinhos, sucos, extratos, óleos, etc.) ainda são poucos os estudos existentes sobre a atividade biológica de VE, entre os quais: a) *Cavas* e VE elaborados pelo método *Charmat* a partir de uvas cultivadas no Brasil (Chardonnay e Pinot Noir) mostraram-se capazes de inibir a peroxidação lipídica *in vitro* (Satué-Gracia *et al*., 1999) e *in vivo* (Auger *et al.*, 2005), b) *Champagnes* aumentaram a concentração de vitamina E no plasma sangüíneo (Cartron *et al*., 2003), c) extratos aquosos e orgânicos de *Champagnes* apresentaram efeito

neuroprotetor ao estresse oxidativo (Vauzour *et al*., 2007), e d) VE portugueses (DOC *Bairrada*) demonstraram importante capacidade antioxidante (Jordão *et al*., 2010).

Neste sentido, a enologia é frequentemente definida como a ciência da elaboração do vinho. Entretanto, na prática, ela é resultado da combinação entre ciência, tecnologia e engenharia de processos. Esta interdisciplinaridade entre os conhecimentos oriundos da química, bioquímica, microbiologia, engenharia e nutrição pode ser considerada a essência da enologia (Boulton, 1995) e, portanto, a responsável pelas características intrínsecas ao universo do vinho, demonstrando a necessidade de uma maior compreensão sobre a dinâmica dos aspectos apresentados acima.

2.3 Materiais e Métodos

2.3.1 Análises Físico-químicas

Para obter vinhos de boa qualidade é fundamental a realização de análises físico-químicas para que seja possível acompanhar o andamento do processo, identificar possíveis alterações e intervir, caso necessário, realizando as devidas correções**,** sendo que para a realização destas análises existem vários métodos que podem ser utilizados (Cesca, 2009). Segundo Zoecklein *et al*., (2000), existem quatro principais razões para realizar estes controles: a) controle de qualidade geral; b) redução da acidez volátil; c) melhoramento do processo; e d) requerimentos legais. Portanto, as análises físico-químicas dos vinhos são um dos aspectos mais importantes do controle de qualidade enológico. Todas as fases da elaboração de vinhos são, atualmente, controladas mediante ensaios químicos e sensoriais, desde a determinação da colheita até o momento adequado para o envase (Zoecklein *et al*., 2000; Cesca, 2009).

Em relação a esse estudo, as variáveis físico-químicas foram quantificadas, pois elas são a base para a elaboração de vinhos de qualidade, tornando dinâmico o processo

de fermentação (Bevilaqua, 1995), o que permite avaliá-lo tanto qualitativamente (relacionando os componentes-base que são responsáveis por caracterizar os vinhos de regiões peculiares com a tecnologia de vinificação empregada (Ough, 1992); quanto sensorialmente (Smyth, 2005; Castilhos & Del Bianchi, 2011). Além destes aspectos, a caracterização físico-química demonstra de uma maneira simples os fatores que influenciam no momento do consumo deste produto, do ponto de vista de verificar as conformidades legais (Reis & Souza, 2004).

Entre as principais variáveis físico-químicas, está o álcool etílico, responsável por promover qualidade à bebida e tornar o meio impróprio para o desenvolvimento de microrganismos patogênicos. Seu teor é diretamente proporcional ao teor de açúcares fermentescíveis, enquanto que a presença de açúcares redutores no vinho refere-se aos que não sofreram fermentação por ação das leveduras e são responsáveis por promover a sensação de doçura, extremamente importante para o equilíbrio com o pH e a acidez total, os quais influenciam na coloração e na estabilidade dos vinhos. Já a acidez volátil está diretamente ligada com a sanidade do produto (Rizzon & Gatto, 1987; Mazzochi & Ide, 1994; Behrens & Silva, 2000). O extrato seco total é obtido do peso do resíduo seco após a evaporação dos compostos voláteis e está relacionado ao corpo e estrutura do vinho (Rizzon & Miele, 1996). O teor de ácido ascórbico é importante, especialmente em vinhos brancos e espumantes, pois age como antioxidante, prevenindo contaminações microbiológicas, inativando enzimas e influenciando de forma positiva no sabor do vinho (Vogt *et al*., 1984). O dióxido de enxofre (SO_2) é amplamente empregado em enologia, devido à capacidade de bloquear a ação de enzimas oxidásicas (evita turvamentos), à alta afinidade pelo oxigênio (protege polifenóis e ésteres da oxidação e, portanto, preserva a qualidade geral e a longevidade dos vinhos) e à inibição

da ação de bactérias (reduz a produção de ácido acético e tem ação direta sobre o perfil sensorial final dos vinhos) (Flanzy, 2003; Ribéreau-Gayon *et al*., 2003; Blasi, 2004).

Por fim, é importante salientar que a estabilidade e a evolução do vinho estão diretamente ligadas a fenômenos bioquímicos. Esta definição permite compreender a extrema complexidade da sua composição e também o interesse pelo seu estudo, devido à grande diversidade de matérias que podem ser abordadas, como por exemplo, auxiliar na definição do valor alimentar do vinho, produto proveniente de células vivas e que contém tudo o que é necessário à vida (Peynaud, 1996).

2.3.2 Quantificação de Polifenóis

Na composição química da uva existem vários compostos orgânicos, entre os quais os compostos fenólicos possuem importante papel, uma vez que participam da cor do vinho e das características gustativas de maciez, dureza e adstringência. Além desta importância sensorial, o teor de polifenóis pode auxiliar na diferenciação varietal, permitindo a regulamentação de parâmetros para a comercialização dos vinhos, como forma de impedir adulterações (De Freitas, 2006). Por fim, o potencial antioxidante dos vinhos é amplamente conhecido, em geral associado aos compostos fenólicos presentes e, os benefícios do seu consumo moderado incluem, entre outros, a diminuição na incidência de neoplasias, cardiopatias e doenças neurodegenerativas (Stefenon, 2006).

Diversos pesquisadores têm trabalhado na separação, identificação, quantificação e aplicação dos compostos fenólicos em alimentos (King & Young, 1999; Angelo & Jorge, 2007). Os métodos de determinação podem ser classificados em determinação de compostos fenólicos totais, quantificação individual e/ou de um grupo ou classe de compostos fenólicos (Moure *et al*., 2001; Angelo & Jorge, 2007), sendo a

espectrofotometria UV e a cromatografia líquida de alta eficiência (CLAE) amplamente utilizados (Angelo & Jorge, 2007).

A extração de compostos fenólicos em bioativos da uva foi realizada segundo Bonoli *et al*. (2003) e Rockenbach (2008). Para a determinação espectrofotométrica do teor de polifenóis foram usadas metodologias reconhecidas, sendo que a leitura da absorbância a 280nm foi utilizada para quantificar Polifenóis Totais (PT), a leitura a 320nm para hidroxicinamatos totais (HCT), o teor de flavonóides totais (FT) foi obtido através da fórmula FT = $[A_{280} - 4] - (0,66)$ x $[A_{320} - 1,4]$ (Iland *et al*., 2000; Zoecklein *et al,* 2000; Ribéreau-Gayon *et al*., 2003), enquanto que o teor de OPC e de Antocianinas Totais (AT) foi determinado através da técnica proposta por Fukui & Nakahara, 2006. Para a identificação de compostos isolados por CLAE foram utilizadas colunas cromatográficas e fases móveis apropriadas (Roggero & Archier, 1989; Lamuela-Raventós & Watherhouse, 1994; McMurtrey et al., 1994; Jeandet *et al*., 1995; OIV-Resolution OENO 22/2003; Sun *et al*., 2006).

2.3.3 Perfil Mineral

No que diz respeito à composição mineral dos diferentes vinhos e espumantes, cabe salientar que o potássio, o cálcio e o magnésio, são os cátions mais importantes no vinho e são classificados como macro-elementos em relação ao teor. O ferro está presente nos vinhos em pequena quantidade, na forma de íon ferroso, mais facilmente assimilável. Existe também pequena quantidade de lítio, ao qual é atribuído efeito antidepressivo. Outros cátions classificados como micro-elementos (sódio, manganês, zinco e rubídio), além de alumínio, silício, titânio, cromo e níquel (Rizzon, 2005; Chen *et al*., 1994; Monaci *et al*., 2003; Lara *et al*., 2005), podem estar presentes nos vinhos em concentrações reduzidas.

Atualmente, muitas descobertas foram feitas na determinação de elementos-traço em materiais biológicos e ambientais utilizando técnicas nucleares. Entre estas, pode-se citar a técnica de PIXE (Particle-Induced X-Ray Emission), baseada na emissão de raios-X característicos dos elementos presentes em uma amostra quando esta é irradiada por feixes de íons carregados (prótons, partícula α) (Maxwel *et al*., 1995; Kertész *et al*., 2005). Esse procedimento possibilita a identificação e a quantificação simultânea dos elementos da tabela periódica (do sódio ao urânio) de forma rápida e precisa. Além disso, essa técnica exige uma pequena quantidade de material para a análise e não é destrutiva, ou seja, as amostras irradiadas por PIXE podem posteriormente ser analisadas por outras técnicas complementares. É uma técnica com alta sensibilidade, permitindo medir a concentração de elementos presentes em nível de traços (Johansson *et al*., 1995; Kennedy *et al*., 1999; Uzonyi & Szabó, 2005).

2.3.4 Determinações Enzimáticas em Vinhos Espumantes

As enzimas Superóxido Dismutase (SOD) e Catalase (CAT) integram as defesas endógenas dos organismos vivos no combate ao estresse oxidativo e são consideradas como os mais potentes antioxidantes encontrados na natureza. Como alguns compostos fenólicos têm apresentado atividades miméticas a estas enzimas e têm sido reportados como coadjuvantes na manutenção da homeostase celular (Halliwell & Gutteridge, 1999; Ratnam *et al*., 2006; Lanza & Vecchio, 2009; Tomaino *et al*., 2010), torna-se importante verificar este aspecto também em vinhos, devido aos vários grupos fenólicos que eles contêm.

A atividade mimetizadora à enzima SOD nos VE foi medida espectrofotometricamente, segundo descrito por Bannister & Calabrese (1987). O método baseia-se na inibição da formação de adrenocromo decorrente da auto-oxidação

da adrenalina e os resultados estão expressos em U SOD.mg^{-1} de proteína, sendo que uma unidade de SOD será definida como o volume (μL) da enzima capaz de inibir 50% da reação de formação de adrenocromo. A medida da atividade mimetizadora à CAT em VE foi realizada de acordo com Aebi (1984). O método baseia-se na velocidade de decomposição do peróxido de hidrogênio, a qual é medida espectrofotometricamente. Para o cálculo, foi utilizado o coeficiente de extinção molar do peróxido de hidrogênio (40×10^{-6} μM^{-1} cm^{-1}) e os resultados estão expressos em μmols H_2O_2 mg $prot.^{-1}min.^{-1}$, o que corresponde a 1U de Catalase.

O emprego de enzimas com atividade β-glicosidásica em enologia é amplo, devido à sua capacidade de modificar a composição aromática e fenólica (Delcroix *et al*., 1994; Vrhovsek *et al*., 1997; Krasnow & Murphy, 2004; Dhake & Patil, 2005). Em relação aos compostos fenólicos, podem ocorrer reações reversíveis ou não, entre as agliconas e seus derivados glicosilados, em função de sua estrutura (Aguié-Béghin *et.al*., 2008). A atividade β-glicosidásica em leveduras já foi reportada em *S. cerevisiae* (Hernández *et al*., 2003), sendo que existem evidências da transferência dessa enzima a partir do meio intracelular da levedura para o meio extracelular (via autólise). Isto poderia contribuir para a formação/transformação dos aromas complexos normalmente encontrados em VE, como por exemplo, terpenos glicosilados não-voláteis hidrolisados pela ação das β-glicosidases (Boulton *et al*., 1995; Flanzy, 2003). É importante salientar que estes aromas são considerados uma importante característica qualitativa quando somados a um paladar untuoso. Neste sentido, o teor de manoproteínas (polissacarídeos presentes na parede celular das leveduras) também está relacionado aos níveis de β-glicosidase e ao *sur lie* (Feuillat *et al*., 1989; Dupin *et al*., 2000). O estudo dos mecanismos que envolvem estas variáveis é importante, pois elas podem contribuir para a melhoria da qualidade geral dos vinhos. A atividade β-glicosidásica em VE foi

monitorada através da união dos métodos descritos por Delcroix *et al*., (1994), Riou *et al*., (1998) e Dhake & Patil (2005), e os resultados estão expressos em unidades de β-glicosidase que correspondem a liberação de 1 µmol de *p*-nitrofenol.min^{-1}.

2.3.5 Avaliação Sensorial

O conceito moderno de qualidade, em um mercado consumidor competitivo e multinacional, é inteiramente baseado na satisfação das expectativas do consumidor, e contrariar esta tendência significa comprometer o sucesso do produto junto ao mercado (Stone & Sidel, 1993). Neste sentido, a caracterização sensorial vem sendo cada vez mais utilizada para definir as melhores práticas enológicas capazes de evidenciar diferenças sensoriais entre os diversos *terroirs* (Fischer *et al*., 1999; Bosch-Fusté *et al*., 2009; Torrens *et al*., 2010), sendo que para dirimir as diferenças entre os métodos e a expertise dos degustadores, faz-se necessário o uso de tratamentos estatísticos, os quais levam em conta as diferenças inter-individuais para que todos os aspectos tenham o mesmo peso na configuração final da avaliação (Escoffier & Pagès, 1998).

A análise sensorial de um vinho é realizada por "experts" treinados, através de padrões de qualidade desenvolvidos por tradicionais escolas de enologia ou associações de degustadores profissionais. A principal finalidade desta avaliação é o enquadramento da bebida dentro de padrões pré-estabelecidos. Estes refletem, na maioria dos casos, as características tradicionais de um vinho e compõem sua identidade junto a consumidores das localidades de origem do vinho (Behrens & Silva, 2000). Neste trabalho, os VE foram degustados por enólogos (pertencentes à Associação Brasileira de Enologia), em taças oficiais (Figura 10) e as impressões foram registradas em uma ficha de análise sensorial (pág. 54) que segue os padrões da OIV (*Office International de la Vigne et du Vin*).

Figura 10. Taça oficial do espumante brasileiro (Strauss Cristais, 2012).

Segundo Miele (2006), os órgãos dos sentidos utilizados na analise sensorial são o tato, a audição, a visão, o olfato e o gosto. O tato relaciona-se as sensações táteis, como a consistência, a fluidez e a untuosidade. A audição compreende a sonoridade, a intensidade e a persistência das borbulhas de dióxido de carbono que emanam das taças dos VE e a sonoridade em verter o vinho numa taça. A visão trata da limpidez, da

intensidade e da tonalidade da cor, além da fluidez e da efervescência no caso dos VE. O olfato é o sentido mais complexo do organismo humano. As substâncias aromáticas devem ser voláteis, solúveis e estarem presentes em concentrações suficientes para que possam ser percebidas. Avaliam-se a intensidade, a fineza e a persistência do aroma, sendo que este é classificado em quatro tipos: a) primários: que provém da uva e permanece no vinho; b) pré-fermentativos: que se desenvolve entre a colheita da uva e o inicio da fermentação; c) secundários: que se formam durante as fermentações (alcoólica e malolática); e d) terciários: que se formam durante o amadurecimento/envelhecimento do vinho. Por fim, o gosto está relacionado com as sensações percebidas pela língua, que diz respeito também às sensações da via retronasal. Ou seja, ao avaliar o vinho, destacam-se determinados aspectos relacionados à sua composição e à sua característica, entre os quais se citam o ataque, a intensidade dos gostos e sabores, o corpo (estrutura), a evolução, a persistência, o equilíbrio, a tipicidade, o fim de boca, o retrogosto, possíveis defeitos. Todos estes aspectos juntos formam a identidade e qualidade geral do produto.

2.3.6 Avaliação da Atividade Antioxidante

Os Radicais Livres (RL) são formados em todas as organelas e compartimentos intracelulares e sua geração é fundamental para a homeostase de células e tecidos (Valko *et al*., 2007; Tahara *et al*., 2001; Finkel & Holbrook, 2000), sendo que cada organela e/ou compartimento possui alvos de dano oxidativo, além de abrigarem mecanismos próprios para eliminação do excesso destes RL (Valko *et al*., 2007). A formação de RL ocorre tanto de forma endógena (a mitocôndria é tida como a principal fonte) quanto exógena, através de estímulos ambientais, como por exemplo, radiação solar, agentes químicos, entre outros (Finkel & Holbrook, 2000).

Ao desequilíbrio entre as espécies reativas produzidas e as reservas antioxidantes denomina-se estresse oxidativo. Esta condição pode provocar danos importantes em nível celular: a) RL atacam as membranas celulares; b) danificam a mitocôndria, prejudicando a produção de energia; c) interagem com as moléculas de proteína, danificando sistemas metabólicos, e d) fragmentam o ácido desoxirribonucléico (ADN), provocando mutações variadas e, muitas vezes, divisões celulares descontroladas (Valko *et al*., 2007; Silva, 2009).

Sabe-se que o desenvolvimento de inúmeras patologias crônicas severas, como, por exemplo, câncer, diabetes, doenças cardiovasculares, desordens neurodegenerativas e doenças auto-imunes estão relacionadas aos danos não reparados e que contribuem definitivamente para o envelhecimento precoce do organismo (Bitel *et al*., 2010; Mekki *et al*., 2010; Saito *et al*., 2010; Yusuf & Frenkel, 2010). Neste sentido, diversos estudos têm demonstrado que são observadas diferenças importantes quanto à incidência de certas doenças entre etnias que possuem estilos de vida e vivem em condições ambientais distintas. Isto tem sido explicado, pelo menos em parte, pela dieta antioxidante adotada pela população (Silva, 2009), como por exemplo, o conhecido Paradoxo Francês (consumo de vinho tinto *versus* baixo índice de mortalidade decorrente de doenças cardiovasculares) (Ratnam *et al*., 2006).

Conforme já foi citado anteriormente, as enzimas SOD e CAT fazem parte do sistema de defesa dos organismos (além do complexo glutationa) e já existem estudos que demonstram a capacidade de determinados compostos, como por exemplo, os polifenóis, em exercer atividade mimética a elas. A Figura 11 mostra de forma esquemática, a interação das enzimas antioxidantes no combate ao estresse oxidativo, resultando finalmente em H_2O.

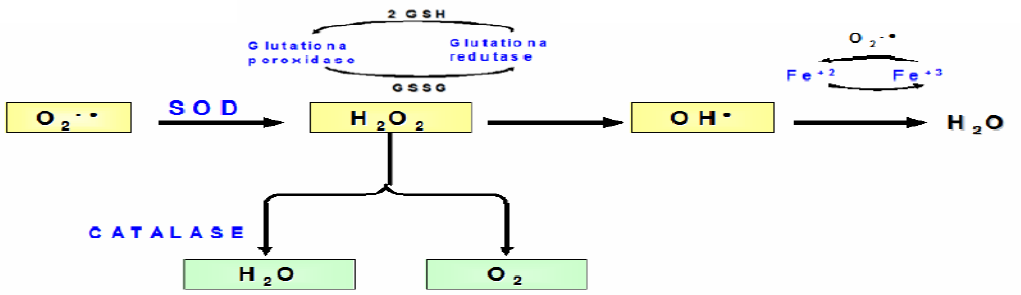

Figura 11. Conversão do oxigênio em água em sistemas enzimáticos biológicos (Silva, 2009).

No combate ao estresse oxidativo, os polifenóis podem agir como protagonistas e/ou coadjuvantes das variantes exógenas de defesa do organismo, pois são capazes de quelar metais, sequestrar o radical superóxido e o oxigênio *singlet*. Estas ações estão relacionadas com diversas atividades atribuídas aos polifenóis: a) antifúngica e antiinflamatória (Yilmaz & Toledo, 2004); b) antibacteriana e antiviral (Trueba & Sanchez, 2001); c) antioxidante *in vivo*, antiaterogênica, apoptótica e de redução dos riscos de câncer (Leiro *et al*., 2004; Yilmaz & Toledo, 2004); d) protetora da membrana celular e inibidora da peroxidação lipídica (Roig *et al*., 2002); e) contra espécies reativas do nitrogênio e do ácido hipocloroso (Cotelle *et al*., 1996; De Groot & Rauen, 1998; Van Acker *et al.*, 1998). Neste contexto, os vinhos possuem quantidades significativas de polifenóis (ácidos fenólicos, estilbenos, flavonóides, etc.), além de outros compostos antioxidantes, como por exemplo, o ácido ascórbico (Ribéreau-Gayon *et al*., 2003).

A seguir estão relacionados alguns estudos que ressaltam a importância dos compostos antioxidantes que fizeram parte deste estudo: a) a incubação de células humanas endoteliais demonstrou que o resveratrol (Figura 12) pode atuar na regulação da SOD e da CAT (Li *et al*., 2009); b) o ácido gálico (Figura 13) tem grande afinidade pelo oxigênio, o que gera os radicais quinona e/ou semiquinona e resulta em um grande poder antioxidante devido à capacidade destes em quelar metais, possibilitando desta

forma, uma proteção aos danos que os RL podem causar ao ADN (Barreto *et al*., 2007; Ferk *et al*., 2007; Eslami *et al*., 2010). c) a atividade anticarcinogênica do ácido ascórbico (Figura 14) pode estar relacionada à sua ação frente às espécies reativas do nitrogênio (Mikirova *et al*., 2008); d) propriedades anticoagulantes e antiinflamatórias do ácido caféico (Figura 15) úteis na proteção do tecido cardíaco frente aos efeitos do diabetes foram descritos por Chao *et al*, 2009; e) além dos conhecidos efeitos antioxidantes relacionados à proteção cardíaca (Gonzáles-Santiago *et al*., 2006), o tirosol (Figura 16) mostrou-se capaz de exercer efeitos neuroprotetores (Bu *et al*., 2007; Vauzour *et al*., 2007); f) Segundo Neiva *et al*., 2008, a catequina (Figura 17) pode atuar como coadjuvante no combate à agregação plaquetária devido à sua capacidade em manter a homeostase celular; g) a biodisponibilidade dos polifenóis vem sendo discutida, sendo que estudos conduzidos por Rondini *et al*. (2004) e De Mira *et al.* (2008) demonstraram que o ácido ferúlico (Figura 18) insolúvel, ou seja , ligado a estruturas da parede celular ou esterificados com outras moléculas, pode ser absorvido no intestino delgado e liberado no plasma sanguíneo; g) efeitos bronco dilatadores, antidepressivos e anti-hipertensivos podem ser atribuídos às procianidinas (Figura 19) presentes em extratos vegetais (Tanae *et al*., 2007); h) a epicatequina (Figura 18) pode ser capaz de exercer potente proteção contra a isquemia miocárdica (Yamazaki *et al*., 2010); e finalmente, i) o picied (Figura 20) pode atuar como um excelente varredor de RL e quelador de metais (Medina et al., 2010).

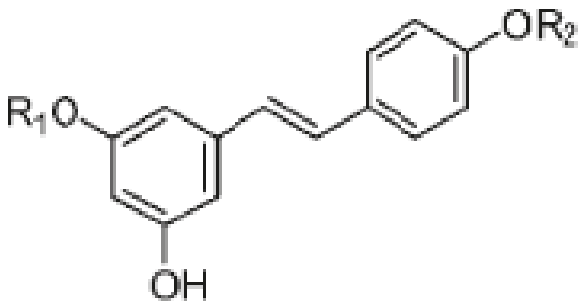

Figura 12. Estrutura do resveratrol (R1 = H e R2 = H; Medina *et al*., 2010).

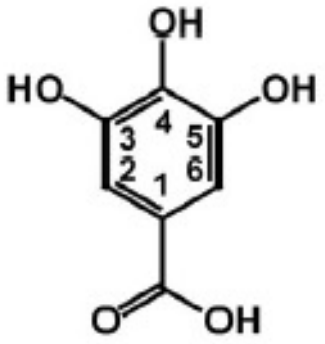

Figura 13. Estrutura do ácido gálico (Eslami *et al*., 2010).

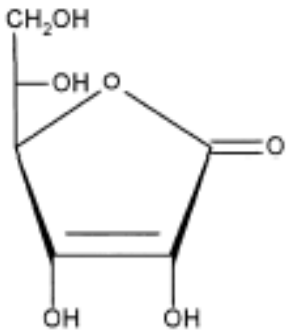

Figura 14. Estrutura do ácido ascórbico (Mirikova *et al*., 2008).

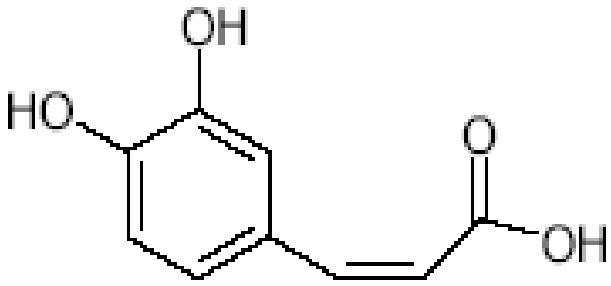

Figura 15. Estrutura do ácido caféico (Mirikova *et al*., 2008).

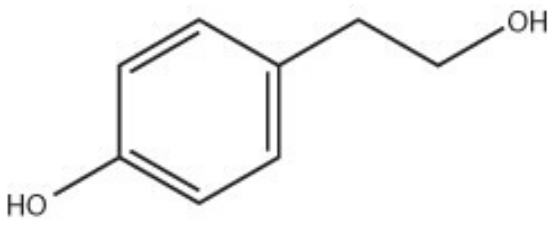

Figura 16. Estrutura do tirosol (Guimarães *et al*., 2009).

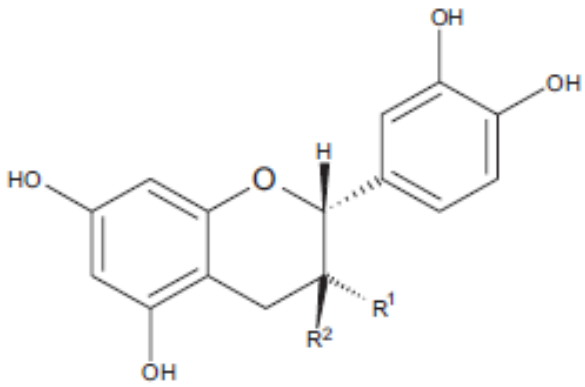

Figura 17. Estrutura do catequina e da epicatequina (R1 = OH e R2 = H; Souza *et al*., 2007).

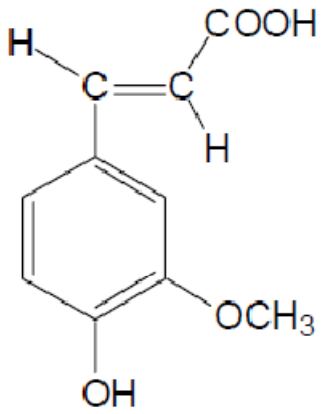

Figura 18. Estrutura do ácido ferúlico (Flanzy, 2003).

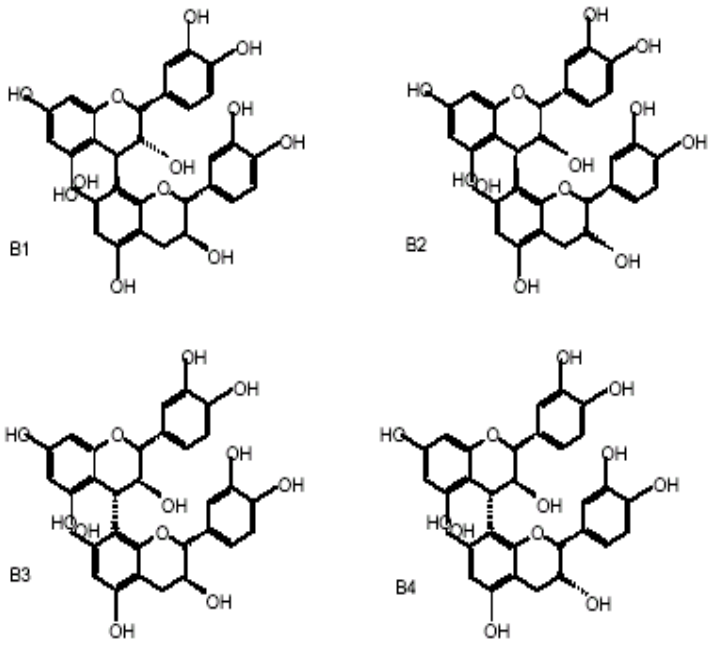

Figura 19. Estruturas das procianidinas B_1, B_2, B_3 e B_4 (Cabrita *et al*., 2003).

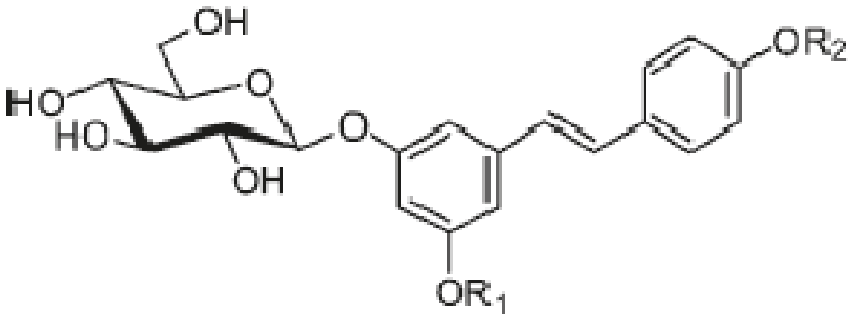

Figura 20. Estrutura do piceid (R1 = H e R2 = H; Medina *et al*., 2010).

Diferentes metodologias têm sido desenvolvidas para obter uma medição, qualitativa ou quantitativa, da capacidade antioxidante de compostos, com base em testes químicos ou utilizando-se culturas celulares. Dentre os testes *in vitro* existentes, a medida de capacidade de varredura do radical $DPPH^{\bullet}$ (1,1 difenil 2-picrilhidrazil) vem sendo bastante utilizada. O $DPPH^{\bullet}$ é um radical estável que pode ser reduzido por um antioxidante, resultando na diminuição de coloração que é, então, determinada espectrofotometricamente a 517nm (Yamaguchi *et al*., 1998).

Além dos ensaios *in vitro*, os resultados obtidos em sistemas biológicos são amplamente empregados (Soares *et al*., 2003), pois em células, a expressão da atividade antioxidante é mais complexa por envolver questões de permeabilidade celular e possível metabolização dos compostos ensaiados, além de um complexo sistema regulatório enzimático de defesas antioxidantes endógenas celulares (Gralla & Valentine, 1991; Halliwell & Gutteridge, 1999). A levedura *S. cerevisiae* é um dos melhores modelos de sistema biológico para estudos de citotoxicidade, avaliação de capacidade antioxidante e genotoxicidade de inúmeros compostos, mostrando-se um ensaio rápido, sensível, econômico e reprodutível, apresentando resultados confiáveis na identificação da atividade biológica. (Raspor, *et al*., 2005; Fragoso *et al*., 2008; McCue & Phang, 2008; Machado *et al*., 2009).

Embora os aspectos que envolvem o assunto vinho e saúde venham sendo estudados já há muito tempo, estudos recentes e inovadores continuam demonstrando avanços na elucidação dos mecanismos pelos quais, provavelmente, o consumo moderado de vinho pode aportar benefícios à saúde humana. A Figura 21 apresenta alguns exemplos.

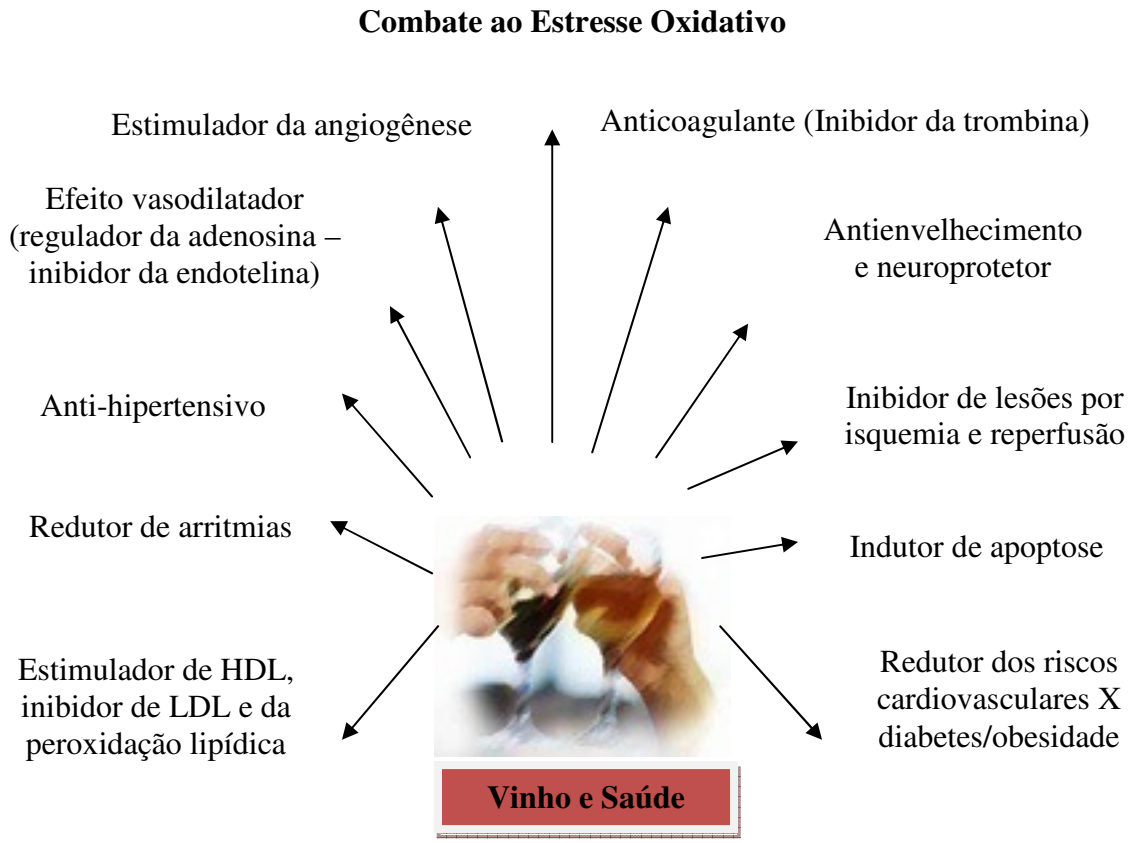

Figura 21. Diversos efeitos benéficos atribuídos aos vinhos (Adapatado de Das *et al*., 2010).

Estes vários efeitos benéficos à saúde (em função das propriedades exercidas pelos diferentes compostos fenólicos presentes nas uvas e vinhos) permitem inferir que seus bioativos possam apresentar atividades similares (Pereira, 2008; Maier *et al*., 2009; Nichols & Katiyar, 2010; Reuter *et al*., 2010). Neste sentido, a indústria farmacêutica e cosmética está em constante mudança para atender a demanda de um mercado em plena expansão, com consumidores que buscam produtos sofisticados obtidos através de fontes naturais, sustentáveis e que possuam sua eficácia garantida através de

comprovação científica (Burgess, 2009). A Figura 22 apresenta um resumo das principais ações descritas para bioativos da uva e do vinho.

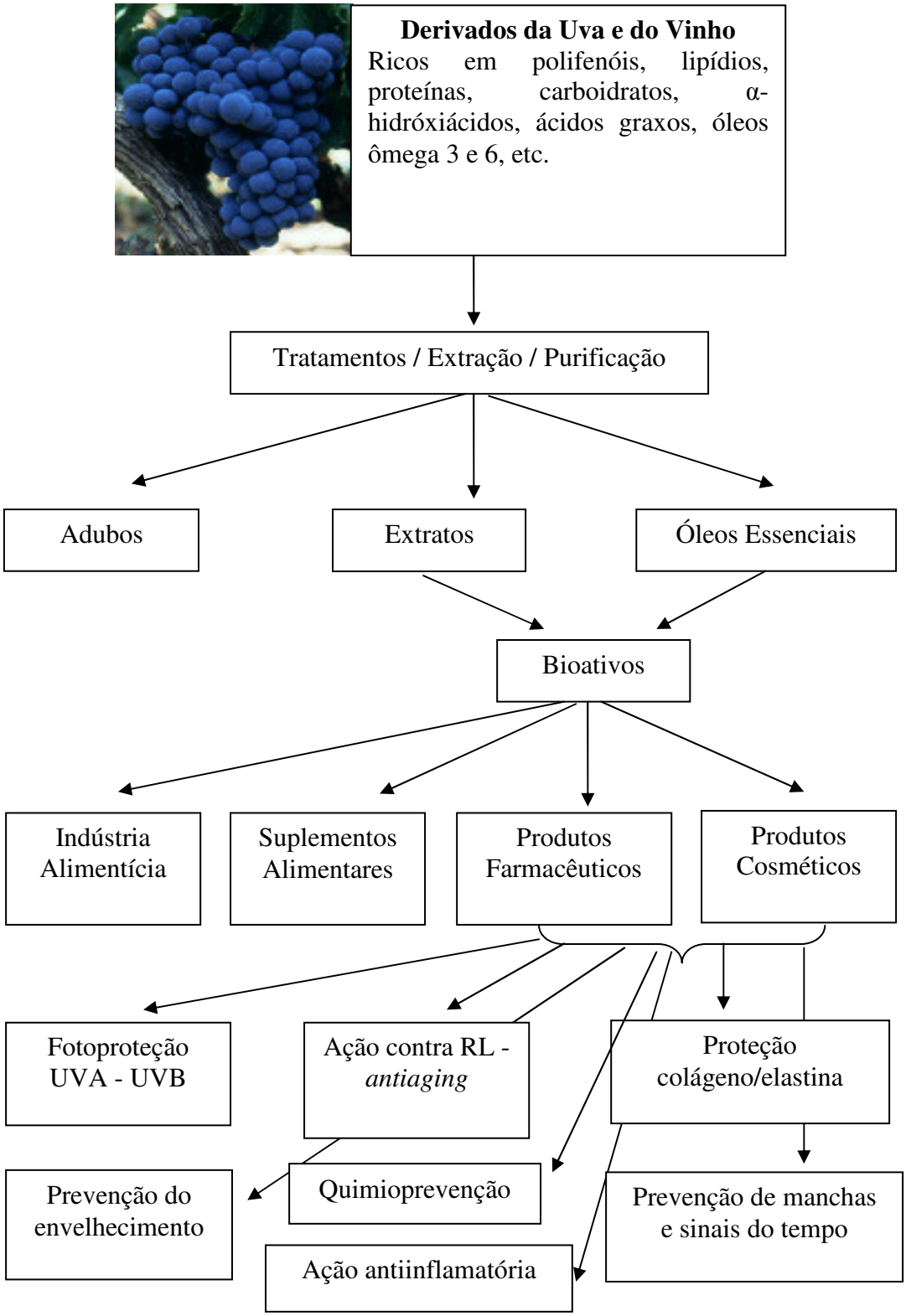

Figura 22. Propriedades de bioativos da uva e do vinho (Adaptado de Bicheron, 2006).

3. RESULTADOS E DISCUSSÃO

Este capítulo é apresentado na forma de artigos científicos, que abrangem diversos aspectos sobre o estudo dos vinhos espumantes.

3.1 – Artigo 1 – Stefenon, C. A.; De Martini Bonesi, C.; Marzarotto, V.; Barnabé, D.; Agostini, F.; Perin, J.; Serafini, L.A. & Vanderlinde, R. (2010). Sugar level in *Charmat* Sparkling wines affects quality and resveratrol levels? **Redox Report.** 15(6): 243-249.

O objetivo principal avaliar a influência dos diferentes níveis de glicose nos teores de *trans*-resveratrol e *trans*-piceid, na atividade β-glicosidásica e na capacidade antioxidante de VE *Charmat.*

Research article

Sugar levels in *Charmat* sparkling wines can affect the quality and resveratrol levels

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Sparkling wines contain CO_2 obtained through a second fermentation by natural processes (*Charmat* method); they may be prepared with variable final sugar concentrations, resulting in physicochemical compositions and phenolic profiles different to those obtained with other natural methods. The purpose of this study was to verify the influence of sweetness on enological parameters, *trans*-resveratrol and *trans*-piceid levels, antioxidant capacity (power of scavenger the radical DPPH• (1,1-diphenyl-2-picrylhydrazyl) and mimetic enzymatic activities of superoxide dismutase (SOD-like) and catalase (CAT-like) assays, and β-glucosidase activities on *Charmat* sparkling wines. The interaction of polyphenol levels and sweetness was observed. Levels of *trans*-piceid and *trans*-resveratrol showed a decrease in function of glucose concentration up to 40 g/l. All samples showed antioxidant capacity and β-glucosidase activity was stable even in the presence of sugar. A positive correlation between SOD-like and DPPH• was observed. This work shows an approach able to clarify important aspects for the wine industry with regard to world-wide consumer demand for sweetened products.

Keywords: sparkling wine, Charmat method, *trans*-resveratrol, *trans*–piceid, antioxidant capacity, β-glucosidase

Introduction

Sparkling wines contain CO_2 obtained through a second fermentation by natural processes. The *Charmat* method (large containers) is widely used world-wide due to the high quality of the products obtained by this process. They may be prepared with variable final sugar concentrations.[1,4] According to Brazilian legislation, sparkling wines are made with *Vitis vinifera* varieties in accordance with similar international legislation.[5,6]

Sparkling wines have a variable physicochemical composition, including phenolic profiles, which depends on several factors, including grape variety, fruit growth and ripening conditions, quality of the base wine, yeast used and *sur lie* (time needed to mature).[4,7–10,12] Many studies have already shown that: (i) resveratrol is a potent phenolic compound with known biological activity associated with a decrease in the incidence of several illnesses such as cardiovascular diseases and cancer; and (ii) sugar levels influence certain aspects, including formation of adducts with polyphenols and antioxidant activity.[13–15] In addition, the *terroir* – the winemaking philosophy –

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and, consequently, the phenolic and biochemical qualities (color, bitterness, sweetness, flavor, longevity, antioxidant properties, *etc*.) can influence the acceptance of the products by consumers around the world.[16–18]

In vitro tests to assess 1,1-diphenyl-2-picryl-hydrazyl-radical (DPPH) scavenging activity, and superoxide dismutase (Sod-like) and catalase (Cat-like) activities are widely used due to their accuracy, including in the wine industry.[19–21] The results are usually related to the phenolic profile of the sample. Commercial products that contain β-glucosidase are widely used in enological practices, due to their ability to modify the aromatic composition and the phenolic profile.[22–24]

Preliminary studies with sparkling wines conducted by our group showed a potential influence of sweetness grade on the aspects mentioned above.[25] Therefore, the purpose of this study was to verify the influence of sweetness on enological parameters, *trans*-resveratrol and *trans*-piceid levels, antioxidant capacity, and β-glucosidase activities on *Charmat* sparkling wines made by Möet Hennessy do Brasil – Vinhos e Destilados with grapes grown in the vineyards of the Serra Gaucha – a new wine land located in the highlands of the state of Rio Grande do Sul, Brazil.

Materials and methods

Samples

The grapes used in this process were obtained in the region of Garibaldi, in the Serra Gaucha, Brazil, in 2006. Sparkling wines were made from a base wine obtained by the assemblage of Chardonnay (10%), Italic Riesling (42%) and Pinot Noir wines (48%), by using the *Charmat* process in 2007. This base wine was stored in stainless-steel tanks until the beginning of the second fermentation. The second fermentation was carried out with *Saccharomyces bayanus*, in triplicate. At the time of bottling, unique *liqueur d'expédition* previously prepared only with the same base wine (Table 1), a small addition of SO_2 and different levels of sugar (10, 20, 30, 40, 50, 60, and 70 g/l) were added. In order to perform the assays, the sparkling wines were previously degassed, using a vacuum pump with a valve for air removal, coupled to a workbench agitator. All analyses were performed 30 days after bottling.

Chemical reagents

Reagents for the enological assays and DPPH• were acquired from E. Merck, Darmstadt, Germany, while the reagents for the phenolic high-performance liquid chromatography (HPLC) and enzyme analyses were obtained from Sigma-Aldrich (except for piceid HPLC grade, which was obtained from Polyphenols Laboratories AS, Sandnes, Norway). All other reagents were acquired from Extrasynthese, Gennay, France.

Enological analyses

Alcohol content, total acidity, pressure, volatile acidity, pH, free and total SO_2, dry extract and reduced dry extract concentration were determined using the methods described by Zoecklein *et al*.[26] The levels of ascorbic acid was measured by titrating with 2,6-dicholorophenolindophenol dye in accordance with Iland *et al*.[27] The sugar concentration was obtained through an enzymatic measure (reagents from Chema Italia, Rome, Italy) using a multiparametric analyser Enochem (Tecnologia Difusion Ibérica, Barcelona, Spain).[27–29]

Table 1 Information about base wine

Information	Base Wine
Grapes harvest period	10 January to 14 February 2006
Juice collection	Direct pressing in pneumatic presses
Enzymes	Pectolytic activity (2.50 ml.h/l)
Débourbage	Previous addition of silica sol. and gelatin – centrifugation
Temperature of fermentation	16 ± 2 ºC
First fermentation	14 ± 1 days
Sugaring	25.0 g/l
Yeast	*Saccharomyces cerevisiae* EC 1118
Clarification before *assemblage*	Centrifugation
Clarification after *assemblage*	Bentonite (18 g.h/l)
Filtration	Earth filter with low permeability
Acidity	90.6 (g/l)
Sugar	1.4 (g/l)
Alcohol	10.8 (% v/v)
Free SO_2	16.9 (mg/l)
Total SO_2	87.5 (mg/l)
Start *Charmat* process	28 February 2007

Determination of polyphenols by UV spectrophotometry and HPLC

Total polyphenols (TP) and total hydroxycinnamates (THC) were quantified by measuring the absorbance at 280 nm and 320 nm (Shimadzu UV-1700 spectrophotometer), respectively. TP results were expressed as milligram per litre of catechin and THC results as milligram per litre of caffeic acid equivalents. The total flavonoids (TF) were calculated using the following formula:[26,27]

$$TF = [(A_{280} - 4) - 0.66] \times (A_{320} - 1.4) \qquad \text{Eq. 1}$$

Results were expressed in milligram per litre from a catechin standard curve ($R = 0.99997$).

The extraction of the phenolic compounds present in the sparkling wine samples was performed by solid–liquid extraction with ethyl acetate as follows: 20 ml of sparkling wines were extracted with 20 ml of ethyl acetate with agitation, at room temperature under darkness, three times. After centrifugation at 10,000 *g* for 10 min, the supernatant was evaporated at less than 35ºC to dryness and recovered in 1 ml of 50% ethanol in water prior to HPLC analysis. Just before analysis by HPLC, each sample was filtered through a cellulose membrane of 0.20 μm pore size. A Hewlett-Packard (Palo Alto, CA, USA) liquid gradient LC 1100 series, a Zorbax 300 SB C18 (12 mm × 4.6 mm × 5 μm) pre-column, and a C18ODS (150 mm × 4 mm × 5 μm; Agilent Technologies, USA) column were used.

Results were expressed in milligram per litre of *trans*-resveratrol and *trans*-piceid.[30] The optimized chromatographic conditions were as follows: flow rate was fixed at 1.0 ml/min; injection volume was 20 μl; column temperature was 30ºC; the appropriated gradient elution was used with acetonitrile (solvent A) and Milli-Q ultra pure water (solvent B) for 50 min, followed by washing to restore the column to initial conditions. *Trans*-resveratrol was quantified by a standard curve (0.051–2.537 mg/l) through the dilution of an initial solution of 10.15 mg/l in ethanol. *Trans*-piceid was quantified in the same way with the following alterations (standard curve, 0.075–3.750 mg/l; initial solution, 15 mg/l). Both curves showed a good *r* value (0.99954). The peaks of *trans*-resveratrol and *trans*-piceid were measured at 307 nm and 285 nm, respectively.

Evaluation of antioxidant activity in vitro

The antioxidant activity of the sparkling wines was measured by *in vitro* assays: 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•) scavenging activity, and superoxide dismutase (SOD-like)- and catalase (CAT-like) activities.

DPPH• radical scavenging activity was measured using a method modified from Yamaguchi *et al*.,[19] in which sparkling wines pure or diluted in distilled water (1%, 10% and 50% [v/v]) were added to Tris-HCl buffer solution (100 mM, pH 7.0) containing 250 μM DPPH• dissolved in ethanol. In the control tube, sterilized distilled water was used in lieu of sparkling wines. The tubes were kept in the dark for 20 min and the absorbance was measured at 517 nm (Shimadzu UV-1700 spectrophotometer). Results were expressed in IC_{50} (50% inhibition of DPPH• radical).[19]

The potential CAT and/or SOD activities in the sparkling wines studied were assessed as previously described by measuring the rate of decrease in H_2O_2 absorbance at 240 nm and the inhibition of adrenaline auto-oxidation, respectively. SOD-like results were expressed in U. Sod. (a unit of SOD-like activity is defined as the volume [μl] of sample able to inhibit 50% of the adrenochrome-formation reaction), while CAT-like results were expressed in μmoles of H_2O_2 decomposed per minute (a unit of CAT-like activity).[20,21]

Evaluation of enzymatic activity in vitro

The β-glucosidase assay was based on the procedures described by Riou *et al*.[31] and Dhake and Patil[22] by mixing 0.1 ml of 5 mM *p*-nitrophenyl-β-D-glucopyranoside (pNPG) and 0.4 ml of 0.1 M sodium acetate buffer at pH 5.5. After incubation for 10 min at 50ºC, the reaction was stopped by adding 2 ml of 1 M sodium carbonate, and the released *p*-nitrophenol was monitored at 420 nm. One unit of β-glucosidase activity corresponds to the release of 1 mol of *p*-nitrophenol per minute under the assay conditions.

Data analysis

Data were analyzed using the following tests: Kruskal–Wallis H, Spearman correlation and principal component analysis (PCA), with SPSS v16.0 software for Windows.

Results and discussion

Enological analyses and polyphenols by UV spectrophotometry

The average levels of pressure, alcohol content, total and volatile acidity, free and total SO_2, ascorbic acid, and pH were 5.00 ± 0.20 atm, 11.54 ± 0.15% v/v, 6.80 ± 0.12 g/l of tartaric acid, 0.318 ± 0.09 g/l of acetic acid, 15.86 ± 1.44 mg/l, 126.64 ± 3.24 mg/l, 5.75 ± 0.61, and 3.01 ± 0.01, respectively. Values lower than the allowed levels for volatile acidity, free SO_2 and

Table 2 Average concentration (± SD) of sugar, dry extract (DE), reduced dry extract (RDE), ascorbic acid (AA), total polyphenols (TP), total flavonoids (TF), total hydroxycinnamates (THC), *trans*-piceid (P) and *trans*-resveratrol (R) analyzed in sparkling wines

Sugar (g/l)	DE (g/l)	RDE (g/l)	AA (mg/l)	TP** (mg/l)	TF** (mg/l)	THC*** (mg/l)	P (mg/l)	R (mg/l)
1 10.4 ± 0.2[a#]	25.5 ± 0.1[a]	16.1 ± 0.2	5.8 ± 0.0	444.4 ± 0.1[a]	117.6 ± 0.1[a]	23.8 ± 0.1[a]	0.88 ± 0.06[a]	0.40 ± 0.06[a]
2 20.1 ± 0.4[b]	36.2 ± 0.5[b]	17.0 ± 0.2	6.5 ± 0.6	444.4 ± 0.1[a]	117.6 ± 0.1[a]	24.5 ± 0.1[a]	0.55 ± 0.03[b]	0.37 ± 0.01[a]
3 31.6 ± 1.0[c]	46.7 ± 0.2[c]	16.4 ± 1.6	5.0 ± 0.6	444.4 ± 0.1[a]	117.6 ± 0.1[a]	24.2 ± 0.1[a]	0.55 ± 0.04[b]	0.27 ± 0.04[b]
4 41.3 ± 1.4[d]	57.9 ± 0.6[d]	17.6 ± 1.6	6.5 ± 0.9	442.8 ± 0.1[b]	112.6 ± 0.1[b]	23.8 ± 0.1[b]	0.39 ± 0.03[c]	0.26 ± 0.03[b]
5 51.3 ± 0.9[e]	68.6 ± 0.5[e]	18.3 ± 1.4	5.6 ± 0.5	440.2 ± 0.1[c]	105.0 ± 0.1[c]	23.2 ± 0.1[c]	0.31 ± 0.04[c]	0.28 ± 0.01[b]
6 61.9 ± 1.2[f]	79.0 ± 0.5[f]	18.1 ± 1.4	5.2 ± 0.6	438.6 ± 0.1[d]	100.8 ± 0.1[d]	23.5 ± 0.1[c]	0.34 ± 0.06[c]	0.27 ± 0.04[b]
7 72.3 ± 1.9[g]	90.0 ± 0.5[g]	18.7 ± 1.4	5.6 ± 0.9	436.1 ± 0.1[e]	94.1 ± 0.1[e]	22.9 ± 0.1[d]	0.39 ± 0.01[c]	0.25 ± 0.01[b]

Data are mean ± SD values of three independent experiments.
**Values expressed in catechin; ***values expressed in caffeic acid equivalents.
#Data followed by different letters for each line differ significantly difference according to analysis of variance and Tukey's post hoc test ($P \leq 0.05$) for each parameter evaluated.

total SO_2 were observed in the sparkling wines, indicating that the grapes are healthy and that good vinification practices were used.[1] Table 2 shows sugar values, as well as dry and reduced dry extract. All enological values were found to be within those established by Brazilian legislation and in accordance with the international ranges.[5,6]

Table 2 shows that there were no changes in polyphenols by UV spectrophotometry assay at the 10 g/l, 20 g/l and 30 g/l sugar levels. However, with the increase in sweetness, the concentrations of all analyzed phenolic groups decreased. If necessary, sucrose is used in enology for industrial fermentations. Sucrose is a non-reducing disaccharide which, by hydrolysis, leads to β-D-glucose and β-Dfructose. The results mentioned above may be due to esterification reactions between glucose molecules and hydroxyl groups, *e.g*. ellagic acid and sugar adduct formation with epicatechin.[32,33] In this study, polyphenol levels by the UV spectrophotometry assay were negatively correlated with sugar concentration (TP – $R = -0.935$; $P = 0.01$; TF – $R = -0.934$; $P = 0.01$; and THC – $R = -0.957$; $P = 0.01$).

Trans-*resveratrol and* trans-*piceid by HPLC*

The observed *trans*-piceid levels were higher than the *trans*-resveratrol levels (Table 2), in accordance with the literature.[30,34] The addition of sugar decreased the concentrations of both phenolic compounds. This was observed at doses up to 40 g/l (*trans*-piceid) and 30 g/l (*trans*-resveratrol). From there onwards, under the conditions of this study, we observed that the concentration of these compounds remained constant. Sun *et al*.[30] showed that the *trans*-piceid peaks remained constant during wine maturation. The initial decrease of these concentrations is probably due to the more acidic pH of the sparkling wines, because the glycosylation of stilbenes can increase their solubility, and to the presence of CO_2.[30,34,35] Glucosyltransferases have different K_m values (resveratrol glucosyltransferase has a K_m value 50% less than UDP-glucose, which explains the major decrease observed for *trans*-piceid). Furthermore, we found β-glucosidase activity in the sparkling wines (Table 3).[34] In this case, the *trans*-resveratrol content was negatively correlated with sugar concentration ($R = -0.675$; $P = 0.01$). Conversely, *trans*-piceid level was positively correlated with that same variable ($R = 0.686$; $P = 0.01$), suggesting a possible conversion of the aglycones into their glycosylate derivatives and suggesting a possible reversible reaction between the aglycones and their glycosylated derivative.

Evaluation of antioxidant capacity in vitro

This is the first time that SOD-like and CAT-like activities have been studied in sparkling wines. Table 3 shows that there were no changes in the antioxidant activities *in vitro* (CAT-like, SOD-like and DPPH•) due to sweetness.

Furthermore, we observed a positive correlation between levels of sugar and SOD-like activity ($R = 0.341$; $P = 0.05$), which may be related to sugars, capacity as scavengers of hydroxyl radicals.[36,37] SOD integrates human endogenous antioxidant defenses, being able to catalyze the dismutation of superoxide anion ($O_2^{\bullet -}$) to oxygen and hydrogen peroxide.[38] Therefore, sparkling wines' capacity of scavenging the free radical5 DPPH• and their SOD mimicking activity implicitly shows the potential benefits against oxidative stress of regular and moderate consumption of these wines.[39]

Furthermore, all samples assayed also showed a

Table 3 β-Glucosidase, DPPH•, SOD and CAT-like activity in sparkling wines with different final levels of sugar

Sugar (g/l)	Cat-like[#]	Sod-like*	β-Glucosidase[##]	DPPH•**
10.4 ± 0.2[a#]	6.2 ± 2.2	65.2 ± 2.7	2.1×10^{-5} ± 0.3	72.1 ± 0.5
20.1 ± 0.4[b]	6.2 ± 2.2	65.7 ± 2.2	2.2×10^{-5} ± 0.1	75.2 ± 2.4
31.6 ± 1.0[c]	6.2 ± 2.9	63.3 ± 1.6	2.2×10^{-5} ± 0.1	73.2 ± 1.4
41.3 ± 1.4[d]	6.2 ± 2.9	72.3 ± 3.5	2.2×10^{-5} ± 0.1	76.8 ± 1.6
51.3 ± 0.9[e]	7.5 ± 0.3	72.7 ± 2.4	2.2×10^{-5} ± 0.1	75.6 ± 2.5
61.9 ± 1.2[f]	6.6 ± 1.3	63.0 ± 2.7	2.2×10^{-5} ± 0.1	74.1 ± 2.5
72.3 ± 1.9[g]	6.2 ± 2.2	71.0 ± 2.7	2.2×10^{-5} ± 0.1	78.2 ± 0.2

Data are mean ± SD values of three independent experiments.
[#]μmols of H_2O_2 decomposed per minute; *IC_{50} value (50% inhibition of adrenochrome formation); [##]Unit β-glucosidase (release of 1 mol *p*-nitrofenol/min); **IC_{50} value (50% inhibition of DPPH• radical). Enzymatic assays and DPPH• do not show statistically significant differences according to variance and Tukey's post hoc test ($P \leq 0.05$) for each parameter evaluated.

CAT-like activity (similar to catalase enzyme, being able to convert hydrogen peroxide to water and molecular oxygen). SOD and CAT enzymes have an important role in maintaining the physiological redox equilibrium, avoiding or decreasing oxidative stress.[38,39] The intrinsic relationship between phenols and important enzymatic mimetic activities, *e.g.* the interaction with the sugar content of sparkling wines, is shown in Figure 1. Thus, *demi-sec Charmat* sparkling wines might be used as an antioxidant system capable of acting as an aid in a balanced diet, promoting a healthy life-style, regardless of sugar concentration. Moreover, the positive correlation observed between THC and *trans*-resveratrol (R = 0.535; P = 0.01) corroborates the important role of phenolic compounds of low molecular weight in preventing oxidation of other polyphenols.

Evaluation of enzymatic activity in vitro

This is the first time that the β-glucosidase activity has been studied in sparkling wines. Table 3 shows that there were no changes in this enzymatic activity in relation to different sugar levels. Thus, the cleavage of the *trans*-piceid molecule by this enzyme might explain the maintenance of the levels of this polyphenol and its aglycone, since the glucose free in the medium can promote the glycosilation of resveratrol.[40]

β-Glucosidase activity has been known to exist in *S. cerevisiae* for quite a long time.[41] The present study suggests that there may be a transference of this enzyme from the intracellular compartment of the yeast to the extracellular medium, thus contributing to the formation of the complex aromas usually found in sparkling wines. These aromas are formed during *sur lie* time, as terpenes can be present in the non-volatile sugar-conjugated form and might be hydrolyzed by the action of β-glucosidases.[1,3] It is important to emphasize that the aromas thus developed are considered an important qualitative characteristic of sparkling wines.

Furthermore, mannoproteins are polysaccharides produced by the *S. cerevisiae* yeast (located in the cell wall) during alcoholic fermentation. These polymers are present in significant amounts in the wines, and their concentration depends on the wine-making process, including the *sur lie* time.[42,43] Investigation of mannoprotein levels was not a goal of this study; however, the correlation of these compounds and β-glucosidase levels in the yeasts may explain their contribution to improving the safety and quality of wines, because mannoproteins can protect phenolic compounds responsible for antioxidant activity.[44] Since the β-glucosidase mimetic activity remained stable, even in the presence of sugar, one might consider that the high quality of sweetened sparkling wines is maintained. In summary, the PCA (Fig. 1) clearly shows the interaction between the factors

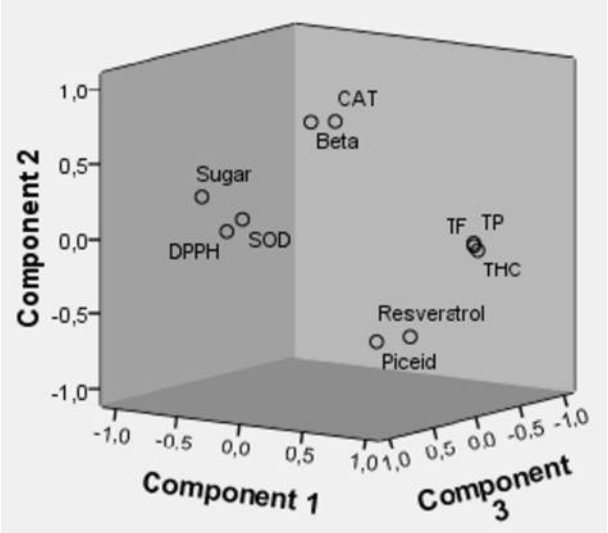

Figure 1 Intrinsic relationships between the phenols and important enzymatic mimetic activities

discussed above, since sweetness (43.5%), enzyme activity of β-glucosidase (21.0%), and SOD-like activity (16.9%) can explain up to 81.4% of the variance observed in the results. Moreover, the potential change in phenolic balance, especially in the levels of *trans*-piceid and *trans*-resveratrol, when sugar is added, is probably later restored due to the buffering effect of wine.[1] The wines are a complex mixture with many interactions, as demonstrated by the results of this study. Unlike other authors, the addition of sugar in sparkling wines did not significantly alter the antioxidant activities *in vitro* (Sod-like, Cat-like and DPPH•), enzyme activity (β-glucosidase), or the levels of important polyphenols analyzed by HPLC (*trans*-piceid and *trans*-resveratrol), which are relevant to the theme of wine and health. Other tests are being conducted to clarify these issues further. This work shows an approach able to clarify important aspects for wine industries with regard to world-wide consumer demand by sweetened products.[15,45]

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O objetivo principal deste estudo foi avaliar as modificações nas características sensoriais e no perfil mineral de vinhos espumantes *Charmat* e *Champenoise*. A atividade antioxidante e o conteúdo fenólico também foram discutidos.

Sensory and Antioxidant Evaluation of Sparkling Wines

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ABSTRACT

The purpose of this study was evaluated sensory properties, phenolic content, mineral profile and antioxidant activities of sparkling wines (SWs). Were studied fifteen SWs produced by seven different wineries located in the *Serra Gaúcha* (south of Brazil), divided into three equal groups (*Charmat brut*, *Charmat demi-sec* and *Champenoise*). The interactions among important characteristics of SWs are still not clarified, and biological activities as auxiliaries in health maintenance have become a goal of consumers around the world. *Charmat* and *Champenoise* methods have different interactions between mineral composition (mainly *demi-sec* samples) and phenolic profile in relationship to biological activity (all groups evaluated showed CAT-like and SOD-like antioxidant activities) and sensorial properties observed. These aspects play an important role in the maintenance of SWs quality and are essential to the sustainability of the wine industry. The results of the present study showed that SWs have bioactive compounds useful in a healthy diet, as manganese, rubidium and zinc. They further allow consumers to enjoy SWs of their choice without giving up any health benefits.

***Keywords*:** sparkling wine, polyphenols, mineral composition, PIXE, sensory evaluation.

INTRODUCTION

Descriptive and sensory tests are widely used to classify, establish quality standards, and assess the level of satisfaction of consumers of various products, such as SWs [1]. Furthermore, tests that show possible functional properties of foods and drinks are increasingly attracting interest not only from the scientific community but also from industry in the spotlight of high global competitiveness and consumers in general, offering products that provide more than pleasure, food safety [2].

While many wine producing regions in world are concerned about how to move their wine stocks, the SWs export market in 2006 showed an increase of 8.77% in relation to 2005 only at Champagne, France [3]; and the SWs export market also increased worldwide [4]. The main methods for producing a SWs are *Charmat* (second fermentation in large containers) and *Champenoise* (second fermentation in the bottle itself [5], and these wines may be classified into many types, according the country of origin law [6]. Furthermore, differences in the sensory and biochemical compositions can be found [5].

SWs are rich in bioactive compounds [2,7]. Many studies have already demonstrated that phenolic compounds present known antioxidant activity [8], which is associated with the reduction in the incidence of several diseases such as atherosclerosis and cancer [9]. Besides, some minerals have an essential role for the human race [10,11], as for example, K, Ca and Mg, considered a major presence in wines [5]. Moreover, the *terroir*, the winemaking philosophy, and consequently, the phenolic and mineral status have real importance for the sensory and biochemical qualities (color,

bitterness, flavors, longevity, antioxidant properties, etc.) and acceptance by consumers around the world [1,2,11,12].

Therefore, the purpose of the present study was to evaluate the sensory characteristics, phenolic contents, mineral profile and antioxidant activities of *Charmat* and *Champenoise* SWs from the *Serra Gaúcha*, the highlands of the state of Rio Grande do Sul, Brazil, a reference in the world's SWs industry.

METHODOLOGHY

Samples

Fifteen SWs (five of each group: *Charmat brut*, *Charmat demi-sec* and C*hampenoise)* made by seven different wineries located in the *Serra Gaúcha* were studied. The samples were produced from three different wine grape varieties: Pinot Noir (PN), Chardonnay (CH), and Italic Riesling (IR), widely used in the world [7,13]. The first fermentation (base wines) was done at 15 °C for an average of 16 days. In the second fermentation, the average temperature was 12 ± 2 °C with the foam formation time varying between 30 and 90 days. In all samples, *Saccharomyces bayanus* was used in both fermentations. In order to perform the assays, the SWs were previously degassed, using a vacuum pump with a valve for air removal, coupled to a workbench agitator.

Chemical reagents

The reagents for the phenolic and mineral assays were acquired from E. Merck, Darmstadt, Germany. The others reagents were acquired from Extrasynthese, Gennay, France.

Enological analyses

Alcohol content, total acidity, pressure, volatile acidity, pH, free and total SO_2, dry extract and reduced dry extract concentration were determined using the methods described by Zoecklein et al. [14]. The sugar concentration was obtained through an enzymatic measure (reactives from Chema Italia, Rome, Italy) using a multi-parametric analyser Enochem (Tecnología Difusión Ibérica, Barcelona, Spain) [15,16,17]. All analyses were performed in duplicate.

Polyphenol profile by UV spectrophotometry

Total polyphenols (TP) and total hydroxycinnamates (THC) were quantified by measuring the absorbance at 280 nm and 320 nm (Shimadzu UV-1700 spectrophotometer), respectively. TP results were expressed as mg/L of catechin and THC results as mg/L of caffeic acid equivalents. Total flavonoids (TF) were calculated using the following formula: TF = [(A280 – 4) – 0.66] x (A320 – 1.4) [14,15]. Results were expressed as mg/L by a catechin standar curve (R= 0.99997). All analyses were performed in duplicate.

Mineral Composition by PIXE (Particle-Induced X-ray Emission)

The quantification of metals in wines and SWs were performed using the PIXE technique [18]. To that end, a 3 MV Tandetron accelerator provided 2.0 MeV proton beams with a 1 nA current. The X-rays induced in the samples were detected using a Si (Li) detector (SLP series, EG&G Ortec, CA, USA), with a resolution of 160 eV at 5.9 keV, positioned at 135° in relation to the beam axis. The standardization procedure adopted in this work is based on the H-value method [18] using apple leaves standard

from NIST (*Standard Material* 1515). The data analysis was performed using the GUPIX code developed at the University of Guelph [19].

Evaluation of antioxidant activity in vitro

Potential catalase (CAT) and/or superoxide dismutase (SOD) activities in the SWs studied were assayed as previously described by measuring the rate of decrease in H_2O_2 absorbance at 240 nm [20] and the inhibition of adrenaline auto oxidation [21], respectively. SOD-like results are expressed in U. Sod. (a SOD-like unit is defined as the volume (μL) of sample able to inhibit 50% of the reaction of adrenochrome formation), and CAT-like results are expressed in μmols of H_2O_2 decomposed per minute (a CAT-like unit).

Sensory Evaluation

The wines were evaluated in October 2007 by a panel of twelve wine experts – male enologists and scientific researchers between 30-42 years of age with previous experience in SWs sensory analysis. The experts were selected to participate based on their interest and availability. Randomized samples were served in official glasses. Distilled water was provided for rinsing of the palate during the testing. Evaluations were conducted at 21 ± 1 °C. The fifteen samples were evaluated according to the descriptive card for SWs developed by Dr. Mauro C. Zanus at the Embrapa-Cnpuv, the Grape and Wine Research Center of the Brazilian Organization for Agricultural Research (Fig. 1). This type of index card consists of the comparison, scoring, and classification of wines of the same class or origin according to their qualities and defects by eighteen parameters accompanied by a scale of five categories (0 = absence to 5 = very intense).

Data analysis

Data were analyzed by using SPSS 16.0 for Windows to perform following tests: *Kruskal-Wallis* H, *Spearman* Correlation, and Principal Component Analysis (PCA).

Sparkling Wines Score Card

Panelists: Sample number:

Intensity (scores: 0 to 5):

0 0.5 1 1.5 2 2.5 3 3,5 4 4.5 5

absent to very intense

DESCRIPTION	
APPEARANCE	
Color	
Effervescence	
Foam	
Bubble size	
AROMA	
Fruity	
Floral	
Yeast /Toasted Bread	
Oaky	
Herbal	
Finesse/Sharp Aroma	
Defects	
TASTE	
Finesse	
Sweetness	
Structure/Body	
Acidity	
Bitterness	
Defects (+)	
Overall Quality (Scale: 0 to 100)	

Notes:

Figure 1. Descriptive card for sparkling wines.**RESULTS AND DISCUSSION**

Enological analysis

Alcohol content of the different SWs analyzed varied from 11.78 to 12.75% (v/v), and total acidity from 5.16 to 8.10 g/L of tartaric acid. The average levels of pressure, volatile acidity, and pH were 5.7 ± 0.2 atm, 0.567 ± 0.061 g/L of acetic acid, and 3.07 ± 0.12 g/L, respectively. Average concentrations of free SO_2 were 20.00 ± 6.17 mg/L and of total SO_2 were 102.00 ± 27.55 mg/L. Values lower than the allowed levels for volatile acidity, free SO_2 and total SO_2 were observed in the SWs, indicating that the grapes are healthy and that good vinification practices were used [22]. Analysis of the dry extract and reduced dry extract showed, respectively, values of 20.25 ± 3.18 and 19.10 ± 2.65 g/L for the *brut* samples, and 57.12 ± 8.74 and 17.05 ± 2.55 g/L for the *demi-sec* samples (data not shown). Table 1 shows the mean sugar values for *brut* and *demi-sec* SWs. All enological values were found within the values established by the Brazilian legislation [23] and are in accordance with the ranges used worldwide [6,24,25].

Table 1 Physical, chemical and biological parameters of sparkling wines studied.

Parameters	**Charmat brut**	**Charmat demi-sec**	**Champenoise**
TP (mg/L)	400.36 ± 136.59	556.88 ± 79.43	562.35 ± 118.40
TF (mg/L)	135.54 ± 94.97	363.82 ± 85.65	306.58 ± 72.13
THC (mg/L)	39.99 ± 6.01	41.86 ± 4.98	56.30 ± 8.79
Cat like (U.Cat.)*	$9.84.10^{-9}$ ± 0.81	$8.78.10^{-9}$ ± 2.35	8.63 ± 0.92
Sod like (IC_{50})**	$40.55 \pm 6.46^{a\#}$	78.89 ± 7.05^{b}	173.65 ± 34.24^{c}
Sugar (g/L)	9.81 ± 2.08^{a}	43.10 ± 7.95^{b}	7.26 ± 1.31^{a}
***Sur lie* (dias)**	90 ± 22^{a}	30 ± 18^{b}	300 ± 60^{c}

*U.Cat. = µmols of H_2O_2 degraded per minute; **IC50 = volume (µL) of the sample able to inhibit 50% of the reaction of formation of adrenocromo $^{\#}$data followed by different letters for each line differ significantly by *Kruskal Wallis H* test ($p \leq 0.05$); n = 15 sparkling wines assayed; TP = total polyphenols; TF = total flavonoids; THC = total hydroxycinnamates.

Polyphenol profile by UV spectrophotometry

While there were no significant differences in phenolic profile among the groups studied, although *Charmat demi-sec* and *Champenoise* samples showed a tendency to have higher concentrations of phenolic compounds (Table 1). In the process of SWs production, the polyphenols concentration may vary depending on the various vinification techniques [22,26].

A major difference between *Charmat* and *Champenoise* is the maturation period on the lees (*sur lie*), which was significantly different between the groups studied. In *Charmat* SWs, this time is usually quite reduced [27]. Therefore, they are light yellow in color, have fruity and floral aromas, and a refreshing flavor. Furthermore, in the traditional method, this period may vary from a few weeks to several years [5], giving the SWs a more intense golden yellow color, stronger aroma (walnuts, toasted-bread, honey, etc.), and a marked flavor [28]. Several authors have suggested that autolytic yeast capacity could be used as a way to improve the quality of SWs by acquisition of aging like characteristics [26].

Mineral Composition by PIXE (Particle-Induced X-ray Emission)

Fig. 2 shows the concentrations of seventeen metals identified in the SWs samples. The mineral compositions of the *Charmat brut* and *Champenoise* were very similar and both differed from the *demi-sec* samples. No Pb was identified in the samples assayed, differently from what was observed in European SWs [29]. Due to the toxicity of this mineral, it is a positive aspect of a Brazilian SWs. So far, there have been no reports on the presence of Br, Cl, Ti, Si, Cr, and Rb in SWs, as seen in this study. As to the other metals assayed, contents were higher than those identified in *Cavas* and *Champagnes* produced by the *Champenoise* method, probably due to their provenance [29; 30].

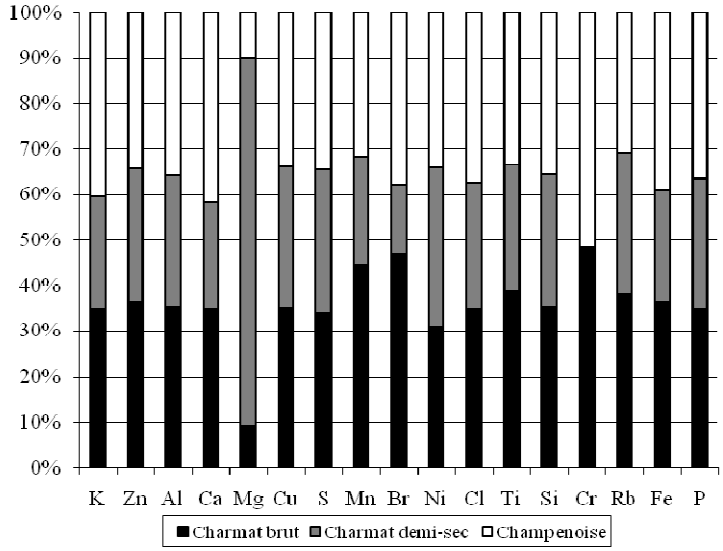

Figure 2. Mineral profile of *Charmat brut, Charmat Demi-sec* and *Champenoise* sparkling wines obtained by PIXE.

Major differences were found in concentrations of K, Ca, Mg, Mn, Br, Cr, and Fe. It is known that the use of clarifying agents (including bentonites) leads to changes of pH; the elevation of alcohol graduation changes the solubility of various ions and tartrates such as Ca and K, and the cold stabilization can improve the precipitation phenomena. The balance between these reactions can predominantly influence the mineral content of these wines [22]. The content of these elements in the *Charmat demi-sec* SWs was lower than in samples of *Charmat brut* and *Champenoise.* This can be explained by several factors, including the need to keep the wines free from physical, chemical, and microbiological changes due to increased presence of sugar (*demi-sec* samples) and to maintain a better-structured taste in the *Champenoise* samples. That is, Charmat SWs are usually produced by enological treatments that are more intensive.

The high Mg levels in the *Charmat demi-sec* samples (Fig. 2) deserve special attention. It is known that fermented drinks have high Mg [31] and that the mineral presence is regarded by consumers as an important item at the time of purchase, because

they play an important role in human nutritional requirements [1]. Furthermore, they can be used as a co-factor in several metabolic routes of the yeast *Saccharomyces cerevisiae*, becoming an indispensable element in the cell balance, participating directly in the modulation of alcoholic fermentation [10]. In addition, the presence of sugar in the wines is also a relevant factor for the consumers [1,32] and they must be informed about it [6]. The sugary type of SWs usually requires a shorter production time (Table 1), resulting in a product that is light and refreshing, and which has the softness required by consumers due to the addition of sugar (expedition liqueur). It is possible that the yeast does not use any Mg present in the SWs, due to their different production process (alcoholic fermentation / maturing X time).

Mineral levels in wines have links with their provenance [29], and although all SWs tested have been produced in the same region, as previously reviewed, the base wines for *Charmat demi-sec* tend to be further clarified and go through vigorous filtration processes. This may explain not only the absence of Cr in the samples, but also a lower concentration of other minerals (except for Mg), since the ability of bentonites of adsorbing these compounds, including Cr, is already well established [5].

Evaluation of antioxidant activity in vitro

Antioxidants are compounds that can act as blockers of oxide-reduction processes by reactive species in excess, which are associated with various diseases, including Parkinson, atherosclerosis, myocardial infarction, ischemia and reperfusion syndrome, and several types of cancer [9]. Previous studies have shown that the presence of phenolic compounds in the diet can be a factor in preventing many of these diseases, and that these bioactive compounds in the wine also have beneficial effects on the human health [9,13,33]. However, the potential antioxidant activity in SWs is still

little known [2,7]. One of the purposes of our study was to verify the possibility of SWs showing activities similar to the human antioxidant enzyme defense system, specifically SOD-like (superoxide anions transforming into H_2O_2 and O_2) and CAT-like (hydrogen peroxide dismutation into water and oxygen) activities [34]. Our results showed that all SWs evaluated presented CAT-like and SOD like antioxidant activities (Table 1).

Although polyphenols, flavonoids and hydroxycinnamates levels (Table 1) were positively correlated with the CAT-like activity (R = 0.829, R = 0.771, R = 0.771, p = 0.01, respectively), we could observe no significant differences in this biological activity between the groups tested (Table 1). This is probably due to the fact that phenolic compounds usually related to the reduction of damage caused by hydrogen peroxide, such as catechin, resveratrol, among others [7,9], suffered a greater oscillation in their concentration because of enological techniques used in each production method [2,7,35]. The CAT-like activity can be also explained by the fact that zinc has the potential of alleviating the toxic effects of heavy metals in rat liver because of its property of inducing the action of metallothionein (S-rich protein) as a free radical scavenger, or its indirect action in reducing the levels of oxygen reactive species [36]. In this study, the CAT-like activity was positively correlated with zinc levels (R = 0.886, p = 0.01). This enzymatic activity can also be explained by manganese presence in the SWs, since the CAT-like activity of this mineral was reported by Tikhonov et al. [37].

Hydroxycinnamates are a group of phenolic compounds of great importance to the sensory and biological characteristics of SWs [7], which in their majority are produced by wine assemblages between white and red grapes, submitted the fermentation without pomace [5]. Table 1 shows that the *Charmat brut* SWs had a greater SOD-like activity than the *Charmat demi-sec* and *Champenoise* samples, respectively, and it was possible to observe the presence of a positive correlation

between this activity and the hydroxycinnamate levels in the SWs studied ($R = 0.943$, $p = 0.01$). It is important to note that the concentration of this phenolic group was more homogeneous in all methods of production tested (Table 1), which may have favored the performance of enzyme-like activity.

The fact that the SOD-like activity declined in the *Charmat demi-sec* SWs can be explain by a lower manganese concentration in these samples. The CAT-like activity of this mineral [37] can thus be explained, since both enzymatic activities are linked. Another possible explanation are reactions between molecules of sugar and polyphenols (adducts formation), proteins, aldehydes and amino acids involved in several reactions during the production and storage process [27,38]. Similarly, important changes in the composition of the wines, including *Champenoise* SWs, can occur during their production due to the autolysis of yeasts during aging on lees [39]. The main changes that may occur are increase in amino acid concentration, reduction in protein levels, appearance of peptides of the different sizes, and increase in polysaccharide content; they may also be influenced by aspects mentioned above, plus other technological parameters [22]. The SOD like activity was also explained by Hegde et al. [40], who showed that rubidium blended with pyruvate might prevent ultraviolet damages in ophthalmology. Therefore, the presence of rubidium (third component in PCA analysis; Fig. 3) in SWs cannot only protect them from oxidative phenomena from light exposure, but it can also act as an antioxidant to help the body's defenses. In our study, the SOD-like activity was positively correlated with the levels of this mineral ($R = 0.828$, $p = 0.01$).

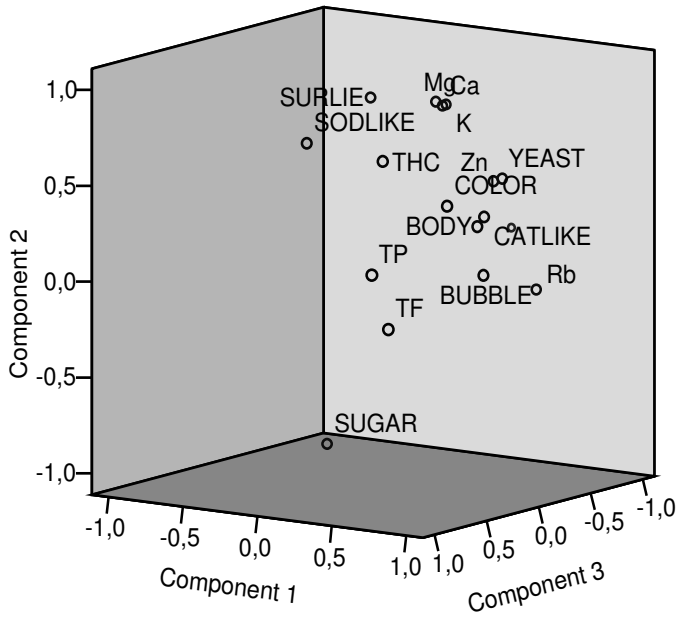

Figure 3. Scores plot of the main characteristics and quality variance the *Charmat* (*brut* and *demi-sec*) and *Champenoise* using the PCA analysis. CP1 = *sur lie*; CP2 = sugar; CP3 = Rb.

The differences in physical, chemical, and biological characteristics at any time decrease the quality of SWs tested, because this is a concept that involves many other factors [1]. In contrast, it shows that all samples tested in the present study have bioactive compounds useful in a healthy diet. It further allows consumers to enjoy SWs of their choice without giving up any health benefits. Moreover, the sugar molecules can display antioxidant capacities that cannot be underestimated [41].

Sensory Evaluation

The panel performances were first controlled and evaluated. Results are not presented in detail in this study, but the panelists were mostly homogeneous and repeatable in their evaluation. There were no significant differences (*t* test) in the sensory evaluation among the samples, which shows once again the high quality of

Brazilian SWs. Fig. 4 shows some different characteristics between different groups, which are commonly linked to the consumer's choice of this type of product at the time of purchase [1].

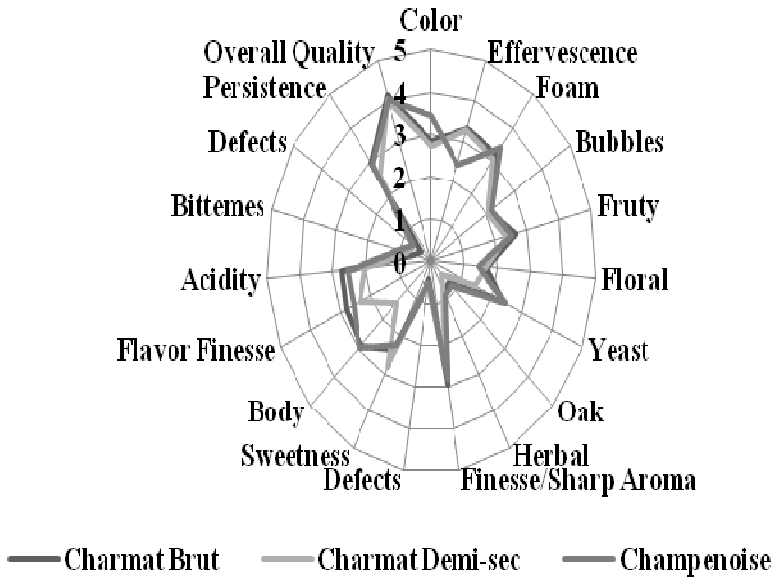

Figure 4. Parameters descriptive in sensory analysis for *Charmat brut*, *Charmat Demi-sec* and *Champenoise* sparkling wines obtained by a panel.

In *Charmat* SWs, all stages occur under isobarometric conditions (from the beginning of second fermentation until bottling), and thus, the loss of carbon dioxide (CO_2) formed naturally by yeasts is minimal. In contrast, during *Champenoise* degorging (technique used to remove sediments formed during autolysis of yeast) small losses of CO_2 may occur [5], which may explain the differences in effervescence between *Champenoise* and *Charmat* SWs reported by the panelists (Fig. 4).

Another parameter directly linked with SWs quality is the presence of CO_2 or bubbles observed [42], which was also positively correlated with the CAT-like activity (R = 0.886, p = 0.01). This shows that a careful method able to minimize CO_2 losses may ensure and even improve this biological activity. This can be then definitely used

as an important quality (marketing) factor with the consumer (i.e., health *versus* pleasure) at the time of purchase [1].

These and other aspects already discussed about the interaction between the CAT-like enzyme activity exerted by zinc and manganese – metals that positively correlate with bubbles – reinforce this idea (R = 0.829 and R = 0.626, p = 0.01, respectively). Furthermore, the principal components analysis (PCA) performed with all samples revealed that the principal components PC1 = *sur lie* (60.22%), PC2 = sugar concentration (21.70%), and PC3 = Rb (14.66%) explain more than 96.58% of the total variance (Fig. 3). The analysis aided our data, since the CO2 variable (foam/*perlage*) appears as one of the major factors influencing the general characteristics of SWs (Fig. 3). These new findings are quite significant since from the point of view of both consumers and winemakers *perlage* is one of the main appeals of these products [42].

As reviewed above, the *Charmat demi-sec* SWs production time is lower [27] and this interfered in the physical, chemical and biological parameters found in the present study (Table 1). The same relation was observed with the sensory characteristics, since yeast aroma and especially body were significantly lower in these samples (Fig. 4). The direct relation between these two aspects – yeast strain used and *sur lie* period – has been widely studied [1,28]. As our results show, it is certainly possible to affirm that these SWs are different. The hierarchical analysis of variables (Fig. 5) clearly shows four clusters, with the sensory characteristics previously related to the CAT-like enzyme activity forming one of these clusters, and the remaining variables (hydroxycinnamate levels, *sur lie*, sugar concentration) related to the SOD-like enzyme activity forming another cluster.

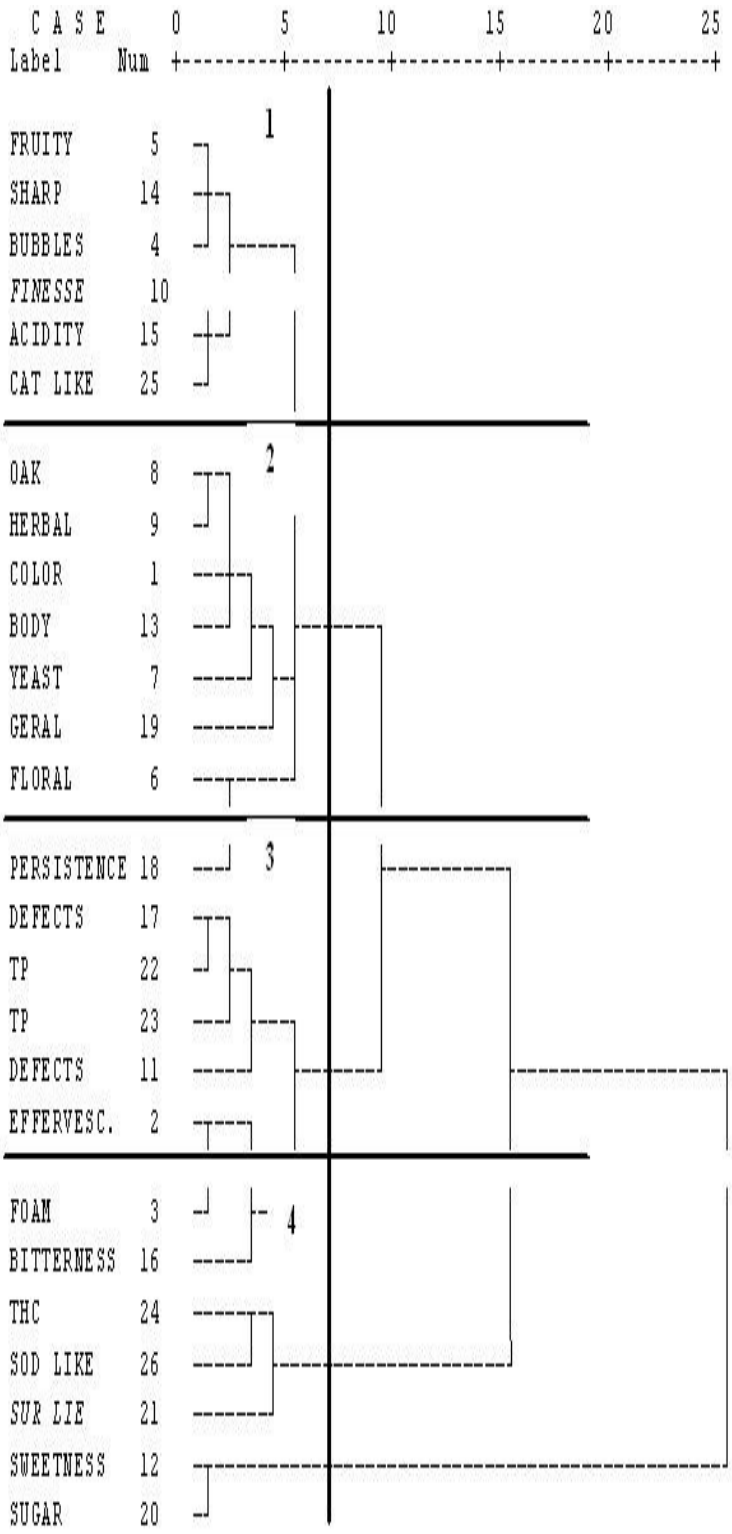

Figure 5. Division into 4 groups by hierarchical cluster analysis dendrogram for 15 sparkling wines using average linkage (between groups).

In summary, the results found and discussed in this study demonstrate – for the first time in *Charmat* and *Champenoise* SWs – the intrinsic relationship between: a) physical factors (production techniques *versus* time); b) enological parameters, mainly sugar concentration, phenolic profile, mineral status (specially Rb levels); and c) biological activity (SOD-like and CAT-like enzyme activity), high lighting the many links between these aspects and the sensorial characteristics that define the style of each group evaluated (aroma, body, *perlage*, etc.). Additional studies are under way to assess the effect of *sur lie* and sugar concentration on the properties of SWs, aiming to provide relevant purchase information to consumers about high-quality products and, further, to help in the quest for a healthier and more enjoyable lifestyle.

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3.3 – Artigo 2 – C.A. Stefenon, C. De M. Bonesi, V. Marzarotto, D. Barnabé, F.R. Spinelli, V. Webber & R. Vanderlinde. 2014. Phenolic composition and antioxidant activity in sparkling wines: modulation by the ageing on lees. **Food Chemistry**. 145: 292-299.

Este trabalho teve como objetivos principais determinar a presença de atividade β-glicosidásica, bem como o comportamento dos compostos fenólicos durante o período de *Sur Lie* em vinhos espumantes *Champenoise* e *Charmat*, identificando qual a variável de maior influência (método de elaboração ou envelhecimento sobre borras) na atividade antioxidante dos mesmos.

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Phenolic composition and antioxidant activity in sparkling wines: Modulation by the ageing on lees

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ABSTRACT

Sparkling wines (SW) have a special biological ageing on lees that is performed using two distinct methods: in the bottle (*Champenoise*) or in isobaric tanks (*Charmat* method). The objective of this study was to compare the levels of phenolic compounds, β-Glucosidase and antioxidant activity during the ageing on lees, in samples of SW produced at industrial scale by both methods. The β-Glucosidase activity has been constant over time, showing a close relationship with all the polyphenols studied (resveratrol, piceid, tyrosol, gallic, caffeic and ferulic acids), which were affected by the *sur lie* time. With these cross-reactions, the biological properties of the SW were also modulated. The results showed that the long period of ageing decreased the antioxidant potential in all samples. This work demonstrates that the *sur lie* is more important than the production method itself, due to its ability to modulate the necessary changes to achieve the specific objective.

1. Introduction

The elaboration of SW consists of two phases. In the first one, the base wine (BW) is obtained after applying white vinification. The second phase is conducted through the *Champenoise* or *Charmat* methods. The principal differences between these methods are the conversion of glucose in ethanol by yeasts (second fermentation) and ageing on lees (*sur lie*) that can take place in the same bottle or in isobaric tanks. During this time of contact, the exchanges between the components present in the medium (wine) and in the yeast cells will serve as the substratum for the chemical and enzymatic reaction forming different biochemical profiles888 (Buxaderas & López-Tamames, 2012; Gallardo-Chacón, Vichi, Urpí, López-Tamames, & Buxaderas, 2010; Pozo-Bayón, Martínez-Rodríguez, Pueyo, Moreno-Arribas, 2009; Torrens, Riu-Aumatell, Vichi, López- Tamames, & Buxaderas, 2010; Bosch-Fusté et al., 2009). Thus, as those reactions are modulated by the technology used, the sensorial and biological characteristics of each one of the products are directly related to the microorganism employed, and the chemical composition of the BW, resulting in unique profiles with many points of interest for the scientific, as well as for the economic and technical communities.

The *Saccharomyces cerevisiae* yeast in dried and active form is widely used in wineries, because it can ensure a homogeneous fermentation, resulting in high quality wines (Buxaderas & López-Tamames, 2012; Valero, Moyano, Millan, Medina, & Ortega, 2002). Reactions of hydrolysis during the winemaking are caused by enzymes of the grapes themselves or from the microorganisms taking part in the process, as the β-Glucosidases. The influence in the wine composition has been studied, mainly because these enzymes are also capable of hydrolysing non-volatile wine compounds (Hernández, Espinosa, Fernández-González, & Briones, 2003). Polyphenols are a wide range of biological molecules which play a protective role in plants and are daily found in many types of foods and beverages (Leopoldini, Russo, & Toscano, 2011; Prokop, Abrman, Seligson, & Sovak, 2006). The chemical structure of the polyphenols determines their physiological actions, including the antioxidant activity, protection against heart diseases, cancer and neuronal disorders (Stefenon et al., 2012a; Fukui, Choi, & Zhu, 2010; Leopoldini et al., 2011). Resveratrol and its derivatives glucosylated, tyrosol and phenolic acids are cited, between others activities, as neuroprotective and anticancer agents (Fukui et al., 2010; Rodrigo, Miranda, & Vergara, 2011; Vauzour, Corona, & Spencer, 2010). To the best of our knowledge, there are few reports

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about β-Glucosidase performance and about the role of phenolic compounds, especially during ageing on lees in SW, both regarding their capacity to help in human health maintenance as well as in improving the quality of products (Gallardo-Chacón et al., 2010; Stefenon et al., 2010b).

In this context, the goal of this study was to show, for the first time, a comparison between the levels of these phenolic compounds, the enzymatic (especially β-Glucosidase) and antioxidant activities during ageing on lees, in samples of SW produced at industrial scale using the *Champenoise* and *Charmat* methods in the South of Brazil. Furthermore, due to the worldwide increase in sales of these products at, the bonds between the aspects cited above, the general quality of the SW and the differences between both methods were also discussed.

2. Material and methods

2.1. Samples

The samples were elaborated in industrial scale in the companies Möet Henessy do Brazil – Vinhos e Destilados Ltda and Cave Geisse Ltda, using the *Charmat* and *Champenoise* methods (Fig. 1) and divided into three groups: (A) 7000 bottles of *Champenoise* 100% Chardonnay (CHC; base wine – BW1); (B) 7000 bottles of *Champenoise Assemblage* with 48% Chardonnay + 42% Italic Riesling + 10% Pinot Noir (CHA – BW2) and (C) 21,000 bottles of *Charmat Assemblage* (BW2 too). Groups A and B were split in pupitres with a capacity of 120 bottles each one. As for Group C, from three tanks with a capacity of 53,000 litres each one, three other blocks of 7000 bottles were separated. The yeast used in both methods was the *S. cerevisiae* EC1118 and the procedures of filtration, tartaric and protein stabilization were performed before the SW elaboration.

2.2. Chemical reagents

Reagents for the enological assays and DPPH• (2,2-diphenyl-1-picrylhydrazyl) were acquired from E. Merck, Darmstadt, Germany, while the reagents for the high-performance phenolic liquid chromatography (HPLC) and enzyme analyzes were acquired from Sigma–Aldrich (except for piceid HPLC grade, which was acquired from Polyphenols Laboratories AS, Sandnes, Norway). All other reagents were acquired from Extrasynthese, Gennay, France.

2.3. Enological analysis

The alcohol content, total acidity, pressure, volatile acidity, pH, free and total SO_2, dry extract and reduced dry extract, concentration of glucose and ascorbic acid were determined using the methods described by Zoecklein, Fugelsang, Gump, and Nury (2000, chap. 7). For each group of samples, those analyzes were performed in six bottles randomly chosen (twice in each one).

2.4. Determination of polyphenols by UV spectrophotometry

Total polyphenols (TP) and total hydroxycinnamates (THC) were quantified by measuring the absorbance at 280 and 320 nm (Shimadzu UV-1700 spectrophotometer), respectively. TP were expressed as mg/L of catechin and THC as mg/L of caffeic acid. The total flavonoids (TF) were calculated using the following formula, as described by Iland, Ewar, Sitters, Markides, and Bruer (2000), and expressed in mg/L of catechin. $TF = [(A_{280} - 4) - 0.66] \times (A_{320} - 1.4)$.

To determine the total amount of oligomeric procyanidins (OPC), first an acid hydrolysis was performed and then the absorbance at 520 nm was measured in a spectrophotometer (Fukui & Nakahara, 2006). The results were expressed in mg/L of OPC. For each group of samples, those analyzes were performed in six bottles randomly chosen (twice in each one).

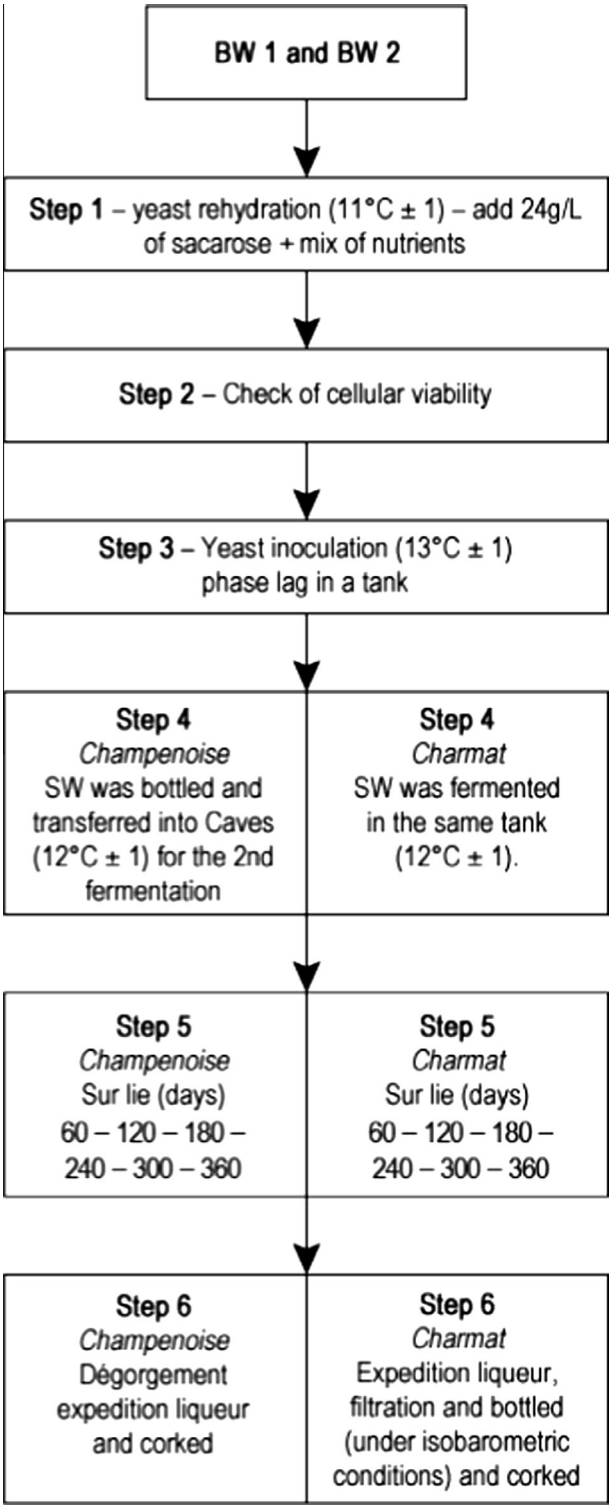

Fig. 1. Schema simplified of sparkling wine production at industrial scale.

2.5. Evaluation of antioxidant activity in vitro

The scavenging capacity of the free radical DPPH• was measured adding a Tris–HCl buffer solution (100 mM, pH 7.0) containing

250 μM of DPPH• dissolved in ethanol to the SW pure or diluted in distilled water [0.1; 1.0; 10 and 50% (v/v)]. In the control tube, sterilized distilled water was used instead of SW. The tubes were kept in the dark for 20 min and the absorbance was measured at 517 nm (Shimadzu UV-1700 spectrophotometer) (Yamaguchi, Takamura, Matoba, & Terao, 1998). The results were expressed in values of IC_{50} (SW needed to reduce 50% of DPPH• free radical), calculated by polynomial regression graphs (Mensor, Menezes, Leitão, Reis, Dos Santos, & Coube, 2001), using the average of triplicates. For each group of samples, those analyzes were performed in six bottles randomly chosen (three times in each one).

2.6. Evaluation of enzymatic activity in vitro

The β-Glucosidase assay was based on the procedures described by (Riou, Salmon, Vallier, Günata, & Barre, 1998) and Dhake and Patil (2005) by mixing 0.1 mL of 5 mM P-nitrophenyl β-D-glucopyranoside (pNPG) and 0.4 mL of 0.1 M sodium acetate buffer at pH 5.5. After incubation for 10 min at 50 °C, the reaction was interrupted by adding 2 mL of 1 M sodium carbonate, and the *p*-nitro phenol released was monitored at 420 nm. One unit of β-Glucosidase activity corresponds to the release of 1 μmol of *p*-nitro phenol/min under the assay conditions. For each group of samples, those analyzes were performed in six bottles randomly chosen (three times in each one).

2.7. Determination of polyphenols by HPLC

Determination of tyrosol, caffeic acid and ferulic acid. The determination of phenolic acids was made in HPLC (Agilent Technologies 1100 Series) with UV detector according to the methodology adapted from Roggero and Archier (1989). The column used was a Zorbax SB-C18 (5a, 4.6 × 250 mm) from Agilent Technologies. The mobile phases were composed of pure water/acetic acid (95:5 v/v) and acetic acid/Milli-Q water/ethanol (5:65:30). The detections were performed in wavelengths of 280 nm for tyrosol, and 313 nm for caffeic and ferulic acids. The samples were filtered with a membrane of 0.2 μm. The injection volume was 20 μL, the column was maintained at 25 °C and the analysis flow was 0.8 mL/min.

Determination of resveratrol and piceid. The determination of resveratrol and piceid was made in HPLC (Agilent Technologies 1100 Series) with DAD detector (diode array detector) according to the methodology adapted from McMurtrey, Minn, Pobanz, and Schultz (1994). The column used was a Zorbax SB-C18 (5a, 4.6 × 250 mm) from Agilent Technologies preceded by a guard column LiChrospher 100RP-18 (5 mm, 4 mm × 4 mm). The mobile phase consisted of water Milli-Q/acetonitrile (50:50 v/v) pH 3, adjusted with orthophosphoric acid and filtered through membrane of 0.45 μm. The detection was performed in wavelength of 254 at 316 nm for resveratrol and piceid. The samples were filtered with a membrane of 0.2 μm. The injection volume was 20 mL, the column was maintained at 25 °C and the analysis flow was 0.8 mL/min.

Determination of gallic acid. The determination of gallic acid was made in HPLC (Agilent Technologies 1100 Series) with DAD detector according to the methodology adapted from Lamuela-Raventós and Waterhouse (1994). The column used was a Zorbax SB-C18 (5a, 4.6 × 250 mm) from Agilent Technologies preceded by a guard column Zorbax 300SB-C18 (5 mm, 12 mm × 4.6 mm). The mobile phases were composed of: Solvent A: $NH_4H_2PO_4$ solution of 50 mmol/L pH 2.6, adjusted with orthophosphoric acid; Solvent B: acetonitrile/solvent A (80:20 v/v); Solvent C: orthophosphoric acid solution 0.2 mol/L pH 1.5, adjusted with ammonium hydroxide. The detection was performed at a wavelength of 204 nm. The samples were filtered with a membrane of 0.2 μm. The injection volume was 5 μL, the column was maintained at 25 °C and the analysis flow was 0.5 mL/min. For each group of samples, those analyzes were performed in six bottles randomly chosen (three times in each one).

3. Results and discussion

3.1. Enological analysis

Total acidity of the SW varied from 4.1–7.33 g/L of tartaric acid. The average levels of pressure, volatile acidity, and pH were 5.6 ± 0.2 atm, 0.41 ± 0.01 g/L of acetic acid and 3.50 ± 0.03, respectively. The average concentrations of free SO_2 were 22.50 ± 0.58 mg/L and of total SO_2 were 95.67 ± 6.08 mg/L. Analysis of the dry extract and reduced dry extract showed, respectively, values (expressed in g/L) of 22.70 ± 0.50 and 17.23 ± 0.15 for CHC, 21.90 ± 3.67 and 16.33 ± 0.38 for CTA and 27.10 ± 0.70 and 18.53 ± 0.06 for CHA. The increase in the concentration of glucose and alcohol in the SW in relation with its BW is a natural consequence of the second fermentation; the small variations in the analysis results over time were not significant and both cases occurred independently of the elaboration method (data not shown). These results show that the grapes were healthy, appropriate vinification practices were used and the values are in the average of the contents normally found worldwide (Pozo-Bayón et al., 2009; Torrens et al., 2010). The presence of L-ascorbic acid into SW and its relationship with many factors such as yeast metabolism, offer of sunlight on the wine, grape variety and maturation degree reported by colleagues were discussed by our group (Stefenon et al., 2010a). In this study the results obtained were similar and the levels of this compound had no significant differences in all SW analysed (data not shown). However, our results suggest that the Chardonnay variety can have more vitamin C than Pinot Noir and Italic Riesling, because BW1 showed 82.76% more L-ascorbic acid than BW2.This grape variety is used in SW production around the world and is considered as responsible for the structure and pleasant citrus aromas that can be found in them (Buxaderas & López-Tamames, 2012; D'Incecco et al., 2004; Sánchez, Díaz-Maroto Hidalgo, González-Viñas, and Pérez-Coello, 2005). Moreover, taking into account that this acid is strongly reactive, we can suggest that the presence of this compound has an important role in the maintenance of aromatic characteristics in these products, due to the changes in the production of higher alcohols and esters by *S. cerevisiae* (Valero et al., 2002).

3.2. Determination of polyphenols by UV spectrophotometry

Table 1 shows a decrease in the levels of TP and TF in both methods, while THC content remained without significant differences. The oxidation reactions taking place in the first steps of the process have strong affinity by small molecules such as the THC, while larger molecules tend to react along the time (Bosch-Fusté et al., 2009; Pozo-Bayón et al., 2009; Stefenon et al., 2010a). The content of OPC shows an increase into CHA and CTA samples, whereas on the CHC, no significant differences were found. This is probably due to the red grape employed, because it is rich in phenolic compounds (Stefenon et al., 2010a). Then, regarding generic phenolic groups, we can assume that the ageing on lees and grape variety were variables with more influence than the production method. In addition, a negative correlation was observed between TP and OPC ($R = -0.687$; $p = 0.01$) as well as between TF and OPC ($R = -710$; $p = 0.01$) only for the *Assemblage* SW (both CHA and CTA). Pozo-Bayón et al., 2009 reported many factors involved in the chemical composition of SW, such as: grape variety, vineyard yield, quality of the base wine and yeast strain for

Table 1
Levels of β-Glucosidase (β-G), DPPH•, total polyphenols (TP), total flavonoids (TF), total hydroxycinnamates (THC) and oligomeric procyanidins (OPC) in SW in different periods of *sur lie* (ageing on lees).

SW	*sur lie* (d)	TP[A] (mg/L)	TF (mg/L)	THC (mg/L)	OPC (mg/L)	DPPH•[B]	β-G (μmol/min)
CHC[C]	Base wine 1	539.96[a,F] ± 8.12	373.80[a] ± 10.01	27.63 ± 2.11	8.80 ± 1.09	47.36[a] ± 2.97	3.58×10^{-5} ± 0.03
	0[G]	467.07[b] ± 23.99	165.62[b] ± 3.90	28.23 ± 4.03	7.92 ± 1.53	45.07[a] ± 1.21	3.64×10^{-5} ± 0.04
	60	463.98[b] ± 16.64	162.88[b] ± 20.84	25.06 ± 1.53	7.08 ± 1.49	51.49[b] ± 3.16	3.66×10^{-5} ± 0.05
	120	452.81[b] ± 12.17	155.31[b] ± 20.70	24.30 ± 1.33	7.03 ± 1.53	76.24[c] ± 1.19	3.65×10^{-5} ± 0.01
	180	450.30[b] ± 8.50	121.06[b] ± 24.23	23.60 ± 0.99	7.23 ± 1.13	84.32[d] ± 1.22	3.60×10^{-5} ± 0.03
	240	451.80[b] ± 3.79	123.84[b] ± 6.24	23.83 ± 0.87	7.08 ± 1.49	84.63[d] ± 2.24	3.57×10^{-5} ± 0.05
	300	451.46[b] ± 5.93	121.11[b] ± 18.18	23.40 ± 0.81	7.27 ± 1.10	87.48[d] ± 0.71	3.62×10^{-5} ± 0.04
	360	468.42[b] ± 12.64	124.95[b] ± 7.92	23.86 ± 3.42	7.20 ± 1.98	90.51[e] ± 1.97	3.68×10^{-5} ± 0.03
CHA[D]	Base wine 2	503.61[a] ± 1.65	377.30[a] ± 12.10	27.96 ± 2.11	7.20[a] ± 0.25	67.68[a] ± 0.73	2.80×10^{-5} ± 0.01
	0	496.11[b] ± 3.49	293.72[b] ± 14.67	30.00 ± 0.57	8.96[b] ± 0.58	72.91[b] ± 0.75	2.89×10^{-5} ± 0.02
	60	497.50[b] ± 2.99	293.99[b] ± 9.89	30.33 ± 1.18	9.20[b] ± 0.69	74.5[b] ± 1.41	2.80×10^{-5} ± 0.07
	120	498.46[b] ± 1.71	300.67[b] ± 21.82	30.03 ± 0.75	11.06[b] ± 2.94	74.97[b] ± 0.80	2.81×10^{-5} ± 0.03
	180	492.47[b] ± 1.16	292.47[b] ± 26.76	29.60 ± 0.55	13.00[b] ± 4.36	77.33[c] ± 1.50	2.73×10^{-5} ± 0.10
	240	496.94[b] ± 2.19	296.32[b] ± 19.28	29.96 ± 1.04	14.23[b] ± 1.33	78.58[c] ± 0.39	2.72×10^{-5} ± 0.07
	300	495.23[b] ± 0.55	294.25[b] ± 12.79	29.23 ± 0.61	16.33[b] ± 3.21	79.96[c] ± 1.20	2.72×10^{-5} ± 0.08
	360	498.06[b] ± 2.03	298.94[b] ± 20.20	30.23 ± 2.51	16.40[b] ± 3.29	83.12[d] ± 1.15	2.80×10^{-5} ± 0.02
CTA[E]	Base wine 2	503.61[a] ± 1.65	377.30[a] ± 12.10	27.96 ± 2.11	7.20[a] ± 0.25	67.68[a] ± 0.73	2.80×10^{-5} ± 0.01
	0	482.42[b] ± 4.43	283.14[b] ± 19.13	29.36 ± 1.15	8.76[a] ± 0.53	68.02[a] ± 1.04	2.81×10^{-5} ± 0.02
	60	471.80[b] ± 2.12	248.47[b] ± 14.28	28.73 ± 1.39	11.70[b] ± 5.02	69.06[a] ± 0.36	2.85×10^{-5} ± 0.07
	120	472.64[b] ± 5.05	216.69[b] ± 10.49	28.83 ± 0.46	13.56[b] ± 4.41	72.29[b] ± 0.99	2.78×10^{-5} ± 0.03
	180	451.41[b] ± 6.18	166.85[b] ± 9.51	27.20 ± 0.21	13.00[b] ± 4.36	72.91[b] ± 0.98	2.65×10^{-5} ± 0.01
	240	457.28[b] ± 2.58	166.18[b] ± 16.15	27.56 ± 0.51	15.07[b] ± 2.40	74.97[c] ± 1.27	2.79×10^{-5} ± 0.07
	300	454.15[b] ± 7.56	163.67[b] ± 10.59	26.66 ± 0.86	17.67[b] ± 1.10	77.10[d] ± 0.60	2.75×10^{-5} ± 0.02
	360	444.15[b] ± 3.96	148.85[b] ± 16.40	27.66 ± 0.60	17.12[b] ± 1.59	77.95[d] ± 0.70	2.73×10^{-5} ± 0.20

[A] Data are mean ± SD (Standard Deviation) values of three independent experiments, to all parameters.
[B] (SW needed to reduce 50% of the free radical DPPH•).
[C] *Champenoise* Chardonnay.
[D] *Champenoise Assemblage.*
[E] *Charmat Assemblage*.
[F] Data followed by different letters for each column differ significantly according to analysis of variance and Tukey's *post hoc* test ($p \leqslant 0.05$) for each parameter evaluated and for each method of SW.
[G] After 6 h of the yeast inoculation.

second fermentation; they agree that the second fermentation and the ageing on lees are the key factors used to explain the quality since both events are involved in the distinctive character of each SW.

3.3. Evaluation of antioxidant activity in vitro

Beyond the general quality of the SW, another points of view are the beneficial effects of these compounds in the human health (Gallardo-Chacón et al., 2010; Stefenon et al., 2010b; Vauzour et al., 2010).It is relevant to remember that the pharmacological, medicinal and biochemical properties of polyphenols were extensively studied in recent reviews (Leopoldini et al., 2011; Rodrigo et al., 2011). However, to the best of our knowledge, this is the first comparison between the generic phenolic groups profile related with the methods *Charmat* and *Champenoise* in controlled samples. Table 1 show an increase on IC_{50} values along the time, i.e., the older the SW is, the lower the antioxidant activity will be. Our results show a greater influence of the ageing over the *Champenoise* than over *Charmat* ones, because the loss of this capacity was 91.12% to CHC, 22.81% to CHA and 15.17% to CTA sparkling wines. Nevertheless, when young, CHC was more antioxidant than the others at the same point of the *sur lie*, around the 120 days. But in the middle of the ageing period studied, this SW was less effective than the *Assemblage* SW in both production methods. In accordance with what was discussed above, these results can be linked with the higher content in ascorbic acid into CHC due to the presence of Pinot Noir grapes into CHA and CTA samples. These responses are modulated by many factors and Table 2 shows the correlations (negative or positive) between some important variables and the antioxidant activity of SW. Many phenols can be grouped into TP, TF, THC and OPC (Iland et al., 2000) and it becomes difficult to assess how and which changes occur in each method. In an attempt to clarify some of these aspects, we discuss below about some important polyphenols, as the resveratrol, to improve the management of SW production.

Table 2
Correlation of Pearson between the IC_{50} values for the different sparkling wines analysed (SW needed to reduce 50% of the DPPH• formation) and levels of some parameters.

Parameters[a]	Sparkling wines antioxidant activity (IC_{50})					
	(IC_{50}) to CHC		(IC_{50}) to CHA		(IC_{50}) to CTA	
	R	*p*	*R*	*p*	*R*	*p*
Caffeic acid	x	x	−0.698	0.01	x	x
Ferulic acid	x	x	−0.690	0.01	−0.645	0.01
Gallic acid	0.810	0.01	0.848	0.01	0.532	0.01
Glucose	0.517	0.01	0.674	0.01		
OPC	x	x	0.758	0.01	0.816	0.01
Piceid	−0.458	0.05	x	x	x	x
Resveratrol	−0.904	0.01	−0.767	0.01	−0.587	0.01
TF	−0.676	0.01	−0.894	0.01	−0.378	0.05
THC	−0.643	0.01	x	x	x	x
TP	−0.576	0.01	−0.824	0.01	x	x
Tyrosol	−0.461	0.05	x	x	x	x
Ascorbic acid	−0.576	0.01	−0.392	0.01	−0.331	0.01
β-G	x	x	x	x	−0.389	0.05

[a] TP = total polyphenols; TF = total flavonoids; THC = hydroxycinnamates; OPC = oligomeric procyanidins; β-G = β-Glucosidase; CHC = *Champenoise* Chardonnay; CHA = *Champenoise Assemblage*; CTA = *Charmat Assemblage*.

3.4. Determination of enzymatic activity in vitro

Table 1 shows for the first time the levels of the β-Glucosidase in SW during the ageing on lees in both production methods for a

period of up to 360 days. Earlier studies by our group showed similar data in commercial samples of SW acquired in supermarkets and wine stores (Stefenon et al., 2010b). Yeast autolysis represents an enzymatic self-degradation of cell components that begins at the end of the stationary growth phase of alcoholic fermentation and is associated with cell death, resulting in the release of cellular components into the wine and their interaction with the wine constituents (Buxaderas & López-Tamames, 2012). The yeast cell wall can also act as an absorptive surface agent, but the β-Glucosidase activity seems to have not been influenced by these aspects, because no changes from the base wine until the end of the second fermentation were verified (data not shown) and the levels remained unchanged over time both in *Champenoise* and in *Charmat* ones. Since the β-Glucosidase integrates the pool of yeast enzymes (Hernández et al., 2003), the demonstration that it remains active during the ageing on lees opens new research possibilities and other experiments are being conducted by our group on this subject. Hence, during the *sur lie*, the method used seems to be less important than the employed varieties, because the CHC showed 28.6% more β-Glucosidase activity than CHA and CTA. This characteristic can be related, at least partially, to the high acceptance of products produced with chardonnay grapes (Buxaderas & López-Tamames, 2012), because this enzyme is linked with an aromatic profile and can explain the changes occurred in them over time (D'Incecco et al., 2004; Sánchez et al., 2005).

3.5. Determination of polyphenols by HPLC

In this study, we investigated the connection between the β-Glucosidase activity with the possible changes on the phenolic profile and its relationship with the antioxidant potential of SW, especially about the balance of resveratrol and piceid levels. Furthermore, SW contains relatively high concentrations of phenolic acids and phenolic alcohols (D'Incecco et al., 2004; Vauzour et al., 2010). The beneficial effects of the caffeic acid and tyrosol in the human vascular system and in the neuroprotective capacity, as well as the therapeutic use of ferulic and gallic acids against oxidative stress and its complications (asthma, coronary diseases, diabetes, e.g.) have been investigated (Leopoldini et al., 2011; Rodrigo et al., 2011; Vauzour et al., 2010). However, assessing the role of these compounds on the biochemical and sensorial profile of the SW in order to offer, at the same time, products of high quality and with bioactive useful to maintain of human health is still necessary.

3.5.1. Determination of resveratrol

The first research was about the levels of resveratrol and its glycosylated derivative during the *sur lie*. CHC (Fig. 2a) shows a decrease on piceid and an increase in resveratrol contents probably due to the β-Glucosidase activity, which was larger in these samples. The positive correlation observed between this enzymatic property and the level of this phenolic compound ($R = 0.412$, $p = 0.05$) for this group of samples corroborate this hypothesis. For the CHA and CTA samples (Fig. 2b and c), the effect of glucose concentration (10.41 g/L ± 0.58) is important too, because in these samples, the content of glucose was on average 45% higher than in CHC (6.88 g/L ± 0.65) and constant levels of piceid and a decrease on the resveratrol concentration were observed. In the first case, the stability can be explained by the occurrence of the reverse reaction, when an aglycone is released it returns to the form of its glucosylated derivative as described by Medina et al., 2010. The negative correlation observed between the glucose and resveratrol levels ($R = -0.454$, $p = 0.05$) reinforce this idea. The reduction in resveratrol contents may be due to photoisomerization and other enzymatic reactions, such as those mediated by phenoloxidases present in the medium or in which cofactors (e.g. metals) are involved in the formation of derivatives previously identified in wine (Prokop et al., 2006; Stefenon et al., 2012). Furthermore, the concentration of both compounds mediated by the presence of β-Glucosidase can have a strong influence in the antioxidant activity as demonstrated by the clear relation between them (Table 2).

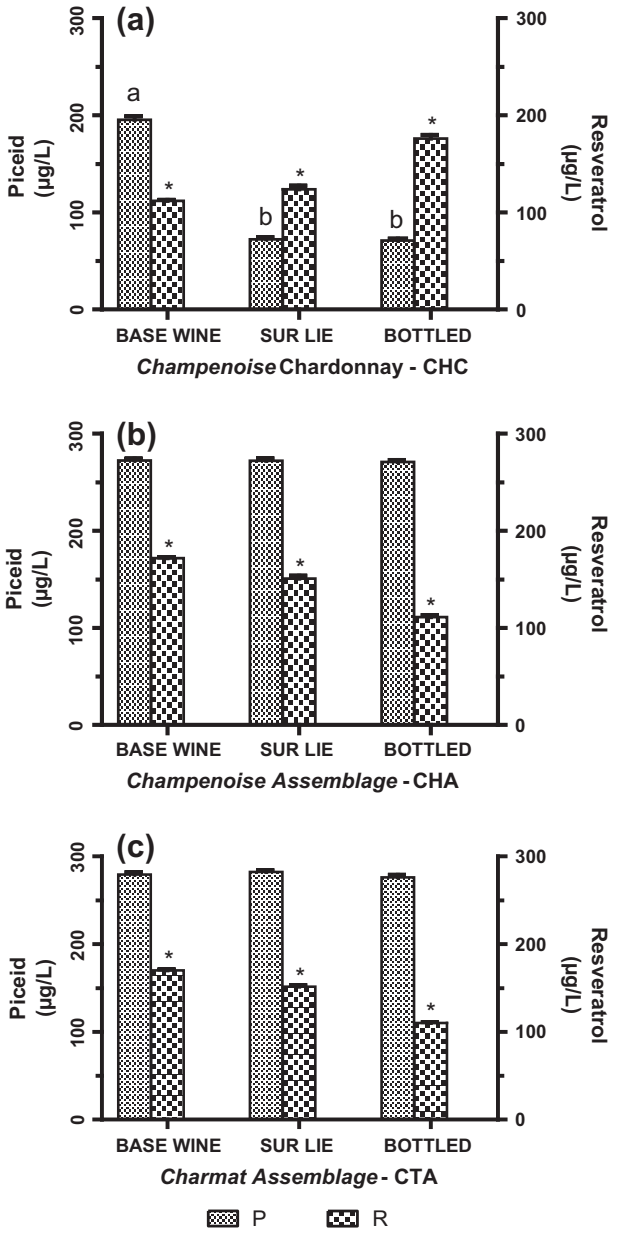

Fig. 2. Comparative levels of piceid (*P*) and resveratrol (*R*) during the *sur lie* to CHC (a), CHA (b) and CTA (c). Data followed by distinct letters or symbols differ significantly according to analysis of variance and Tukey's *post hoc* test ($p \leqslant 0.05$) for each parameter evaluated and for each method of SW.

3.5.2. Determination of tyrosol

The tyrosol is a compound commonly found in chardonnay grapes (D'Incecco et al., 2004) and in the CHC samples the content was initially high (Fig. 3a). Our data were similar to those found in *Champagne* samples (Vauzour et al., 2010). Regarding the *Champenoise* method (varietal/CHC or *assemblage*/CHA) we can consider the level of tyrosol to be constant, because at the end of the ageing period studied, the level is similar to the one of the two analysed blocks. And the slight increase observed in CHC samples until

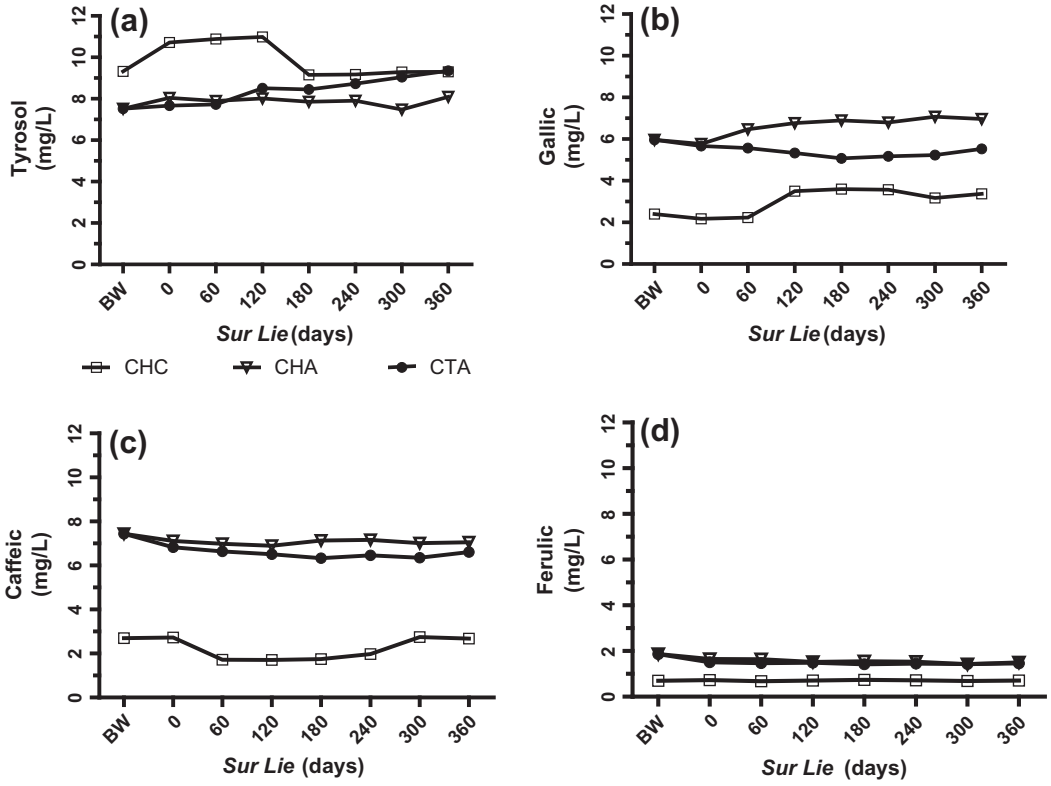

Fig. 3. Evolution of phenol alcohol (a) and phenol acids (b–d) during the *sur lie* to *Champenoise* Chardonnay SW (CHC), *Champenoise Assemblage* SW (CHA) and *Charmat Assemblage* SW (CTA).

120 days can explain the larger antioxidant activity in these SW. The influence of tyrosol over the IC_{50} is clear (Table 2). Regarding the *Charmat* samples, a gradual increase was observed. As it is known, the complex array of aroma and flavour found in SW is largely originated from the grapes, yeast metabolism during the alcoholic fermentations and the ageing on lees (D'Incecco et al., 2004; Torrens et al., 2010). In this case, the most important variable seems to be the elaboration method, because the correlation between the tyrosol content and *sur lie* was opposite: *Charmat* ($R = 0.917$, $p = 0.01$) and *Champenoise* ($R = -0.519$, $p = 0.01$). Since tyrosol is formed from tyrosine by a sequence of three reactions (deamination, decarboxylation and reduction), the reductive ambient in a larger volume (*Charmat* process) can explain this assumption. Furthermore, as discussed previously, the β-Glucosidases, in the presence of glucose on a rich medium, as the wine, are able to modulate the response of many compounds, such as, the transference of the glucose molecule to the tyrosol to form salidroside. On the other hand, salidroside may be degraded into tyrosol and glucose (Ling-Ling, Zhu, Petrovic, & Gonsalves, 2007). More studies will be performed to corroborate this hypothesis, because to our knowledge, the salidroside in wines has not been demonstrated until now.

3.5.3. Determination of gallic acid

The contents of gallic acid (Fig. 3b) into CHC and CHA samples showed a tendency to increase during the *sur lie*. This possibility can be related with the enzymes released during yeast autolysis that could be involved in the hydrolysis of tannins polymers (Pozo-Bayón et al., 2009). This result is reinforced by the positive correlation observed between the *sur lie* and gallic acid (CHC: $R = 0.659$, $p = 0.01$; CHA: $R = 0.603$, $p = 0.01$).The content was similar to the one observed in *Cavas* and white wines (Bosch-Fusté et al., 2009; Esteruelas et al., 2011), and higher than in *Champagnes* (Vauzour et al., 2010). On the contrary, the gallic acid curve at ageing on lees in CTA samples shows a tendency to decrease, although the level has remained in an average range in comparison to the other analysed groups. Since gallic acid is a monomer of the tannins, in the *Charmat* process the OPC hydrolysis can be hindered due to the fact that surface contact between the wine and the lees is smaller. Positive correlation between OPC and gallic acid was observed only in this type of SW (CTA: $R = 0.484$, $p = 0.01$).The differences observed on the gallic acid curves are linked with the response of the antioxidant capacity assay (Table 2).

3.5.4. Determination of caffeic and ferulic acid

Higher levels of caffeic acid (Fig. 3c) were obtained in CHA and CTA samples, indicating a strong influence of the varieties in the concentration of this phenolic compound. Our data is higher than what was observed in *Cavas* (Bosch-Fusté et al., 2009), but similar to other white wines (Esteruelas et al., 2011). The presence of caffeic acid was observed in all samples and the curve during the *sur lie* was similar and constant for the three analysed groups. This aspect is very important, because the browning increase is due to the formation of brown macromolecules coming from the polymerisation of phenols; the decrease in the main hydroxycinnamic acids present in SW is also related with these reactions and can affect the overall quality (Bosch-Fusté et al., 2009). Moreover, the caffeic acid associate with proteins creates an initially soluble molecule, but with the growth, the complex becomes insoluble, generating turbidity into wines (Esteruelas et al., 2011). Additionally, the degree of insolubility is affected by the nature of the sugars present in the medium and in these samples, negative correlation between caffeic acid and glucose was observed (CHC: $R = -0.446$, $p = 0.05$; CHA: $R = -0.477$, $p = 0.05$; CTA: $R = -0.772$, $p = 0.01$). As this compound positively affects the antioxidant activity (Table 2), actions to promote the

balance between concentration and ageing of SW are very important. The same situation was found between ferulic acid and glucose contents (CHA: $R = -0.667$, $p = 0.01$; CTA: $R = -0.885$, $p = 0.01$). Regarding this compound (Fig. 3d), the varieties are also important, because their performance was similar to the one of caffeic acid, including the minimal decrease due to the precipitation linked to natural proteins (Esteruelas et al., 2011). Bosch-Fusté et al. (2009) studied the development of *Cava* sparkling wine during its ageing in contact with lees and the caffeic acid showed content similar to the one of *Champenoise* Chardonnay 100%, whereas the typical Brazilian *assemblage* (Chardonnay, Italic Riesling and Pinot Noir) showed higher concentration of this phenolic acid, in both methods. The *sur lie* should be accurately monitored because in those last mentioned samples, we found a negative correlation between ferulic acid and ageing on lees (CHA: $R = -0.525$, $p = 0.01$; CTA: $R = -0.636$, $p = 0.01$). Changes on the chemical structure of the phenols and the reactions over time may result in easily oxidizable derivatives (Leopoldini et al., 2011), such as the eugenol, which have carnation aroma and can depreciate the sensorial profile of SW.

Finally, the Principal Components of Analysis (PCA) show the *sur lie* as the first component extracted. This variable explains more than 40% of variance for all cases. Together with resveratrol, β-Glucosidase, caffeic acid and tyrosol, more than 80% of the variance was also explained (Fig. 4). This finding is remarkable, because it clearly shows that the ageing on lees is able to modulate many compounds in the medium. The samples used in this work were produced under controlled and equal conditions and the results found were similar to the ones observed in commercial SW previously studied by our group. This fact is important because the compounds discussed above have many enological and biological properties and this statement can result in an approximation of the scientific evidences and its innovations related with the industrial realities and markets demands. Hence, the target of winemakers worldwide is to certify the quality of the product to the consumer and to offer new technologies to improve the enological practices, aiming at the production of SW of increasingly high quality.

To summarise, this work has provided a comparison between the two principal production methods of sparkling wines. Primarily, it is known that *Champenoise* and *Charmat* have important differences, since in the first one, the long period of *sur lie* is associated with sensorial characteristics, such as: more structure, body, marked flavours and aromatic complexity. In *Charmat* ones, freshness and elegance with delicate aromas are usually found, and this is directly related with the short *sur lie*, but these aspects cannot determine the quality level. Yeast autolysis is a slow process that involves the interaction between components released by dead yeast cells and the wine and through this study we can conclude that the volume of wine in contact with the lees surface (bottle or tanks) can affect the sequential reactions involved in the whole process, since the compounds showed different curves to each method, such as the tyrosol and gallic acid ones.

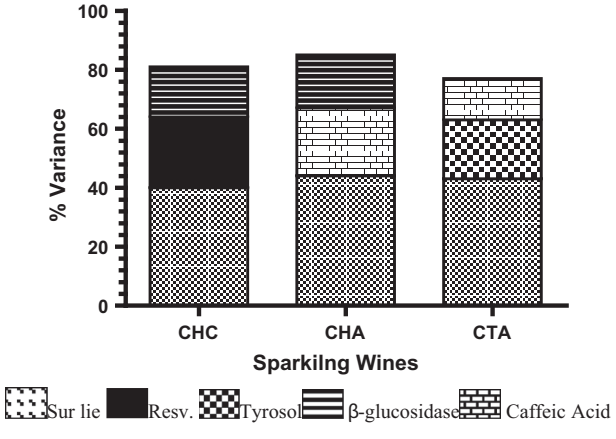

Fig. 4. Variance explained by principal components of analysis to *Champenoise* Chardonnay SW (CHC), *Champenoise Assemblage* SW (CHA) and *Charmat Assemblage* SW (CTA).

Secondly, the grapes are the matrices of the SW profile and we showed that the chardonnay grape has more β-Glucosidase activity than the *assemblage* used. The metabolism is triggered by enzymes and we proved that this activity not only exists into SW, but also that it remains unchanged while the ageing happens. Therefore, we can conclude that the β-Glucosidase activity is stable in the wine conditions. This is important because the reactions that involve this enzyme, the levels of resveratrol and piceid plus the glucose concentration, may be able to maintain or improve the SW antioxidant capacity. Besides, caffeic and ferulic acids play significant roles in this context and are also affected by the glucose levels in the medium, acting in this way on the overall quality of the SW.

Our results showed that the older the SW is, the smaller the antioxidant activity is too. As white and red wines can act against the oxidative stress in distinct ways, the choice for a short or long ageing on lees will determine the response of the SW, because the *sur lie* is able to modulate the necessary changes to achieve a specific objective. Therefore, we can conclude that the ageing on lees becomes more important than the production methods of SW due to, mainly, its close relationship with the phenolic profile.

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4. CONSIDERAÇÕES FINAIS E CONCLUSÕES

Durante a Idade Média, os vinhos de *Champagne* possuíam uma efervescência passageira devido à incompleta fermentação do suco de uva (Méheut & Griffe, 1997). O início de um processo mais metódico e preciso nasceu do trabalho desenvolvido por Pierre Pérignon, um abade de Hautvilles (de 1668 até sua morte em 1715). Se a efervescência era vista como um inconveniente no século XVI, no milênio seguinte, passou a representar um sinal de personalidade e elegância, apesar da turbidez causada pela presença das borras da fermentação secundária (Díaz de Mendívil *et al*., 1999). A primeira evidência de uma segunda fermentação surgiu por volta da metade do século XVIII, devido à adição de licor de tiragem (uma prática empírica usada para garantir a efervescência necessária; método *Champenoise*). A quantidade exata de açúcar adicionado para gerar uma pressão de gás particular passou a ser calculada no século XIX, quando Louis Pasteur esclareceu a origem das borbulhas (*perlage*). A partir disto, atribuí-se à Madame Veuve Clicquot a invenção dos pupitres, criados para permitir que as garrafas pudessem ser colocadas em vários ângulos até que as borras se depositassem próximo ao gargalo. Assim, com a descompressão na abertura da garrafa, a turbidez foi eliminada e o resultado foi a obtenção de um vinho espumante límpido e brilhante (Olavarrieta, 1995). Os desenvolvimentos científicos do século XX revolucionaram todos os campos do conhecimento, e a enologia não se constituiu em uma exceção. As inúmeras etapas envolvidas no processo vêm sendo estudadas uma a uma. Os procedimentos físicos (como por exemplo, o controle de temperatura), químicos (uso de aditivos) e microbiológicos (leveduras selecionadas, enzimas, etc.) formam uma gama

de opções para aumentar a qualidade e a competitividade do produto final. Uma das alternativas de maior impacto foi o surgimento do método *Charmat*, no qual o controle é contínuo e o vinho espumante pode ser filtrado e engarrafado sob pressão constante. As vantagens econômicas são óbvias. Por esta razão, tem sido cada vez mais usado e estudado em muitos países, incluindo França, Espanha e Itália (Buxaderas & López-Tamames, 2012). Dentro deste entendimento, o período de amadurecimento sobre as borras (*sur lie*) vem ganhando destaque ao longo dos últimos anos, sendo que através do método *Champenoise*, os períodos longos estão associados a uma maior complexidade aromática e corpo mais acentuado. Já no método *Charmat*, usualmente empregam-se os termos curto e longo para designar o tipo de *sur lie* adotado, quando normalmente buscam-se vinhos espumantes mais frescos e jovens (Alexandre & Guilloux-Benatier, 2006). Uma vez que a autólise da levedura é uma auto-degradação enzimática dos componentes celulares (especialmente a hidrólise dos glucanos em manoproteínas e liberação de compostos aromáticos) que se inicia no final da fermentação e está associada à morte celular (Charpentier, 2010), a liberação dos compostos pode variar de acordo com diversos fatores, como por exemplo, a estirpe de levedura utilizada, temperatura de fermentação, graduação alcoólica, pressão de CO_2 e duração do *sur lie* (Gallardo-Chacón *et al*., 2010). Os constituíntes do vinho, a lise celular e o tempo de *sur lie* são considerados os principais fatores que regulam as características sensoriais dos vinhos espumantes (Moreno-Arribas & Polo, 2009; Pozo-Bayón et al, 2009), embora ainda existam lacunas significativas em relação às demais interações com outras aspectos importantes, como por exemplo, a capacidade antioxidante apresentada por este tipo de produto. As conclusões obtidas a partir destas considerações e através dos resultados desta pesquisa estão apresentadas no Quadro 1.

Quadro 1. Avaliação de parâmetros enológicos, sensoriais e biológicos em vinhos espumantes (VE): conclusões pontuais.

Parâmetro Estudado	**Conclusões**	**Variáveis com maior influência**		
		Método de Elaboração	***Sur Lie***	**Outras**
Análises Enológicas	Os VE brasileiros possuem nível de qualidade internacional (padrões analíticos dentro dos limites legais). Capítulos 1, 2, 3 e 4*	-	-	-
Ácido Ascórbico	A concentração deste ácido depende do vinho base e aumenta após a segunda fermentação. Capítulos 1 e 4*	-	X	Variedade de uva
Compostos Fenólicos	Primeiro estudo a avaliar estes compostos em VE *Charmat* e a comparar os dados com os obtidos nos *Champenoise.* Capítulo 1*	X	X	Caso a caso
Polifenóis Totais, Flavonóides Totais e Hidroxicinamatos Totais	A concentração destas três classes fenólicas em VE pode variar, principalmente, em função do vinho base, da classe e do tipo de VE. Capítulos 1, 2, 3 e 4*	X	X	Variedade de uva Teor de açúcar
Oligômeros de Procianidinas (OPC)	A concentração destes polifenóis aumenta após a segunda fermentação, especialmente quando há participação de variedades tintas. Capítulo 4*	-	X	Variedade de uva
Ácido Gálico	A concentração deste ácido aumenta após a segunda fermentação, em função da classe e do tipo de VE avaliado (*Champenoise* apresentam maior concentração). Capítulos 1 e 4*	X	-	Teor de açúcar OPC β-glicosidase
(+)-catequina	Este monômero apresentou comportamento variável dentro do desenho experimental, para o grupo amostrado. Capítulo 1*	X	X	Diversos fatores
(-)-epicatequina	Ocorre aumento no teor deste composto em relação ao respectivo vinho base, exceto para os VE *demi-sec*. Capítulo 1*	-	-	Teor de açúcar
trans-resveratrol *trans*-piceid	Os níveis de ambos os compostos variam de acordo coma as técnicas enológicas aplicadas e são claramente afetados pelo teor de açúcar no meio e pela atividade enzimática. Capítulos 1, 2 e 4*	X	X	Clarificantes β-glicosidase Teor de açúcar

Tirosol	VE *Charmat* apresentam maior concentração deste álcool fenólico, o qual está relacionado com o aumento na atividade antioxidante. Capítulo 4*	X	X	Variedade de uva
Ácido Caféico e Ferúlico	Os níveis destes polifenóis foram semelhantes e mantiveram-se constantes nos três grupos analisados. Capítulo 4*	-	X	Variedade de uva
Atividade Antioxidante	Primeiro estudo a avaliar estes compostos em VE *Charmat* e a comparar os dados com os obtidos nos *Champenoise.* Capítulo 1, 2, 3 e 4 *	X	X	Caso a caso
Atividade Antioxidante *in vitro* ($DPPH^{\bullet}$)	Esta atividade foi maior quanto menor foi o *sur lie*. Capítulos 1 e 4*	-	X	Variedade de uva, Polifenóis Teor de açúcar
Atividade Antioxidante *in vitro* (SOD-like, CAT-like)	Foi possível estabelecer uma relação entre as atividades CAT-like e os níveis de zinco e manganês, bem como, de SOD-like com os teores de rubídio e hidroxicinamatos. Não houve influência da concentração de açúcar sobre este parâmetro. Capítulos 2 e 3*	X	X	Polifenóis Minerais
Atividade Antioxidante *in vivo* (*S. cerevisiae*)	Todas as amostras foram capazes de proteger as células dos danos causados pelo agente estressor; o resultado e o comportamento foram os mesmos verificados nos testes *in vitro*. Capítulo 1*	-	X	Variedade de uva, Polifenóis Teor de açúcar
Atividade Enzimática (β-glicosidase)	O vinho base pré-determina os níveis desta atividade. Capítulos 2 e 4*	-	X	Teor de açúcar Variedade de uva
Composição Mineral	VE brasileiros, *brut*, apresentaram, em geral, teores superiores de minerais em relação aos *demi-sec* e aos produtos elaborados na Espanha e França (também *brut*); Capítulo 3*	X	X	*Terroir* Clarificantes Teor de açúcar
Análise Sensorial	A presença de CO_2 (espuma/*perlage*) atua positivamente sobre a atividade CAT-like; os 3 principais aspectos sensoriais (visual, olfativo e gustativo) são diretamente influenciados pelo tipo e classe de VE. Capítulo 3*	X	X	Minerais Teor de açúcar

* Capítulo no qual se encontra a discussão pertinente a cada parâmetro.

As avaliações que geraram esta série de conclusões permitiram responder às questões que formaram os objetivos desta Tese:

1. Vinhos espumantes, *Charmat* e *Champenoise*, *Brut* e *Demi-sec*, apresentam importante atividade antioxidante.
2. Foram observadas significativas correlações entre a composição mineral (em especial Rubídio, Zinco, Cromo e Magnésio) e os polifenóis presentes em vinhos espumantes com a atividade antioxidante exercida pelos mesmos.
3. É o *sur lie* (tempo de amadurecimento sobre as borras) e não o método de elaboração *(Charmat* ou *Champenoise*) que determina a variação na atividade biológica, principalmente, devido a sua maior influência sobre os teores dos compostos relacionados com a atividade antioxidante observada em vinhos espumantes.
4. A presença de açúcar pode interferir na capacidade antioxidante, aumentando-a ou diminuído-a, em função da presença de atividade β-glicosidásica em vinhos espumantes, a qual permanece constante durante o período de *sur lie* e, portanto, está relacionada com as reações bioquímicas envolvidas no processo.
5. Os padrões de identidade e qualidade dos vinhos espumantes analisados atendem à legislação nacional vigente e se equiparam ao perfil dos demais produtos comercializados mundialmente.

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ANEXOS

Além dos trabalhos apresentados na Tese, durante o período do doutorado, outros estudos foram publicados, oriundos da fase de mestrado e da parceria com outros pesquisadores.

Anexo I - **Antioxidant activity of sparkling wines produced by *Champenoise* and *Charmat* methods -** foi publicado na revista *Food Chemistry* e teve como objetivo principal avaliar a atividade antioxidante de vinhos espumantes brasileiros elaborados pelos métodos *Charmat* e *Champenoise*, levando-se em consideração a influência da concentração de açúcar sobre esta atividade. As amostras foram provenientes de conceituadas vinícolas da Serra Gaúcha, no Rio Grande do Sul, estado responsável por mais de 90% da produção deste tipo de vinho. As principais características enológicas, a composição fenólica e o teor de ácido ascórbico também foram discutidos.

Anexo II - **Elemental characterization of cabernet sauvignon wines using particle-induced X-ray emission (PIXE)** - foi publicado na revista *Food Chemistry* e teve como objetivo o uso da técnica de PIXE para caracterizar vinhos Cabernet Sauvignon do Vale dos Vinhedos na Serra Gaúcha/RS, identificando a variabilidade na concentração de metais e comparando estes resultados com os de outras regiões do estado.

O Anexo III - **Determinação de compostos fenólicos em bioativos de uva *Vitis vinifera* utilizados na indústria cosmética e avaliação de sua atividade antioxidante** – foi publicado na revista *Cosmetics & Toiletries* e teve como objetivo determinar e quantificar compostos fenólicos presentes no extrato e no óleo de semente de uva, no leite de uva e no extrato de vinho, bem como avaliar a atividade antioxidante destas matérias-primas.

ANEXO I

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Antioxidant activity of sparkling wines produced by *Champenoise* and *Charmat* methods

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ABSTRACT

The objective of this study was to evaluate the antioxidant activity of 19 Brazilian sparkling wines produced by *Champenoise* and *Charmat* methods. All sparkling wines tested showed significant antioxidant activity, both *in vivo* and *in vitro* assays. In general, the *Charmat brut* possessed more antioxidant activity than *Charmat demi-sec* and *Champenoise* samples. In most of the sparkling wines studied, the majority compound found was gallic acid, although *trans*-resveratrol, (+)-catechin, (−)-epicathechin and procyanidins B_1, B_2, B_3 and B_4, were also identified. Significant differences were observed in the concentrations of these compounds, when considering the *assemblage* used and the production methods.

The wine industry around the world uses similar oenological technologies and the wines are divided into categories, for example, in relation to sugar concentration or elaboration methods. The findings of this study would help the wineries to determine the sugar contents and time to mature (*sur lie*) appropriate for sensorial characteristics desired by the winemakers and consumers. Furthermore, the data can offer an improvement in the biological properties of the sparkling wines.

1. Introduction

The process of making sparkling wines begins by obtaining the base wine from white grapes (*blanc de blancs*) or from white and red grapes (*blanc de noirs*). White base wines are obtained when fermentation takes place without contact between the must and the grape skins. The wines obtained will be red or *rosés*, depending on the time and intensity of this maceration (Hidalgo et al., 2004). The base wine is then submitted to a second fermentation, in order to produce carbon dioxide naturally by *Charmat* (in large containers) or *Champenoise* methods (in the bottle). Sparkling wines may be varietals (a single grape) or *assemblage*/*coupage* (two or more varieties and vintages) (Ribéreau-Gayón, Glories, Maujean, & Dubourdieu, 2003). The second fermentation can be followed by ageing of the wine with yeasts and the sparkling wines may be classified according to sugar content.

Sparkling wines are rich in phenolic compounds (Chamkha, Cathala, Cheynier, & Douillard, 2003; Ibern-Gómes et al., 2000; Pozo-Bayón, Hernández, Martín Álvares, & Polo, 2003), with known antioxidant activity (Cartron et al., 2003; Roig, Cascón, Arola, Bladé, & Salvadó, 2002; Satué-Garcia, Andrés-Lacueva, Lamuela-Raventós, & Frankel, 1999; Yilmaz & Toledo, 2004). The content of these compounds, however, depends on several factors, including variety of grape, fruit growth and ripening conditions, quality of the base wine, yeast used and *sur lie* (time needed to mature) (Cortell, Halbleib, Gallagher, Righetti, & Kennedy, 2005; Delgado, Martín, del Álamo, & González, 2004; Mazauric & Salmon, 2005, 2006).

Several studies have already been performed in order to evaluate the antioxidant activity of red and white wines (Cartron et al., 2003; De Beer, Joubert, Gelderblom, & Manley, 2003; Jamroz & Beltowski, 2001; Landrault et al., 2001); however, there are few data on the antioxidant capacity of sparkling wines (Cartron et al., 2003; Satué-Garcia et al., 1999). Furthermore, there are no reports on the influence of the different methods for making and/or sugar concentration on the biological activity of these wines.

Therefore, the purpose of this study was to determine the antioxidant capacity of Brazilian sparkling wines *in vitro* (scavenging capacity of the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH·) and *in vivo* (in eukaryotic cells of the *Saccharomyces cerevisiae* yeast). The influence of the different methods used in manufacture (*Charmat* or *Champenoise*), the concentration of sugar (*brut* or *demi-sec*) and the phenolic composition on the antioxidant activity of these wines was also evaluated.

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2. Material and methods

2.1. Samples

Nineteen sparkling wines were studied: 12 *Charmat* (seven *brut* and five *demi-sec*) and seven *Champenoise*, made by seven different wineries in the "Serra Gaúcha", the mountains situated in the south of Brazil. In each of these groups, the performance in relation to its respective base wine was evaluated too. The main characteristics of the sparkling wines used are shown in Table 1. These samples were made from 12 varieties: Pinot Noir (PN), Chardonnay (CH), Italian Riesling (IR), Semillon (SE), White Muscat (WMu), Merlot (ME), Cabernet Sauvignon (CS), Prosecco (PR), White Malvasia (WMa), Candia Malvasia (CMa), Canelli Muscat (CMu) and Alexandria Muscat (AM). The first fermentation (base wines) was performed at 15 °C for an average of 16 days. In the second fermentation, the mean temperature was 12 ± 2 °C with the foam formation time varying between 30 and 90 days. The ageing period varied from zero to 540 days (Table 1). Except for sparkling wines **9**, **10** and **11**, obtained from fermentations with *S. cerevisiae*, in all the others *S. bayanus* was used in both fermentations. In order to perform the assays, the sparkling wines were previously degassed, using a vacuum pump with a valve for air removal, coupled to a workbench agitator.

2.2. Chemical reagents

DPPH·, *trans*-resveratrol, (+)-catechin, (−)-epicatechin, gallic acid and procyanidin B_3 were acquired from Sigma–Aldrich, St. Louis, MO. The procyanidins B_1, B_2 and B_4 were kindly provided by Dr. Regina Vanderlinde (Instituto Brasileiro do Vinho, Bento Gongalves, Brazil). The anthocyanins cyanidin-3-glycoside, delphinidin-3-glycoside, peonidin-3-glycoside and malvidin-3-glycoside were acquired from Extrasynthese, Genay, France. The other reagents were acquired from E. Merck, Damstadt, Germany.

2.3. Oenological analysis of sparkling wines

The alcohol content, total acidity, pressure, volatile acidity, pH, free and total SO_2, dry extract and reduced dry extract, concentration of sugar and ascorbic acid were determined using the methods described by Zoecklein, Fugelsang, Gump, and Nury (2000). All analyses were performed in duplicate.

2.4. Determination of polyphenols by UV spectrophotometry

Total polyphenols and total hydroxycinnamates were quantified by measuring the absorbances at 280 and 320 nm (UV-1700 spectrophotometer, Shimadzu, Kyoto, Japan), respectively. The results of total polyphenols were expressed as mg/l of catechin and those of hydroxycinnamates as mg/l of caffeic acid. The total flavonoids (*TF*) were calculated using the following formula, as described by Iland, Ewart, Sitters, Markides, and Bruer (2000):

$$TF = [(A_{280} - 4) - 0.66] \times (A_{320} - 1.4).$$

The results were expressed in mg/l of catechin. All the analyses were performed in duplicate.

2.5. Determination of polyphenols by HPLC

A 5 ml aliquot of each sample was filtered through a cellulose membrane with a 0.20 μm diameter just before the analysis of the major phenolic compounds by high performance liquid chromatography using a Hewlett–Packard (Palo Alto, CA) 1100 series

Table 1
Main characteristics of the sparkling wines (SW) studied.

SW	*Assemblage*	Wineries	*Sur lie*[1] (days)	Ascorbic acid ± SD[2] (mg/l)	Sugar ± SD (g/l)	TP[3] (mg/l of C[6]) ± SD	*TF*[4] (mg/l of C) ± SD	THC[5] (mg/l of CA[7])* ± SD
Charmat brut								
1	40% PN, 60% CH	A*	30	33.3 ± 0.03[a**]	12.5 ± 0.23[a]	354 ± 4.19[a]	156 ± 2.94[a]	39.8 ± 0.22[a]
2	60% PN, 40% CH	B	126	21.9 ± 0.01[b]	5.48 ± 0.26[b]	185 ± 7.13[b]	52.3 ± 4.19[b]	26.6 ± 0.61[b]
3	50% IR, 30% SE, 20% WMu	C	No ageing	28.9 ± 0.03[c]	11.4 ± 0.08[c]	164 ± 4.19[c]	6.16 ± 1.68[c]	31.5 ± 0.50[c]
4	50% IR, 33% PN, 17% CH	B	60	11.4 ± 0.04[d]	9.33 ± 0.20[d]	193 ± 1.68[b]	23.8 ± 1.67[d]	33.9 ± 0.55[d]
5	30% IR, 70% CH	D	60	21.8 ± 0.03[b]	8.94 ± 0.17[d]	520 ± 0.00[d]	302 ± 1.67[e]	43.7 ± 0.33[e]
6	100% CH	E	30	22.6 ± 0.06[b]	10.5 ± 0.22[e]	420 ± 3.35[e]	220 ± 12.15[f]	40.1 ± 3.05[a]
7	40% IR, 10% ME, 50% CS	B	193	32.6 ± 0.04[a]	10.6 ± 0.29[e]	1350 ± 3.35[f]	780 ± 6.70[g]	113 ± 0.66[f]
Champenoise								
8	40% PN, 60% CH	A	180	11.9 ± 0.03[d]	5.84 ± 0.14[b]	362 ± 0.84[a]	192 ± 1.67[h]	34.1 ± 0.22[d]
9	100% PR	F	150	20.5 ± 0.02[e]	7.28 ± 0.15[f]	483 ± 5.86[g]	234 ± 5.87[f]	50.0 ± 0.05[g]
10	100% CH	F	540	16.7 ± 0.03[f]	8.04 ± 0.19[d]	620 ± 4.19[h]	365 ± 4.19[i]	51.3 ± 0.00[h]
11	20% PN, 80% CH	F	270	21.2 ± 0.01[b]	7.24 ± 0.11[f]	493 ± 2.93[g]	242 ± 5.03[f]	50.4 ± 0.39[g]
12	10% CH, 60% ME, 30% PN	F	365	79.4 ± 0.02[g]	9.91 ± 0.07[d]	2790 ± 41.90[i]	1870 ± 22.63[j]	185 ± 3.89[i]
13	50% PN, 50% CH	F	150	43.6 ± 0.04[h]	6.59 ± 0.20[g]	616 ± 37.71[h]	301 ± 21.37[e]	63.3 ± 3.33[j]
14	20% PN, 80% CH	G	365	46.5 ± 0.03[i]	5.89 ± 0.19[b]	375 ± 0.84[j]	216 ± 0.42[f]	31.8 ± 0.11[c]
Charmat demi-sec								
15	50% IR, 30% SE, 20% WMu	C	No ageing	29.0 ± 0.03[c]	37.5 ± 0.39[h]	185 ± 0.84[b]	15.4 ± 1.68[k]	34.2 ± 0.16[d]
16	30% IR, 70% CH	D	60	19.1 ± 0.07[j]	51.1 ± 0.14[i]	509 ± 3.35[k]	306 ± 9.22[e]	42.8 ± 0.44[e]
17	100% CH	E	30	19.5 ± 0.04[j]	54.4 ± 0.09[j]	523 ± 3.35[d]	297 ± 1.68[e]	45.4 ± 0.33[k]
18	56% WMa, 25% CMa, 10% CMu, 9% AM	B	8	61.3 ± 0.03[k]	36.2 ± 0.13[k]	340 ± 0.84[l]	146 ± 0.42[l]	38.9 ± 0.05[a]
19	74% IR, 14% PN, 12% CH	B	30	15.7 ± 0.03[l]	36.4 ± 0.04[k]	217 ± 0.42[m]	32.6 ± 0.42[m]	36.9 ± 0.05[l]

[1] Time needed to mature.
[2] Standard deviation.
[3] Total polyphenols (TP).
[4] Total flavonoids (*TF*).
[5] Total hydroxycinnamates (THC).
[6] Catechin (C).
[7] Caffeic acid (CA).
* Distinct letters corresponding to different wineries in the Serra Gaúcha/Rio Grande do Sul/Brazil.
** Data followed by different letters for each column differ significantly by Kruskal–Wallis *H* test ($p \leqslant 0.05$).

LC liquid gradient, with a Diode Array Detector (DAD). A Zorbax 300 SB C18 (12 mm × 4.6 mm × 5 μm) pre-column and a C18-ODS (150 mm × 4 mm × 5 μm) (Agilent Technologies, Santa Clara, CA) column were used. The specific phenols quantified were *trans*-resveratrol (Jeandet et al., 1995), anthocyanidins (OIV – Resolution OENO 22/2003), procyanidins B_1, B_2, B_3 and B_4, (+)-catechin, (−)-epicatechin and gallic acid (Lamuela-Raventós & Waterhouse, 1994).

2.6. Evaluation of antioxidant activity in vitro

The scavenging capacity of free radical DPPH· was measured by adding to the sparkling wines, pure or diluted in distilled water [0.1%; 1.0%; 10% and 50% (v/v)], a tris–HCl buffer solution (100 mM, pH 7.0) containing 250 μM of DPPH· dissolved in ethanol. In the control tube, sterilised distilled water was used in lieu of sparkling wines. The tubes were kept in the dark for 20 min and the absorbance was measured at 517 nm (Shimadzu UV-1700 spectrophotometer) (Yamaguchi, Takamura, Matoba, & Terão, 1998). The results were expressed in values of IC_{50} (quantity of sparkling wine needed to reduce 50% of the free radical DPPH·), calculated by polynomial regression graphs (Mensor et al., 2001), using the mean of triplicates.

2.7. Evaluation of antioxidant activity in vivo

The assays *in vivo* were performed using cells of *S. cerevisiae* XV 185-14c yeast (MAT α ade 2-1, arg 4-17, his 1-7, lys 1-1, trp 1-1, trp 5-48, hom 3-10), kindly provided by Dr. R.C. Von Borstel (Department of Genetics, University of Alberta, Canada). Cell suspensions of 2×10^6 cells/ml obtained from the exponential growth phase were treated with hydrogen peroxide (75 mM), in the presence and absence of sparkling wines. The tubes were incubated for 1 h at 28 °C. Then the samples were diluted in a 0.9% (w/v) sodium chloride solution, seeded into a YPD culture medium (10 g/l of yeast extract, 20 g/l of peptone, 20 g/l of dextrose and 20 g/l of agar–agar) and incubated for 72 h at 28 °C. After incubation, the colonies were counted, and 100% survival was considered the total of colonies observed on the control plate (untreated cells) (Wilmsen, Spada, & Salvador, 2005).

2.8. Data analysis

The data were analysed using the following tests: Kruskal–Wallis *H*, Spearman Correlation and Principal Components Analysis (PCA), using SPSS 12.0 for Windows (SPSS Inc., Chicago, IL).

3. Results and discussion

3.1. Oenological analysis and determination of polyphenols by UV spectrophotometry

The alcohol contents of the different sparkling wines analysed varied from 11.23% to 13.05% (v/v), and total acidity from 5.08 to 8.21 g/l of tartaric acid. The mean levels of pressure, volatile acidity and pH were 5.7 ± 0.2 atm, 0.588 ± 0.091 g/l of acetic acid and 3.29 ± 0.14, respectively. The mean concentration of free SO_2 was 20.0 ± 8.12 mg/l and total SO_2 was 122 ± 37.3 mg/l. The values were below the allowed level for volatile acidity, free SO_2 and total SO_2, indicating that the grapes were healthy and that good vinification practices were used (Boulton, Singleton, Bisson, & Kunkee, 1995). The analysis of the dry extract and reduced dry extract showed, respectively, values of 23.5 ± 3.57 and 19.1 ± 2.95 mg/l for the *brut* samples, and 58.3 ± 9.93 and 16.1 ± 2.69 mg/l for the *demi-sec* sparkling wines (data not shown). The sugar concentration varied from 5.48 to 12.5 g/l, for the *brut* samples, and from 36.2 to 54.4 g/l for the *demi-sec* sparkling wines (Table 1). These values are within the range established by Brazilian law (Brasil, 1990) for sparkling wines.

The ascorbic acid content of the sparkling wines studied varied from 11.4 to 79.4 mg/l (Table 1). Up to the present, there are few data in the literature concerning the content of this acid in sparkling wines. We know that its concentration is the result of the variety of grape, degree of maturity (Ribéreau-Gayón et al., 2003) and the amount of sunlight on the vine (Valpuesta & Botella, 2004), for example. Fig. 1a shows that sparkling wines had an increase in ascorbic acid concentration, in comparison to the base wine (samples **2**, **15** and **19**). Although the use of the ascorbic acid in wines is a well-known practice (Marks & Morris, 1993), we believe that this result is probably due to yeast metabolism (Hancock, Galpin, & Viola, 2000; Sauer, Branduardi, Valli, & Porro, 2004; Smir-

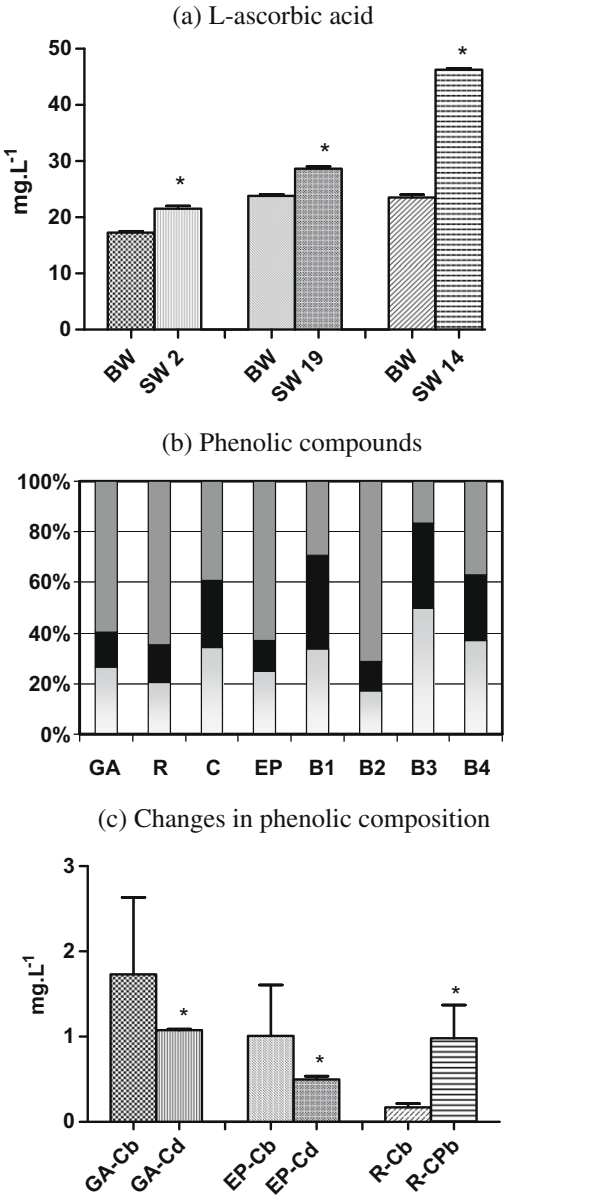

Fig. 1. Profiles of L-ascorbic acid (a) in base wine (BW) and respective sparkling wine (SW), polyphenols (b) by HPLC: □; *Charmat brut* (Cb), ▒; *Champenoise* (CPb), ■; *Charmat demi-sec* (Cd), gallic acid (GA), *trans*-resveratrol (R), catechin (C), epicatechin (EP), and procyanidins (B_1–B_2–B_3–B_4); and changes in phenolic composition (c) mediated by *sur lie* and sugar concentration. (a) L-Ascorbic acid, (b) phenolic compounds, and (c) changes in phenolic composition.

noff, Conklin, & Loewus, 2001), as there was no ascorbic acid addition to the sparkling wines assayed. Furthermore, in sample **14**, for which the *sur lie* period was 360 days (Table 1), the concentration of vitamin C practically doubled. New tests to evaluate the biosynthesis of ascorbic acid by oenological yeasts are currently being carried out as a result.

Higher values of total polyphenols, total flavonoids and total hydroxycinnamates were found, obviously, in red sparkling wines (samples **7** and **12**). As for the whites, major variations were observed, depending partially on the method by which the sparkling wine was made (Table 2). Sample **14** (*Champenoise*) showed a higher concentration of total polyphenols and total flavonoids compared to the base wine of origin. On the other hand, the concentration of these compounds diminished in *Charmat* samples (both *brut* and *demi-sec*) compared to their respective base wines (Fig. 1b). The mean reduction of total polyphenols and total flavonoids levels observed in *Charmat* sparkling wines was 24.58 ± 0.72% and 57.19 ± 4.22%, respectively. The concentration of total hydroxycinnamates diminished after the second fermentation, independent of the method by which they were made (Fig. 2), probably due to the action of yeasts, which can metabolise and/or adsorb up to 20% of the content of these compounds (Ribéreau-Gayón et al., 2003; Zoecklein et al., 2000).

3.2. Polyphenols analysis by HPLC

Fig. 1b shows the percentile differences on the phenolic profile of all sparkling wines. Some specific differences have been noted: sample **12** had the highest contents of all polyphenols analysed; larger amounts of *trans*-resveratrol, (+)-catechin and procyanidins B_1, B_3 and B_4 were observed in sample **7**; higher values of (−)-epicatechin were obtained in sample **1** and of procyanidin B_2 in sample **10**; in all sparkling wines analysed, the main phenolic component was gallic acid (data not shown).

Table 2
DPPH· mean values for the different sparkling wines analysed and mean survival values of the *Saccharomyces cerevisiae* yeast treated with hydrogen peroxide (H_2O_2) 75 mM in presence and absence of different sparkling wines.

Sparkling wines	DPPH·		Survival	
	IC_{50}* ± SD**	Rank#	±SD (%)	Rank
Charmat brut				
1	11.80 ± 1.28[a]	2	93.55 ± 0.25[a]	1
2	13.03 ± 1.19[b]	3	93.65 ± 4.75[a]	1
3	1.83 ± 0.49[c]	1	96.90 ± 3.10 [a]	1
4	80.76 ± 2.75[d]	8	100.00 ± 0.00[a]	1
5	20.76 ± 5.12[e]	4	78.70 ± 3.70[b]	3
6	20.26 ± 0.32[e]	4	96.75 ± 0.35[a]	1
7	31.50 ± 0.42[f]	5	62.43 ± 8.30[c]	4
Champenoise				
8	9.05 ± 1.04[a]	2	84.22 ± 1.91[d]	2
9	19.63 ± 0.63[e]	4	56.80 ± 1.00[e]	5
10	30.83 ± 2.01[f]	5	53.58 ± 0.95[e]	5
11	11.09 ± 1.81[a]	2	34.80 ± 0.50[f]	6
12	32.83 ± 0.13[f]	5	85.50 ± 0.90[d]	2
13	16.77 ± 0.54[b]	3	65.41 ± 1.21[c]	4
14	39.53 ± 1.53[g]	6	85.05 ± 3.35[d]	2
Charmat demi-sec				
15	26.87 ± 0.58[e]	4	65.45 ± 1.05[c]	4
16	32.56 ± 0.43[f]	5	64.85 ± 2.35[c]	4
17	25.04 ± 0.13[e]	4	79.20 ± 3.60[b]	3
18	23.73 ± 0.57[e]	4	100.00 ± 0.00[a]	1
19	26.10 ± 1.93[e]	4	95.30 ± 4.70[a]	1
Catechin (control)	63.68 ± 1.42[h]	7		
H_2O_2 (control)			27.95 ± 0.25[g]	7

* IC_{50} (% of amount of samples necessary to scavenge 50% of DPPH·).
** Standard deviation.
# Rank in crescent order according to statistical significance (Kruskal–Wallis *H* test, $p \leqslant 0.05$) among the values of each parameter.

So far no studies have been performed on the phenolic composition of *Charmat* sparkling wines. As to the *Champenoise*, it was observed that the Brazilian sparkling wines possessed similar phenolic profiles to those reported for Spanish sparkling wines (Ibern-Gómes et al., 2000; Pozo-Bayón et al., 2003; Satué-Garcia et al., 1999), as well as in French Champagnes (Chamkha et al., 2003). To our knowledge this is the first report on the presence of procyanidins B_1, B_2 and B_4 in sparkling wines.

For the red and *rosé* sparkling wines the contents of four important anthocyanins found in red grapes and wine (Zoecklein et al., 2000) were also quantified, and as expected, sample **13** (*rosé*) possessed lower concentrations among the four compounds analysed compared to samples **7** and **12** (red). The main and most plentiful anthocyanin found in red varieties, malvidin monoglycoside (Ribéreau-Gayón et al., 2003), was the main compound in the three sparkling wine samples evaluated (data not shown).

Fig. 2 shows the phenolic profile of the samples assayed. The second fermentation of base wines increased the concentration of gallic acid in the *brut* sparkling wines, probably due to the hydrolysis of procyanidins that are esterified with this acid (Jordão, 2000; Stevens et al., 2002). Conversely, in *demi-sec* sparkling wines this type of reaction seems to be a disadvantage, suggesting that sugar concentration affects the phenolic composition. The same relationship was verified on (−)-epicatechin values. A higher concentration in *brut* (*Charmat* or *Champenoise*) sparkling wines than in their respective base wines were observed (Fig. 2), and no changes were observed in the *demi-sec* sparkling wines compared to the respective base wines.

The *trans*-resveratrol contents (Fig. 1b) were lower in the *Charmat* sparkling wines (both *brut* and *demi-sec*), compared to the base wines, probably due to the finishing stages, such as clarification and or filtration (Threlfall, Morris, & Mauromoustakos, 1999; Vrhovsek, Wendelin, & Eder, 1997). With the *Champenoise* method, however, there was a higher content of this compound than in its base wine. Both the malolactic fermentation (Pezet & Cuenat, 1996) and the action of yeasts with over expression of the β-glycosidase enzyme (Vrhovsek et al., 1997) may increase the concentration of *trans*-resveratrol in wines. Furthermore, sample **5** was obtained with a longer time *sur lie* compared to the other samples (Table 1). A positive correlation was observed between the concentration of *trans*-resveratrol and the *sur lie* time ($r = 0.456$; $p = 0.05$), suggesting an effect of contact time between the yeasts and the sparkling wine in the *trans*-resveratrol concentration. New assays to evaluate the β-glycosidase performance during the vinification are currently being carried out.

Comparing the base wines, it is observed that the (+)-catechin concentration changes in relation to the *sur lie* (Table 1). Samples **3**, **15** and **19**, elaborated with minimal *sur lie* times did not show differences. For a medium *sur lie* (sample **2**), the values of this phenolic compound increased. Conversely, for greater periods of *sur lie*, the (+)-catechin levels were lower (sample **14**). A negative correlation was found between the reduced dry extract content (which had a direct relationship with the yeast metabolism) and the concentration of (+)-catechin ($r = -0.619$; $p = 0.01$). Procyanidin B_1, B_2, B_3 and B_4 contents were lower after the second fermentation (data not shown). It is possible that, as seen in red wines, this reduction is due to condensation reactions, e.g., of procyanidins with proteins and polysaccharides, to the polymerisation reactions among the different procyanidins and to oxidative degradation phenomena (Lopez-Toledano, Mayen, Merida, & Medina, 2002; Ribéreau-Gayón et al., 2003). These interactions may be influenced by the different techniques adopted during the manufacturing/maturing process (Boulton et al., 1995; Flanzy, 2003; Mazauric & Salmon, 2005; Ribéreau-Gayón et al., 2003), accounting, at least in part, for the differences found.

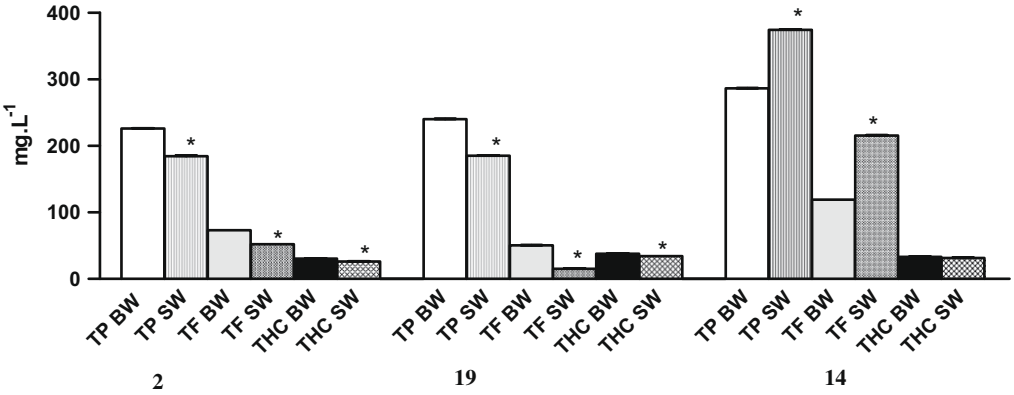

Fig. 2. Level of total polyphenols (TP), total flavonoids (*TF*) and total hydroxycinnamates (THC) in base wines (BW) (**2**, **19** and **14**) and in their corresponding sparkling wine (SW) *Charmat brut* (**2**), *Charmat demi-sec* (**19**) and *Champenoise* (**14**).

3.3. Evaluation of antioxidant activity in vitro

Table 3 shows that sample **3**, which was elaborated without *sur lie* (Table 1) possessed the highest degree of antioxidant activity (IC_{50} = 1.83 ± 0.49%). Furthermore, sparkling wines **6** (*Charmat*) and **10** (*Champenoise*) were prepared exclusively with the Chardonnay variety, and the greatest capacity for scavenging of free radical DPPH· (Table 3) was observed in sample **6**, which was produced with a small *sur lie*, and possessed a higher ascorbic acid content (Table 1) and a higher concentration of major phenolic compounds analysed by HPLC than sample **10** (Table 2).

Sparkling wines **11** and **14** were prepared using the *Champenoise* method by different wineries. Sample **11** possessed a greater capacity to scavenge free radical DPPH· than sample **14** (Table 3). The latter showed lower (+)-catechin, (−)-epicatechin, gallic acid and procyanidin B_1, B_2 and B_4 contents (Table 2), indicating the influence of the vinification techniques (as for example the *sur lie* period) adopted by each winery on the phenolic composition and antioxidant activity of the sparkling wines. Interestingly, the similar *assemblage* (40% Pinot Noir and 60% Chardonnay) submitted to different methods of vinification in the same winery showed small differences in the antioxidant capacity. Sample **8** *sur lie* (Table 1; *Champenoise*), which presented higher antioxidant activity than sparkling wine **1** (*Charmat*) was only a little greater (Table 2), suggesting one more time the influence of this technique. Higher values of total flavonoids and procyanidins B_2 and B_3 (Table 2) were found in sparkling wine **8**. New assays about the influence of the *sur lie* time on the antioxidant activity and phenolic profile are being currently carried out. Beyond *sur lie*, the sugar concentration also influenced results.

Table 3
Variance explained by the first principal components (PC).

PC	Eigenvalue	Explained variance (%)	Cumulative variance (%)
Charmat brut			
1 – *TF*[a]	11.83	51.45	51.45
2 – DE[b]	4.70	20.44	**71.89**
Champenoise			
1 – *Sur lie*	15.94	69.29	69.29
2 – TP[c]	3.92	17.03	**86.32**
Charmat demi-sec			
1 – Sugar	12.41	53.95	53.95
2 – Alcohol	4.37	19.00	**72.95**

[a] Total flavonoids.
[b] Dry extract.
[c] Total polyphenols.

Samples of *Charmat brut* **3**, **5** and **6** showed a higher antioxidant capacity than their respective *demi-sec* peers (samples **15**, **16** and **17**) (Table 2). Higher values of (+)-catechin, procyanidin B_2 and gallic acid (Table 1) were found in these *brut* sparkling wines, compared to their *demi-sec* peers.

Apparently, antioxidant activity does not depend exclusively on the total polyphenols content. Samples **7** and **12** (red sparkling wines) with significant amounts of phenolic compounds (Tables 1 and 2) did not show the highest antioxidant activity. Studies have already demonstrated that the biological activity of resveratrol, specifically the inhibition of the tyrosinekinase protein p56, is diminished when glycosilation of the hydroxyl groups occurs (Soleas, Diamandis, & Goldberg, 1997). Therefore, it is possible that some polyphenols, when connected to carbohydrates (for instance the anthocyanidins of red wines) possess less antioxidant activity than their respective aglycones, which might account at least in part for the results observed. Among the red sparkling wines, the presence of a negative correlation between the antioxidant capacity *in vitro* and the concentrations of cyanidin-3-glycoside, peonidin-3-glycoside and malvidin-3-glycoside (all with a value of r = 0.985) and delphinidin-3-glycoside (r = 1), at a level of significance of p = 0.01 corroborates this hypothesis. New assays examining the sugar influence on antioxidant activity are being currently carried out.

3.4. Evaluation of the antioxidant activity in vivo

In order to determine antioxidant activity *in vivo*, the highest non-cytotoxic concentration of sparkling wines was used, i.e., 10.0% (v/v) (data not shown). All the samples evaluated were able to protect the yeast cells against damage caused by hydrogen peroxide (Table 3).

Of the two red sparkling wines studied, sample **12**, which possessed the highest polyphenol and ascorbic acid contents of all the sparkling wines evaluated (Tables 1 and 2), showed higher antioxidant activity *in vivo* than sparkling wine **7** (Table 2).

Differences in the antioxidant activities of sparkling wines because of the *Charmat* and *Champenoise* methods used to make them are shown in this study. Similarly to what was observed *in vitro*, the *Charmat brut* sparkling wines possessed on average a higher antioxidant capacity (88.85 ± 1.79) than those prepared by the *Champenoise* method (60.77 ± 3.31), including the cases in which the sparkling wines were made using the same varieties/*assemblages* and/or by the same wineries (i.e., samples **1** and **8**, and **6** and **10**).

The greatest differences between the two methods of making sparkling wines are the *sur lie* time and the area of contact between

the sparkling wine, the yeasts, vinary containers and sugar concentration. It has already been demonstrated that the yeasts are able to adsorb different compounds present in the wines, including catechin and epicatechin (Mazauric & Salmon, 2005; Ribéreau-Gayón et al., 2003). The samples of *Charmat brut* (**1** and **6**) prepared with a shorter *sur lie* time than their respective peers *Champenoise* (samples **8** and **10**) (Table 1), possessed higher (+)-catechin and (−)-epicatechin contents (Table 2). A negative correlation was observed between the *sur lie* period and antioxidant activity *in vivo* ($r = -0.519$; $p = 0.01$), i.e., the longer the *sur lie* period, the smaller was the antioxidant capacity of the sparkling wines. This correlation was even greater in the group of *Champenoise* sparkling wines ($r = -0.842$; $p = 0.05$). The statistical analysis of the principal components (PCA), for all sparkling wines, revealed that the first two principal components explain more than 70% of the total variance (Table 3). This analysis corroborated our data, since the *sur lie* variable appears as one of the factors of biggest influence on the antioxidant activity in the *Champenoise* (Fig. 3a), when compared with *Charmat* sparkling wines (Fig. 3b and c). Furthermore, the *Charmat demi-sec* sparking wines (samples **15**, **16** and **17**) possessed lower antioxidant activity (Table 2) than observed for their respective *Charmat brut* peers (samples **3**, **5** and **6**), similarly to what was observed *in vitro*. Negative correlations between the *in vivo* antioxidant capacity and the sugar contents ($r = -0.476$; $p = 0.01$) and dry extract ($r = -0.346$; $p = 0.05$) were found in these two groups of sparkling wines. This is the first time that differences are shown in the antioxidant potential of sparkling wines as a function of its sugar concentration. Orange juices with added sugar showed less antioxidant activity than juices without added sugar (Franke et al., 2004), corroborating the results found in our work. The PCA analysis showed that, for the group of *Charmat demi-sec* sparkling wines, the sugar concentration was one of the main variables that had influenced the antioxidant response of these wines (Fig. 3b).

The phenolic compounds have an acknowledged antioxidant capacity, and can scavenger free radicals, chelate metals, and diminish lipid peroxidation (Cartron et al., 2003; Jamroz & Beltowski, 2001; Roig et al., 2002; Yilmaz & Toledo, 2004). Polyphenol formation by the vine is influenced by natural factors, such as variety of grape, genetic susceptibility to diseases, climate and soil, besides viticultural management (rootstock, vigour, exposure to sunlight as a function of the conduction system, fertilisation, etc.; Cortell et al., 2005; Delgado et al., 2004; Ribéreau-Gayón et al., 2003). Oenology also plays an important role in determining the phenolic profile of the final product, using techniques such as industrial maturing, press yield, maceration time and temperature, yeast used, fermentation period, clarification, stabilisation, filtration, maturing, ageing, etc. (Mazauric & Salmon, 2005; Ribéreau-Gayón et al., 2003).

In this study, the phenolic compounds showed an important role in the antioxidant activity. Interestingly, the PCA analysis showed a stronger association between the phenolic compounds and the antioxidant activity for the *Champenoise* than *Charmat* sparkling wines (Fig. 3). Therefore, the role of phenolic compounds may be greater in the antioxidant activity of the sparkling wines made by the *Champenoise* method.

Summarising, the results presented in this study show that: (a) the *Charmat* and *Champenoise* sparkling wines, both *brut* and *demi-sec*, possess significant antioxidant activity, which is associated with the presence of phenolic compounds; (b) *Charmat brut* sparkling wines possess higher antioxidant activity than *demi-sec Charmat* and *Champenoise*; and (c) there are major differences in the concentrations of phenolic compounds in sparkling wines as a function of the type of grape/*assemblage* used and the method used for manufacture.

New hypotheses about sparkling wine antioxidant activity, in relation to phenolic composition, were discovered with our tests and they will be evaluated individually. However, data shown in this work suggest that it is possible to obtain a specific phenolic profile in sparkling wines by the oenological practices adopted, thus enabling the production of sparkling wines with greater antioxidant activity and thus with a higher added value. Furthermore,

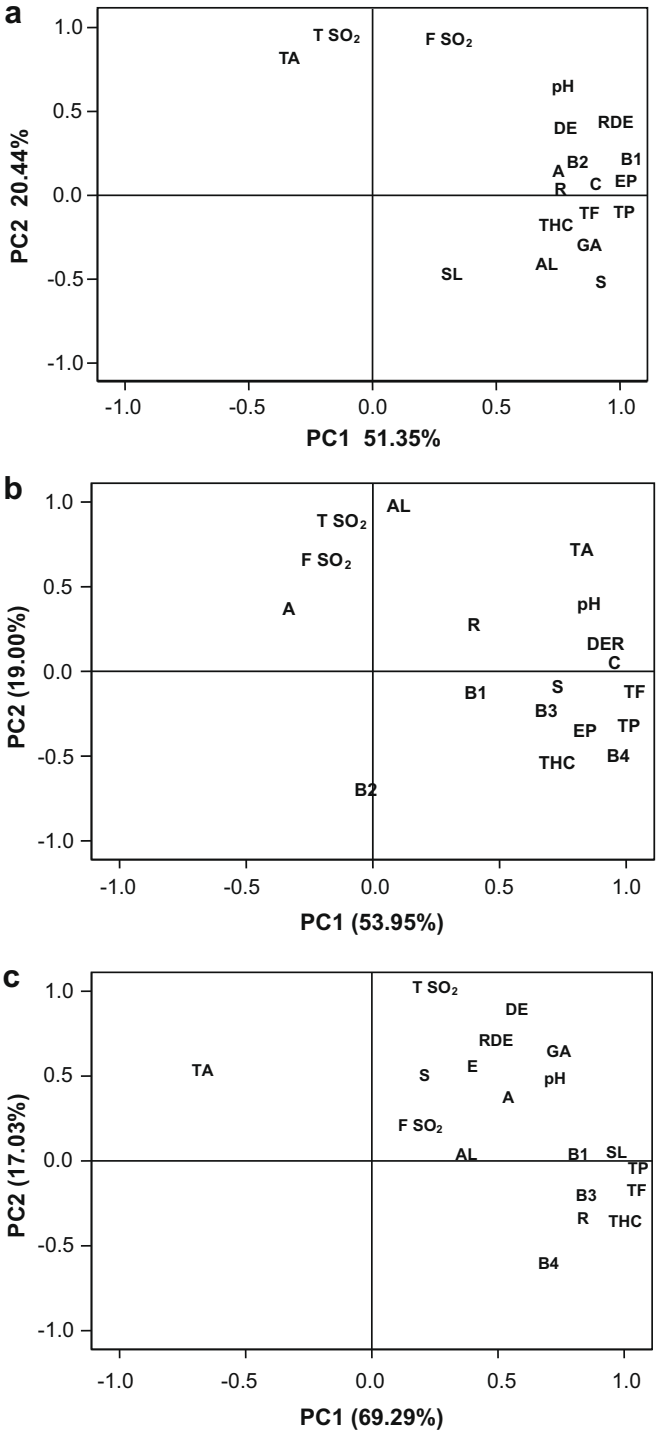

Fig. 3. Scores plots (PC1 vs. PC2) of the phenolic compounds and main characteristics of sparkling wines (a) *Champenoise*, (b) *Charmat demi-sec* and (c) *Charmat brut* (pH, *trans*-resveratrol (R), L-ascorbic acid (A), sugar concentration (S), catechin (C), procyanidins (B_1–B_2–B_3–B_4), gallic acid (GA), epicatechin (EP), total polyphenols (TP), total flavonoids (*TF*), total hydroxycinnamates (THC), dry extract (DE), reduced dry extract (RDE), total acidity (TA), *sur lie* (SL), alcohol (AL) and free and total SO_2 (FSO_2–TSO_2)).

the moderate/guided consumption of sparkling wines may be a positive choice in seeking a healthy life.

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Analytical Methods

Elemental characterisation of Cabernet Sauvignon wines using Particle-Induced X-ray Emission (PIXE)

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ABSTRACT

The elemental composition of 2002 Cabernet Sauvignon wines from four different regions of Rio Grande do Sul state (Brazil) was determined using Particle-Induced X-ray Emission (PIXE) technique. In total, wines from 13 different vineyards were studied. Particular attention was given to wines stemming from Vale dos Vinhedos which is one of the most important wine producing regions in Brazil. Typical PIXE spectra consisted of elements with atomic number between 11 and 38 such as P, S, K, Ti, Mn, Fe, Cu, Zn and Rb. Physicochemical variables such as volatile acidity, alcohol, pH and dry extract were also determined for some wines. Variations in the elemental concentrations among wines from Vale dos Vinhedos and from different regions were observed. In general our results are in good agreement with previous measurements of Brazilian wines. With respect to European wines, our results are characterised by relatively low concentrations of Cu and Zn and high concentration of Rb.

1. Introduction

The Brazilian wine industry is ranked 15th among the world wine producers (Mello, 2008) and fifth in the southern hemisphere (IBRAVIN, 2009) with an average production over 300 million litres/year of wine, juice and others derivatives of grape. The production standards have improved substantially since 1995 when the country joined the *Organisation Internationale de la Vigne et Vin* (O.I.V.). In terms of volume, the Brazilian exports of wine and sparkling wine increased more than six times in the past 5 years reaching 10.7 million litres and a revenue of 7.7 million dollars in 2008 (IBRAVIN, 2009). Russia, Paraguay, United States and The Netherlands are the leading importers of Brazilian wines.

The Brazilian viticulture industry is concentrated in the country's southern state of Rio Grande do Sul (RS), home of about 90% of the national production. The remaining 10% is shared among other states in southern Brazil (Santa Catarina, Paraná and São Paulo) and, in recent years, in northeastern Brazil (Pernambuco and Bahia). Some of the best wineries in Rio Grande do Sul are concentrated in an 82 km^2 region called Vale dos Vinhedos. This region is located in the city of Bento Gonçalves (29°10′17″S and 51°31′09″W) which belongs to the mountainous region known as Serra Gaúcha. It was settled by Italian immigrants from northern Italy more than one century ago and was the first Brazilian region to have the origin of the wines certified by the National Institute of Industrial Property in 2002. In order to obtain the *Indicação de Procedência Vale dos Vinhedos* (I.P.V.V.), the Brazilian equivalent to "Controlled Term of Origin – CTO" certificate, the winemakers from Vale dos Vinhedos had to comply with a number of criteria covering the production, control and marketing issues. In this way a Regulatory Committee ensures that the product meets high quality standards during the winemaking process. This control has contributed to the development of vine cultivation as well as the introduction of new technologies in this region (APROVALE, 2008).

Cabernet Sauvignon is the most important variety among red wines produced in Vale dos Vinhedos. For this reason, in the past 10 years this variety has been the subject of several studies aiming at improving quality and production. For instance, Santos, Silva, Miele, and Franco (2005) have investigated the impact of geographic origin on the sensory profile and acceptance of Brazilian Cabernet Sauvignon wines, while Mandelli (2002) has analysed the importance of climatic factors for winemaking in Serra Gaúcha. Concerning the composition of Cabernet Sauvignon wines, Rizzon and Miele (1997, 2007) studied the analytical (1997) and physicochemical (2007) characteristics of this variety as a function of the vintage. Despite these studies, research in this field in Brazil can be considered scarce if compared to wine-related research

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pursued by other wine producing countries such as Spain, Portugal, France and Australia. For this reason, it is very important to characterise this regional product by its vintage, variety, geographic origin, metal content and physicochemical parameters. Although the content of inorganic components in wine accounts for a small percentage of its total composition, they play an important role in the wine making process and therefore in the final quality of the product. Moreover, as a worldwide consumed beverage, wine becomes an important source of macro and trace elements that are essential to human beings. Given the fact that metal ions play an important role for the oxidative stress in cells which in turn might be related to ageing (Finkel & Hobrook, 2000), the analysis of elemental concentration in wine becomes an important issue.

Several analytical techniques are used to measure trace elements in wine. Most of them are optical-based techniques such as Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Flame Atomic Absorption Spectrometry (FAAS) among others (Saitoh, Sera, Gotoh, & Nakamura, 2002). In general, the direct determination of inorganic elements employing ICP methods is difficult because wine consists of a complex matrix that contains ethanol, sugars and other organic compounds. Therefore, the methods for the determination of metals in wine by these techniques requires some steps on sample preparation like elimination of alcohols, acid digestion, evaporation to dryness and dissolution of the residue in nitric acid.

Another class of analytical techniques, which has been practically unexplored for the analysis of wine, is based on ion beam analysis. Among them, Particle-Induced X-ray Emission (PIXE) (Johansson, Campbell, & Malmqvist, 1995) is one of the most important ones. PIXE is based on the induction of characteristic X-rays by energetic ions (usually protons) when interacting with target nuclei. PIXE has a truly multielemental capability, that is, all elements with atomic number generally higher than 11 can be simultaneously detected in a single measurement and without any prior knowledge of the elements present in the sample. Its sensitivity compares to those provided by optical-based techniques, i.e. in the range of a few parts per million (ppm) or even tens of parts per billion (Kocsonya et al., 2002). In addition, PIXE has two important advantages: (i) the analysis is relatively fast, usually just a couple of minutes; and (ii) the sample preparation in its solid form does not require either any sophisticated handling or any chemical treatment, thus reducing drastically any chance of contamination. Since this technique is nondestructive, it preserves the original samples, allowing extra measurements if required. Due to all these features, PIXE is widely used to characterise a great variety of materials including biological samples (Franke et al., 2006).

In this context, the aim of this work is to use the PIXE technique in order to perform the elemental characterisation of Cabernet Sauvignon wine (2002 vintage) from Vale dos Vinhedos and compare it with the results of other regions located in Rio Grande do Sul. A statistical analysis was carried out in order to identify the variability in metal concentration among wines from distinct regions. Moreover, physicochemical parameters such as total and volatile acidity, alcohol content, pH and dry extract were also measured.

2. Materials and methods

2.1. Studied area

Four different wine production regions of Rio Grande do Sul (RS) were studied: Serra Gaúcha, Vale dos Vinhedos, Campanha Gaúcha and Centro (Fig. 1). A brief description of each region follows:

Serra Gaúcha: This region is located in the northeastern lands of RS state at 29°S and features average altitudes from 400 up to 700 m. The climate is mild, subtropical and damp. Serra Gaúcha comprises approximately seventeen sub-regions including Campos de Cima da Serra and Vale dos Vinhedos. Due to its high quality wines and production yield, Serra Gaúcha is the most prominent viticulture region of Brazil.

Vale dos Vinhedos: It is located in Serra Gaúcha, starting from the western border of Bento Gonçalves city (known as the Brazilian capital of wine) and ending in Rio das Antas River. It has an area of 81.123 km^2 that includes neighbouring towns of Garibaldi and Monte Belo do Sul. Its geographical denomination takes into account the soil profile, topographic and climatic characteristics. The vineyard comprises 26% of the total area.

Campanha Gaúcha: This region is located next to the Uruguayan border at 31°S with an average altitude of 100–300 m. The climate is temperate with relatively dry and hot summers. Thanks to these features viticulture activities has developed rapidly in the past few years.

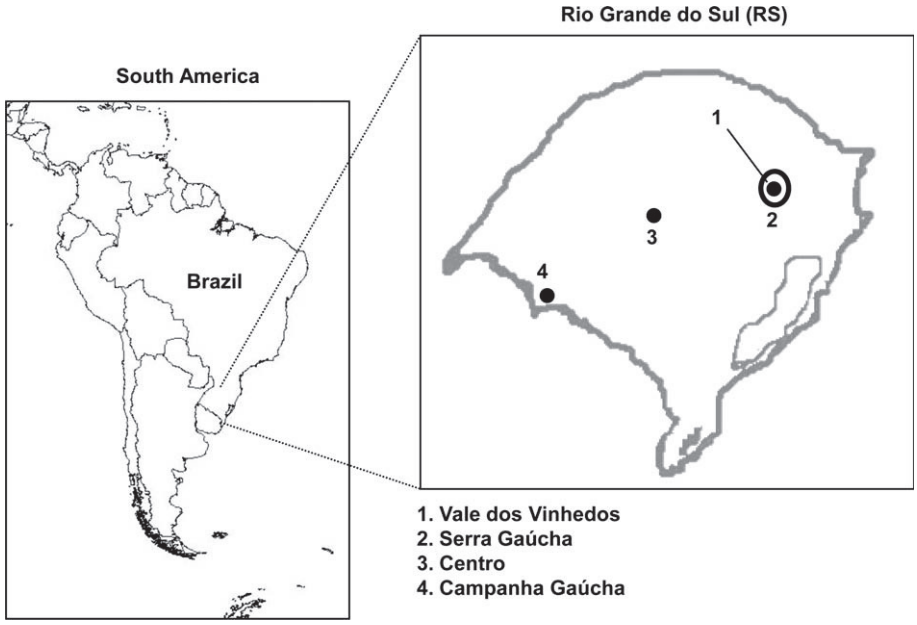

Fig. 1. Wine production regions from Rio Grande do Sul studied in this work. Vale dos Vinhedos (1) belongs to a larger mountain chain known as Serra Gaúcha (2). Centro (3) is located in the central part of the state while Campanha Gaúcha (4) is close to the border between Brazil and Uruguay.

Centro: it is located in the central area of RS state at 29.6°S with an average altitude of 425 m. This region is characterised by temperate and humid climate.

2.2. Samples

Thirteen different brands of Cabernet Sauvignon wines (2002 vintage) were purchased in the local market. The samples consisted of wines produced in different vineyards representing the following regions: Vale dos Vinhedos (eight vineyards, all with I.P.V.V. certificate), Serra Gaúcha (two vineyards), Campanha Gaúcha (two vineyards) and Centro (one vineyard). In general, two 750 mL bottles per vineyard were used for sample preparation.

Since the PIXE technique requires the use of solid samples, we opted for a thermal procedure where the wines are dried until a solid residue was obtained. In order to prevent any substantial evaporation of inorganic elements during the thermal procedure, the samples (500 mL) were put in a beaker inside an oven at a relatively low temperature and for a long period of time. The temperature of the wine samples inside the oven was monitored and typically reached 77 °C during the drying process, which is below the boiling point of the wine. The drying procedure lasted about 7 h, thus ensuring that an adiabatic regime was reached. The samples were removed from the oven as soon as they dried out in order to prevent any sudden rise of their temperature. The whole procedure was tested by measuring the vapours emitted during the thermal treatment and no loss of inorganic elements was observed. The dried residues were homogenised and pressed into pellets of 25 mm diameter and 2 mm thick. The dry weight of the samples varied from 1.6 to 2 g. Two samples were obtained for each bottle.

2.3. Physicochemical variables

For the sake of completeness, the physicochemical characterisation of several wines was carried out. The aim of such measurements is to put the wines studied in this work in a broader perspective with other Brazilian wines. The measurements followed an official protocol established by the Brazilian Agriculture Ministry (MAPA, 2009). Eleven variables were determined for each wine: pH, total and volatile acidity, alcohol content, total and free sulphur dioxide, reducing sugars, density, dry extract, reduced dry extract and alcohol/reduced dry extract. For instance, density, alcohol content and dry extract were determined by densimetry, while pH was obtained using the potentiometric method, and volatile acidity by titulometry.

2.4. PIXE instrumentation

The measurements were carried out at the Ion Implantation Laboratory of the Physics Institute (UFRGS) using a 3 MV Tandetron accelerator. All PIXE measurements were performed with 2 MeV proton beams since the performance of the PIXE system is optimised at this energy. Typically, each target was irradiated with a density current of 22 nA/cm^2 during 400 s, and the respective count rates varied from 1000 and 1500 counts per second. The low density current employed in the experiments ensures a low dead time of the data acquisition system and, more importantly, preserves the integrity of the samples during the irradiations (Reis, Alves, & Jesus, 1996). The reaction chamber accommodated 10 samples at a time and was kept at a pressure of 10^{-6} mbar throughout the experiments. The samples were positioned in the proton beam by an electro-mechanical system coupled to a video camera. In order to avoid charge buildup in the samples, an electron flood gun was installed inside the reaction chamber. Characteristic X-rays induced by the proton beam were detected by a lithium-doped silicon detector positioned at 135° with respect to the beam direction. The energy resolution of the detector was 155 eV at 5.9 keV. A mylar absorber (340 μm thick with a 1 mm diameter hole) was placed in front of the detector window in order to decrease the count rate due to low energetic X-rays. In this way a good balance between the count rates of light and heavier elements was achieved.

2.5. Data analysis

The PIXE system of the Ion Implantation Laboratory is calibrated with several reference materials including apple leaves, bovine liver and fish muscle among others. The standardization procedure adopted in this work relies on the comparison of standards with the samples under study (Johansson et al., 1995). All experimental parameters like geometric factors, detectoŕs solid angle, absorbers, beam energy and accumulated charge are taken into account. Several independent studies have proved the reliability of the standardization procedure adopted in this work (see, for instance, Franke et al. (2006) and Giulian et al. (2009)). Although all standards provided consistent results among them, the present work made use of apple leaves from the National Institute of Standards and Technology (NIST reference material 1515). The choice of apple leaves is due to the similarity of its matrix with those of the wine samples.

Each experimental PIXE spectrum was fitted using the GUPIX-WIN software (Campbell, Hopman, & Maxwell, 2000), which employs the least-square fitting procedure according to Marquardt. In short, all X-ray peaks appearing in the X-ray spectrum are simultaneously fitted, and peak areas are converted to elemental concentrations using the standardization procedure described above. Moreover, this software handles the continuum background using a top-hat filter operation including discreet and continuum pileup effects. Physical processes like secondary fluorescence and self-absorption in the target are evaluated as well. As a result, each element present in that sample is assigned to a particular elemental concentration.

For a set of samples, the concentration of a particular element is obtained through the mean, while the standard deviation is taken as representative value related to the variance of the results. The statistical analysis was carried out using ANOVA and Tukey's Post hoc test (5%) in order to compare the mean values obtained among wines from different regions as well as among wines from Vale dos Vinhedos.

3. Results and discussion

3.1. Physicochemical variables

The results of the physicochemical variables of 2002 Cabernet Sauvignon wines are shown in Table 1. Although the overall results are in agreement with measurements carried out by Souza, Theodoro, Souza, Motta, and Glória (2005) for 1999 vintage and Rizzon and Miele (2007) for 1999, 2000 and 2001 vintages, there are clear discrepancies for some variables. In particular, our results are characterised by higher values of alcohol content when compared with previous years. The higher alcohol value for 2002 vintage may be explained by a better grape maturation (Mandelli, 2002). Parameters such as dry extract and reduced dry extract are also very important to be monitored because they affect the structure of the wine. Our results for these variables are systematically higher than those quoted by Rizzon and Miele (2007).

We have also measured the free and total sulphur dioxide. SO_2 is used as food preservative and is an indispensable compound for the quality of wine because it acts as antimicrobial agent, antioxidant and binder for acetaldehyde during different stages of wine-

making and before bottling (Huang et al., 2008). On average, the level of total sulphur dioxide observed in the present work is lower than the one measured by Souza et al. (2005).

3.2. Elemental composition of the wine

The elemental concentration of thirteen Brazilian Cabernet Sauvignon wines (2002 vintage) was determined through the PIXE technique. Elements such as Mg, Si, P, S, Cl, K, Ca, Mn, Fe, Zn and Rb were present in all samples. Some of the samples did not contain Al, Cu, Br and Sr. Chromium was detected only in wines coming from the central part of the state (Centro). Traces of Ti and Ni were detected just in few samples and therefore these two elements were excluded from further analysis. The results are expressed in milligram per litre. Fig. 2 shows a typical PIXE spectrum of a wine sample as a function of energy. The amplitudes of the X-ray peaks are related to the concentration of the elements in the sample.

3.2.1. Elemental concentration by region

Table 2 gives the elemental concentration of the wines for distinct regions of Rio Grande do Sul. For some samples the elemental concentrations were not significantly above the limit of detection (LOD) and therefore the LOD appears as a lower limit of the range for that particular region. In such cases these samples were not included in the calculations of the mean and standard deviation (sd) shown in this table. In Table 3 we present the overall results of all wines measured in this work along with some other results from Brazilian and foreign wines. In general, the results show that among all elements K has the highest concentration (about 1000 mg/L), followed by P, S, Mg and Ca (concentrations in the range of 20–160 mg/L). Indeed, the elements of this group are considered major components in wine (Alvarez, Moreno, Jos, Camean, & Gonzalez, 2007). In particular K plays a key role in pH, organoleptic quality and stability of the wine (Mozaz, Sotro, Segovia, & Azpilicueta, 1999). The K content observed in this work agrees with the results obtained for 1999 and 2000 vintages (Rizzon & Miele, 2007). However, it seems to be 40% lower when compared to the 2001 vintage (Rizzon & Miele, 2007).

Analysis of soil samples from one vineyard belonging to Vale dos Vinhedos and from neighbouring areas (Giulian et al., 2009) indicate that these elements are relatively abundant in soil as well, but in different proportions. Certainly, sources other than soil are contributing to the S and K concentrations observed in this work.

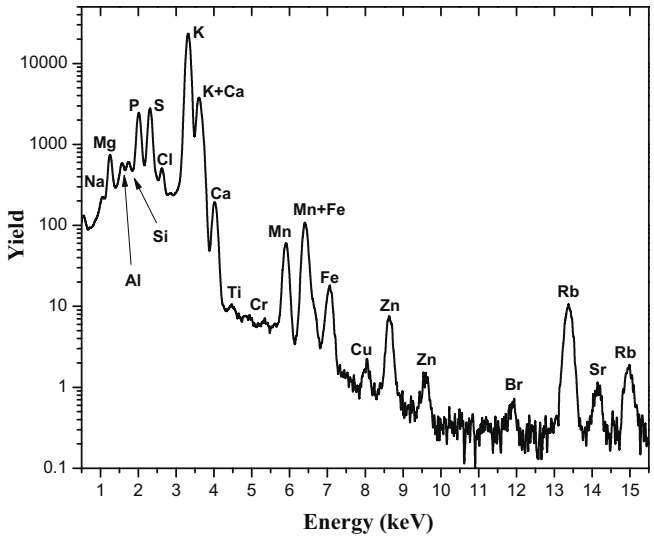

Fig. 2. Typical PIXE spectrum from a Cabernet Sauvignon wine (2002 vintage) as a function of the X-ray energy (in keV).

Potassium metabisulfite ($K_2S_2O_5$) and sulphur dioxide (SO_2) are commonly added during the winemaking process due to their antibacterial and antioxidant properties. The content of sulphur dioxide in wine varies between 12 and 64 mg/L (Jackson, 2008). The sulphur content measured in this work agrees with the values quoted by Anjos et al. (2003).

Calcium, magnesium and phosphorus also appear with substantial concentrations in wine. Except for phosphorus, these elements are abundant in soil as well. In particular, calcium carbonate ($CaCO_3$) is usually added to wine in order to balance acidity and therefore might contribute to its concentration in wine. Moreover, it is important to bear in mind that lime (CaO) is part of a fungicide known as *Bordeaux Mixture* and therefore it constitutes a potential source of calcium as well. The Ca level for 2002 Cabernet Sauvignon vintage measured in this work was found to be lower than previous vintages (Rizzon & Miele, 2007). Higher Ca contents (in the range of 58–93 mg/L) in other Brazilian red wines were also observed (Anjos et al., 2003). Conversely, the Ca concentration in red wines from Argentina (Lara, Cerutti, Salonia, Olsina, & Martinez, 2005) was found to be even lower (10–15 mg/L) than the results observed in this work.

Table 1
Physicochemical variables analysed for 2002 Cabernet Sauvignon wines. Our results are compared to those obtained by Souza et al. (2005) and by Rizzon and Miele (2007).

Physicochemical variable	Cabernet Sauvignon vintage 2002 ($n = 6$)		Souza et al. Vintage 1999 ($n = 4$)	Rizzon et al. Vintage 1999	Rizzon et al. Vintage 2000	Rizzon et al. Vintage 2001	Rizzon et al. Average 99/00/01
	Mean ± sd	Range	Mean ± sd	Mean	Mean	Mean	Mean
Total acidity (meq/L)	90 ± 13	83–116	78.6 ± 6.4	88.4	73.4	75	78.9
Volatile acidity (meq/L)	14 ± 5	10–22	10.4 ± 1.3	8.3	8.8	11.3	9.5
Alcohol (% v/v)	12.9 ± 0.5	12.3–13.6	12.0 ± 0.3	9.92	10.5	10	10.14
Reducing sugars (g/L)	2.3 ± 0.3	2.0–2.6	–	2.03	2.31	2.3	2.21
Density (mg/mL)	0.9954 ± 0.0012	0.9941–0.9975	–	0.9962	0.995	0.9964	0.996
Dry extract (g/L)	32 ± 3	28–35	–	20.69	19.7	20.8	20.4
Reduced dry extract (g/L)	30.9 ± 2.3	28.4–34.3	–	19.67	18.39	19.5	19.19
pH	3.67 ± 0.09	3.57–3.81	3.9 ± 0.1	3.49	3.59	3.8	3.63
Alcohol/reduced dry extract	3.4 ± 0.4	2.9–3.5	–	4.04	4.55	4.1	4.23
SO_2 free (mg/L)	9 ± 6	2–18	–	–	–	–	–
SO_2 total (mg/L)	38 ± 19	12–60	57 ± 27	–	–	–	–

Table 2
Elemental concentration of 2002 Cabernet Sauvignon wines from different regions of the state of Rio Grande do Sul (see Fig. 1 for details). The results of the mean and their respective standard deviation (sd) are expressed in mg/L.

Element	Vale (n = 34)		Serra (n = 8)		Campanha (n = 8)		Centro (n = 4)		All wines	
	Mean ± sd	Range	Mean ± sd	Range	Mean ± sd	Range	Mean ± sd	Range	Mean ± sd	Range
Mg	56 ± 12	31–77	69 ± 19	33–98	66 ± 15	51–98	42 ± 3	40–51	58 ± 15	31–98
Al	3.2 ± 1.4	⩽LOD–6.1	2 ± 1	⩽LOD–3.4	2.7 ± 1.5	1.0–5.4	⩽LOD	0.7–1.2	2.2 ± 1.5	⩽LOD–6.1
Si	4 ± 1	2.4–6.9	4.1 ± 1.2	2.4–6.2	4.7 ± 1.5	2.7–7.0	3.8 ± 0.2	3.3–4.0	4.1 ± 1.0	2.4–7.0
P	91 ± 22	50–142	105 ± 30	63–135	88 ± 23	56–130	72 ± 6	62–89	89 ± 24	50–142
S	85 ± 21	55–139	94 ± 35	43–154	78 ± 13	62–98	75 ± 5	65–88	83 ± 22	43–154
Cl	14 ± 4	8–22	13 ± 4	7–16	14.5 ± 1.1	13–16	8.4 ± 0.5	7.4–9.6	12 ± 4	7–22
K	1032 ± 185	589–1309	1105 ± 283	641–1423	1081 ± 156	872–1331	786 ± 34	736–886	1001 ± 203	589–1423
Ca	40 ± 12	24–71	45 ± 13	24–59	51 ± 9	37–61	31 ± 1	29–33	42 ± 12	24–71
Mn	1.7 ± 0.4	0.8–2.9	1.7 ± 0.6	1.2–2.9	1.7 ± 0.2	1.4–2.0	1.4 ± 0.1	1.1–1.5	1.6 ± 0.4	0.8–2.9
Fe	2.3 ± 1.1	1.1–6.4	2.7 ± 0.4	2.2–3.5	2.8 ± 0.8	1.7–3.7	3.8 ± 1.1	1.7–5.9	2.9 ± 1.2	1.1–6.34
Cu	0.08 ± 0.04	⩽LOD–0.19	⩽LOD	⩽LOD–0.08	0.11 ± 0.07	⩽LOD–0.25	0.09 ± 0.04	⩽LOD–0.13	0.09 ± 0.05	⩽ LOD–0.25
Zn	0.56 ± 0.29	0.15–1.64	0.56 ± 0.20	0.27–0.78	0.49 ± 0.25	0.15–0.86	0.52 ± 0.03	0.48–0.6	0.53 ± 0.26	0.15–1.64
Br	⩽LOD	⩽LOD–0.34	⩽LOD	⩽LOD–0.37	⩽LOD	⩽LOD–0.50	⩽LOD	⩽LOD	⩽LOD	⩽LOD–0.50
Rb	4.0 ± 1.2	1.1–6.6	3.6 ± 1.5	1.3–5.8	3.3 ± 1.5	1.9–6.7	2.6 ± 0.3	1.9–3.3	3.4 ± 1.3	1.1–6.7
Sr	⩽LOD	⩽LOD–1.65	⩽LOD	⩽LOD–0.86	⩽LOD	⩽LOD	⩽LOD	⩽LOD	⩽LOD	⩽LOD–1.65

Table 3
Comparison of the elemental concentrations obtained in this work with those obtained by Anjos et al. (2003) (Brazilian red wine), by Lara et al. (2005) (Argentine red wine), by Alvarez et al. (2007) (Montilla–Morilez Spanish wine), and by Ough et al. (1982) (Californian red wine). All results are expressed in mg/L.

Element	This work (n = 54)		Anjos et al. (n = 13)	Lara et al. (n = 10)	Alvarez et al. (n = 50)		Ough et al. (n = 124)	
	Mean ± sd	Range	Range	Range	Mean ± sd	Range	Mean ± sd	Range
Mg	58 ± 15	31–98	–	–	72.1 ± 9.4	56.7–93.4	129 ± 26	72–245
Al	2.2 ± 1.5	⩽LOD–6.1	–	0.017–0.018	1.9 ± 0.6	0.6–3.1	–	–
Si	4.1 ± 1.0	2.4–7.0	–	–	–	–	–	–
P	89 ± 24	50–142	31.5–87.6	–	72.4 ± 8.2	54.6–88.0	–	–
S	83 ± 22	43–154	70.9–147.6	–	–	–	–	–
Cl	12 ± 4	7–22	13.9–77.8	–	–	–	–	–
K	1001 ± 203	589–1423	–	–	747 ± 130	470–1014	1102 ± 325	125–1750
Ca	42 ± 12	24–71	57.6–92.8	10–15	65.4 ± 16.7	44.7–104.0	79 ± 34	25–310
Mn	1.6 ± 0.4	0.8–2.9	0.8–2.5	–	0.8 ± 0.2	0.1–1.1	–	–
Fe	2.9 ± 1.2	1.1–6.34	1.7–5.2	0.48–0.79	3.7 ± 2.0	0.7–7.2	3.8 ± 1.6	1.3–9.0
Cu	0.09 ± 0.05	⩽LOD–0.25	<LOD–0.4	0.023–0.028	0.24 ± 0.21	0.02–0.82	0.17 ± 0.18	0.03–1.40
Zn	0.5 ± 0.3	0.15–1.64	0.2–1.3	0.024–0.130	0.50 ± 0.30	0.08–1.18	0.93 ± 0.74	0.14–5.60
Br	0.25 ± 0.09	⩽LOD–0.50	–	–	–	–	–	–
Rb	3.4 ± 1.3	1.1–6.7	1.8–4.6	–	–	–	–	–
Sr	0.6 ± 0.3	⩽LOD–1.65	0.4–1.0	–	0.7 ± 0.2	0.35–1.04	–	–

Magnesium and phosphorus are essential micronutrients in plants. Together with calcium they contribute to the structure of cell walls, while magnesium is also a key element in the chlorophyll molecule. The phosphorus content reached values as high as 142 mg/L while the overall average is 89 mg/L. On the other hand, magnesium reached much lower values (see Table 2). The Mg content obtained by Rizzon and Miele (2007) for 1999, 2000 and 2001 vintages fall within the range observed in this work although the Mg content reported here tends to be lower. Moreover our result for Mg is about half of those obtained for California red wines (Ough, Crowell, & Benz, 1982) and for Melbourne white wines (Sauvage, Frank, Stearne, & Millikan, 2002).

Chlorine is a highly volatile element, which hampers its accurate measurement. Therefore our results for this element must be taken with caution despite we have no indication of any losses during the thermal treatment of the samples. Our results indicate that chlorine contributes with 12.5 mg/L and therefore stands between the major and minor elements in wine. The Cl level measured in this work is compatible with the lower limit observed by Anjos et al. (2003) for Brazilian red wines.

The average concentration of minor elements such as Si, Rb, Fe, Al and Mn ranged between 1.6 and 4.1 mg/L. Sub-milligram concentrations were observed for Sr, Zn, Br and Cu. Most of these elements are found in soil as well, with particularly high concentrations for Al, Si and Fe (Giulian et al., 2009; Marengo & Aceto, 2003). Direct contact with different substances and equipment along the wine making process may contribute to the concentrations measured in this work. For instance, the small presence of Cu in wines may be due to surface contamination coming from the use fungicides such as *Bordeaux Mixture* which contains copper sulphate ($CuSO_4$). Moreover it appears that the concentration of Rb in wine might be related to the contact with the grape skin during the making process (Noble, Orr, Cook, & Campbell, 1976). Finally, it is worth mentioning that the values found in this work for Mn, Fe, Zn and Rb are within the ranges reported by Anjos et al. (2003). Particularly for Fe, Cu and Zn the values reported here are systematically lower than those reported by Ough et al. (1982) for Californian red wines.

A singular case was observed for Cr. It was detected only in some samples from Centro wines with a mean concentration of 0.13 mg/L. In general, chromium found in foodstuff has its origin in soil contamination, production practices and technologies like the contact with stainless steel. It can also be related with metallic oxides used in bottles. However, a potential source of chromium to be considered is the leather industry which makes extensive use of chromium-based chemicals for leather tanning and is responsible for most of the solid waste containing this element. Since several small family-based leather industries are located close to Centro

region, the possibility of contamination cannot be discarded. Indeed, if we compare our results with those found e.g. in French red wines (ranging from 7 to 90 μg/L according Vique, Teissedre, Canabis, and Canabis (1997)) our results can be considered high.

In general, comparison of elemental concentrations among wines from Vale dos Vinhedos, Serra Gaúcha and Campanha Gaúcha (Table 2) shows a high degree of compatibility. However, for some elements such as Mg, Al, Cl, K and Ca, the Centro wines tend to have lower concentrations. Moreover, the statistical analysis showed that the content of these elements in Centro wines are significantly different from wines of other regions.

In order to have a broader perspective of the results obtained in this work we can compare our results with those obtained for some European countries (Pohl, 2007). For instance, the concentrations of Mg, Cu and Zn found in the present work stand in the lower limit of the European range. Fe, Mn and Sr fall within the European range while Al and particularly Rb are well above the European range. Moreover, the intrinsic variability (C.V.%) of some elements observed for Vale dos Vinhedos wines are comparable to European ones. For instance, the intrinsic variability for K (18%) and Zn (46%) are similar to those observed by Alvarez et al. (2007) with Montilla–Moriles wines which bear a D.O. certificate.

3.2.2. Comparison among Vale dos Vinhedos wines

Table 4 shows the elemental concentration of Vale dos Vinhedos Cabernet Sauvignon wines (2002 vintage) from eight different trademarks (labelled as wine 1 through 8). The overall averages of the limits of detection (LOD) are shown in this table as well. The LOD values achieved in this work vary smoothly with atomic number and attain its minimum between $Z = 25$ (Mn) and $Z = 30$ (Zn).

In general, there are significant differences in the elemental concentration among wines from this region for some elements. The content of elements such as P and K are significantly different among several wines. The potassium level of wine 4 is significantly lower than the contents in wines 1, 2, 3, 6 and 8, while the phosphorus level of wine 2 is significantly higher than wines 1, 4, 7 and 8. On the other hand, elements such as Mg, Rb, Cu and Fe presented practically no differences among wines.

Although all wines from Vale dos Vinhedos considered in this work comply with the Regulatory Committee requirements, each winery has its own procedures concerning cultivation, wine elaboration and production. In fact, Zoltán and Papp (1997) have shown that the elemental concentrations are affected not only by the climatic conditions but also by cultural practices used in the vineyard, winemaking process and viticulture technology. Taking into account that Vale dos Vinhedos is a small region geographically limited according to its topographic characteristics, such differences may reflect the different methods in the wine making process employed by each winery.

4. Conclusions

The elemental composition and physicochemical parameters of wine are related with several others variables such as climate, soil, cultural practices and winemaking process. These variables change according to the production region and, therefore, differences in the elemental composition are usually observed. Moreover, our results indicate that wines produced in small regions such as Vale dos Vinhedos may have differences in the metal concentrations. If we assume that the climate is basically the same in this microregion then the results obtained in this work reflect differences in winemaking processes adopted by each winery.

Since metals such as Mn, Fe, Cu and Zn are considered essential micronutrients due to their role as metalloenzymes, wine becomes an important dietary source of these and other elements. However, the abusive ingestion of such elements may lead to health problems related to intoxication. In a very recent survey carried out by Naughton and Petroczi (in press) the Target Hazard Quotient (THQ) was evaluated for wines from several European countries and others including Brazil. The THQ provides a rough estimation of the potential risk associated to long term exposure to chemical pollutants. Their results suggest that among several countries, wines from Italy, Brazil and Argentine would pose lower risk due to their relatively low concentration of some elements. In the present work we observed lower concentrations of Cu and Zn when compared to those of some European wines (Pohl, 2007). Certainly a more comprehensive data base is needed to draw any definite conclusion and hopefully the present work will help to shed more light on this matter.

Our results should be considered as an initial survey of a more comprehensive study including different grape varieties and expanding the studies to other regions in southern Brazil. A follow-up of the whole vinification procedure would provide details about the elemental balance of each step in the winemaking process up to the final product. A more comprehensive study including a substantial increase in the number of samples would facilitate the establishment of elemental contents which are influenced by factors such as grape growth, soil type, grape variety and wine making processes. Moreover, such study would probably allow to establish a correlation between the physicochemical variables and the elemental concentrations. These studies could provide further support to fulfil the O.I.V. requirements in order to obtain the

Table 4
Elemental concentration for 2002 Cabernet Sauvignon wines from Vale dos Vinhedos. The results of the mean and their respective standard deviation (sd) are expressed in mg/L. The limits of detection (LOD) are also expressed in mg/L.

Element	Wine 1 Mean ± sd	Wine 2 Mean ± sd	Wine 3 Mean ± sd	Wine 4 Mean ± sd	Wine 5 Mean ± sd	Wine 6 Mean ± sd	Wine 7 Mean ± sd	Wine 8 Mean ± sd	LOD Mean ± sd
Mg	42 ± 8	57 ± 8	53 ± 8	59 ± 13	54 ± 20	63.2 ± 3.6	56 ± 6	66 ± 8	3.2
Al	3.3 ± 0.3	3.6 ± 2.3	4.0 ± 0.3	3.1 ± 0.1	⩽LOD	⩽LOD	4.6 ± 0.1	4.1 ± 0.5	1.9
Si	4.2 ± 0.5	4.2 ± 0.7	5.5 ± 1.1	3.5 ± 1.3	3.5 ± 0.2	4.4 ± 0.3	3.3 ± 0.2	3.9 ± 0.4	0.5
P	89 ± 9	122 ± 21	89 ± 12	65 ± 12	101 ± 28	109 ± 3	80 ± 9	68 ± 11	1.3
S	72 ± 7	87 ± 19	86 ± 36	94 ± 20	97 ± 34	76 ± 10	71 ± 5	101 ± 16	0.8
Cl	20 ± 2	12 ± 2	15 ± 2	10 ± 2	14 ± 3	11.8 ± 1.4	11 ± 3	14.2 ± 1.2	1.0
K	1093 ± 86	1150 ± 176	1082 ± 84	740 ± 116	850 ± 166	1222 ± 57	946 ± 131	1143 ± 40	0.6
Ca	34 ± 5	44 ± 9	46 ± 17	40 ± 21	46 ± 16	37 ± 2	42 ± 7	32.7 ± 1.9	0.7
Mn	2.0 ± 0.3	1.7 ± 0.4	1.52 ± 0.14	1.84 ± 0.04	1.4 ± 0.5	1.30 ± 0.12	1.50 ± 0.27	2.4 ± 0.3	0.08
Fe	2.1 ± 0.4	1.5 ± 0.3	3.4 ± 2.0	2.2 ± 0.6	3.2 ± 1.9	2.0 ± 0.4	1.7 ± 0.7	2.0 ± 0.4	0.09
Cu	⩽LOD	⩽LOD	0.08 ± 0.05	⩽LOD	⩽LOD	0.09 ± 0.05	0.12 ± 0.07	0.08 ± 0.02	0.07
Zn	0.8 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	0.36 ± 0.05	0.5 ± 0.1	1.0 ± 0.5	0.28 ± 0.03	0.3 ± 0.1	0.1
Br	⩽LOD	⩽LOD	⩽LOD	⩽LOD	⩽LOD	⩽LOD	⩽LOD	⩽LOD	0.3
Rb	5.3 ± 1.3	4.7 ± 0.6	3.2 ± 0.9	4.1 ± 0.8	4.3 ± 1.0	3.8 ± 0.9	3.0 ± 0.7	3.2 ± 1.5	1.4
Sr	⩽LOD	⩽LOD	0.9 ± 0.7	⩽LOD	⩽LOD	⩽LOD	1.0 ± 0.3	⩽LOD	0.7

Appellation d'Origine Contrôlée certificate for wines from Vale dos Vinhedos.

Finally, the PIXE technique proved to be a powerful technique providing reliable results with the advantage that samples could be preserved for further analysis. The quantitative values obtained for these elements were in good agreement with those provided by other techniques.

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ANEXO III

Determinação de compostos fenólicos em bioativos de uva *Vitis vinifera* utilizados na indústria cosmética e avaliação de sua atividade antioxidante

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Vários efeitos benéficos à saúde, como a atividade antioxidante, têm sido atribuídos aos compostos fenólicos presentes na uva (*Vitis vinifera*), com isso os seus bioativos apresentam benefícios similares. Neste artigo, são apresentadas a composição fenólica e atividade antioxidante dos bioativos da uva utilizados como matéria-prima na indústria cosmética.

Beneficios a la salud, como la actividad antioxidante, se han atribuido a los compuestos fenólicos presentes en la uva (*Vitis vinifera*), por lo que sus compuestos bioactivos tienen beneficios similares. Este artículo presenta la composición fenólica y la actividad antioxidante de la uva bioactivos utilizado como materia prima en la industria cosmética.

Several health benefits such as antioxidant activity, have been attributed to phenolic compounds present in grape (*Vitis vinifera*), thus their bioactive compounds have similar benefits. This article presents the phenolic composition and antioxidant activity of grape's bioactive used as raw material in the cosmetic industry.

Introdução

Inúmeros são os benefícios à saúde humana associados ao consumo de frutas que possuam em sua composição compostos fenólicos, capazes de agir como queladores de íons metálicos e/ou seqüestradores de espécies reativas, o que justifica a atividade antioxidante destes compostos.[1]

Entre algumas frutas ricas em compostos fenólicos, destaca-se a uva que pode ser consumida *in natura* ou utilizada como matéria-prima na fabricação de vinhos, sucos e geléias.[2] Em um estudo, determinou-se o potencial antioxidante de frutas, onde a uva apresentou a maior atividade antioxidante quando comparada com laranja, maçã e morango.[3] Muitas pesquisas têm sido realizadas avaliando os efeitos antioxidantes dos compostos fenólicos presentes no vinho, entretanto, alguns autores verificaram, em sucos de uva, atividade antioxidante similar à encontrada em vinhos tintos.[4,5]

O processo de fabricação do vinho gera uma quantidade de subprodutos (semente, casca, engaço) que corresponde a 20% do peso inicial da uva onde as sementes representam em torno de 15%. A partir das sementes, cascas e engaços podem ser extraídos/preparados o óleo de semente de uva, o extrato de semente de uva, o leite de uva e o extrato de vinho, esse obtido após o preparo do vinho, que podem ser processados e reutilizados como matéria-prima pela indústria farmacêutica e cosmética.[6]

O extrato de semente de uva é um constituinte natural da uva e contem lipídios, proteínas, carboidratos e polifenóis. O óleo extraído das sementes de uva contém os ácidos palmítico, palmitoléico, esteárico, oléico, linoléico, assim como os ácidos alfa linoléicos, icosanóico e docosanóicos, fornecendo a este produto alta atividade hidratante e emoliente.[6] Tanto o extrato quanto o óleo de semente de uva da espécie *Vitis vinifera* possuem bioflavonóides, um complexo conhecido como oligômeros de procianidinas (OPC) que contribui para a ampla utilização destes compostos na indústria cosmética, sendo encontrados em cremes hidratantes, xampus, óleos de banho, sabonetes entre outros.

As procianidinas são polímeros de alto peso molecular composto de dímeros ou trímeros de (+)-catequina e (-)-epicatequina (Figura 1).[7] Essa família de moléculas polifenólicas é capaz de combater os radicais livres que contribuem para a formação das linhas de expressão, manchas e outros sinais do tempo. Esta atividade antioxidante está relacionada com a capacidade doadora de elétrons desta substância aos radicais livres neutralizando-os e inativando-os.[8,9] A atividade antioxidante das procianidinas é vinte vezes

mais potente do que a do ácido ascórbico e cinquenta vezes mais potente do que a atividade do tocoferol.[7] As procianidinas podem proteger e reestruturar a pele, estimulando a formação das fibras de colágeno e de elastina e prevenindo a degradação por inativação dos radicais livres formados.[7, 10,11]

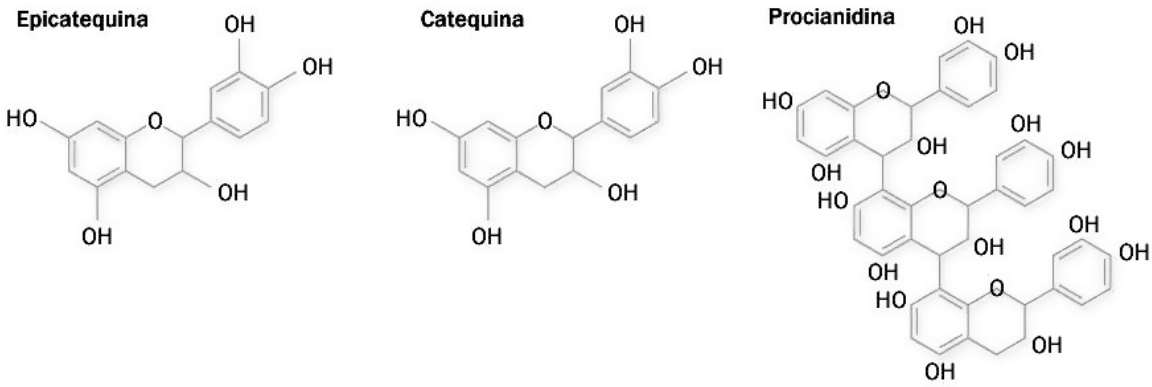

Figura 1. Estrutura da epicatequina, catequina e procianidina.[12]

O envelhecimento celular é provocado por fatores endógenos (cadeia respiratória, má alimentação, fagocitose) e/ou fatores exógenos (poluição, radiação solar, etc.), que causam manchas na pele, doenças dermatológicas, câncer e prejudicam o aspecto das fibras capilares.[13,14] Durante este processo, ocorre a diminuição da solubilidade e a alteração das propriedades físicas do colágeno. Há um acúmulo no número de ligações covalentes cruzadas, provocando aumento na rigidez e na perda da elasticidade do tecido conjuntivo.[11] Além disso, a elastina mostra uma diminuição progressiva no seu conteúdo de lisina e de ácido glutamínico, acarretando a perda da elasticidade cutânea. Também ocorre diminuição da sua produção devido à redução da atividade metabólica e do número de fibroblastos.[11]

Os antioxidantes, presentes nos bioativos da uva (polifenóis), têm um papel vital neste processo, pois auxiliam na minimização destes danos, protegendo a pele e mantendo-a saudável ao longo do tempo. Estudos demonstraram que os polifenóis da uva agem de forma expressiva inibindo os danos oxidativos causados pela radiação solar, prevenindo, desta forma, o envelhecimento precoce e o câncer de pele causado principalmente pela exposição exagerada ao sol.[11,15]

Ainda existe outra preparação provida de subprodutos da uva, o leite de uvas, que consiste em uma emulsão contendo o extrato de semente de uva e o óleo de semente de uva de *Vitis vinifera* e, além disso, lipídeos, proteínas e açúcares da uva. Este produto combina os

efeitos benéficos do extrato com o óleo de semente de uva podendo apresentar atividade antioxidante, e por sua vez, ser incorporado em cosméticos.[16]

Já o extrato de vinho não pode ser considerado como subproduto, pois é obtido após a elaboração do vinho. Através do vinho francês (*Vitis vinifera*) retira-se o álcool e concentra-se o produto garantindo o conteúdo de compostos fenólicos.[17] O extrato de vinho contém ácidos málico e tartárico, sais minerais e compostos fenólicos, como ácidos, fenóis, catequina e tirosol. Estudos demonstram a capacidade desta matéria-prima em combater o envelhecimento capilar, regenerando os fios e ainda mantendo a cor natural ou artificial dos cabelos por mais tempo, sendo utilizado em xampu, máscara capilar, condicionador, entre outras formulações para o cabelo.[17]

Existe um grande apelo para produtos cosméticos com antioxidantes e para isto, testes devem ser realizados para comprovar a atividade proposta nesses produtos. Os testes para a avaliação da atividade antioxidante têm como objetivo detectar produtos ou alterações fisiológicas provocadas por estresse oxidativo. Diferentes metodologias têm sido desenvolvidas para obter uma medição qualitativa ou quantitativa, da capacidade antioxidante de substâncias, com base em testes químicos (sem culturas celulares/ *in vitro*) ou utilizando testes biológicos (culturas celulares/ *in vivo*).[1]

Dentre os testes *in vitro* existentes, a medida de capacidade de varredura do radical $DPPH^{\bullet}$ vem sendo bastante utilizada[1, 18]. Os ensaios *in vitro* geralmente são testes rápidos, sensíveis, econômicos, reprodutíveis, de fácil de execução e apresentam resultados confiáveis na identificação da atividade biológica.[18,19]

Neste contexto, este trabalho tem por objetivo determinar e quantificar compostos fenólicos presentes no extrato e no óleo de semente de uva, no leite de uva e no extrato de vinho, bem como avaliar a atividade antioxidante destas matérias-primas.

Materiais e Métodos

Amostras

As amostras utilizadas para o desenvolvimento deste trabalho foram Extrato de Semente de Uva (ESU) (Vital Especialidades, São Paulo/SP), Óleo de Semente de Uva (OSU) (Lipo do

Brasil, Socorro/SP), Extrato de Vinho Francês (EV) (Brasquim, São Paulo/SP) e Leite de Uvas Verdes e Vermelhas (LUV) (Oh!, São Paulo/SP).

Reagentes

DPPH$^{\bullet}$ adquirido da Sigma-Aldrich (Alemanha), Metanol P.A. (Vetec, Brasil.), Etanol P.A. (Synth, Brasil), Acetona (Vetec, Brasil), Ácido Clorídrico (Vetec, Brasil), Ácido Caféico (Merck, Alemanha), Catequina (Merck, Sigma-Aldrich, Alemanha) e Tampão acetato de sódio (Vetec, Brasil).

Extração de Compostos Fenólicos

Para a determinação de compostos fenólicos e da atividade antioxidante *in vitro* nas amostras de óleo de semente de uva e leite de uva estas forma submetidas a um tratamento prévio, possibilitando a separação dos componentes. Dessa forma, pesou-se 20g de cada amostra e a estas adicionou-se 10mL de acetona, 20mL de metanol/água (60:40, v/v) e 1mL de ácido clorídrico, agitou-se em vórtex por 10 minutos e em seguida centrifugou-se a mistura por 10 minutos a 5366*g*. A fase metanólica foi separada e repetiu-se a extração por mais duas vezes e para as análises foi utilizada esta mesma fase.[20,21] Para as amostras de extrato de semente de uva e extrato de vinho não foi necessária a realização deste tratamento.

Para verificar se a extração dos compostos fenólicos nas amostras foi efetiva realizou-se a mesma metodologia com o óleo de semente de uva e leite de uva acrescentado de 0,02g de padrão catequina.

Determinação de compostos fenólicos

Polifenóis totais (PT) e hidroxicinamatos totais (HCT) foram quantificados nos bioativos da uva através da medida das absorbâncias a 280nm e 320nm (espectrofotômetro Shimadzu UV-1700, Japão), respectivamente. Os resultados de polifenóis totais foram expressos em g/L de catequina e os de hidroxicinamatos em g/L de ácido caféico baseados em uma curva padrão das respectivas substâncias. Os flavonóides totais (FT) foram calculados utilizando-se a seguinte fórmula: Flavonóides totais = [A280 – 4] – (0,66) x [A320 – 1.4] [22], e expressos em g/L de catequina.

Para determinar a quantidade total de oligômeros de procianidinas (OPC) e antocianinas totais (AT) nos bioativos da uva, realizou-se inicialmente a hidrólise ácida, no entanto para o óleo de semente de uva e o leite de uva, fez-se este procedimento após o tratamento prévio

descrito acima, e em seguida, mediu-se a absorbância a 520nm em espectrofotômetro (Shimadzu UV-1700, Japão) e expressou os resultados em g/L de antocianinas e oligômeros de procianidinas.[23] Todas as análises foram realizadas em triplicata.

Determinação da atividade antioxidante *in vitro*

Para determinar a capacidade de varredura do radical livre estável ($DPPH^{\bullet}$) em bioativos da uva, foram misturados 200 μL das amostras com 800 μL de solução tampão acetato de sódio 100 mM, pH 7,0. A essa mistura foram adicionados 1000 μL da solução etanólica de $DPPH^{\bullet}$ (250 μM) para o extrato de semente de uva e para o extrato de vinho, ou solução metanólica de $DPPH^{\bullet}$ (250 μM) para o produto de extração derivado do óleo de semente de uva e leite de uva e os tubos foram mantidos por 20 minutos ao abrigo da luz. A medida da absorbância foi feita em espectrofotômetro UV-visível a 517 nm. Para o branco, as amostras foram substituídas por água destilada. Foram realizadas três repetições para cada concentração (100%, 50%, 25%, 10%, 5% e 2,5%) e os resultados expressos em IC_{50} (concentração percentual capaz de reduzir 50% do radical livre $DPPH^{\bullet}$).[24, 25] Para a comparação dos resultados utilizou-se como padrão o ácido ascórbico e a catequina que foram submetidos à mesma metodologia descrita a cima.

Análise Estatística

Os dados foram expressos em média ± desvio padrão e submetidos à análise estatística específica utilizando-se o programa SPSS 16.0 for *Windows*.

Resultados e Discussão

Extração de Compostos Fenólicos

Para a identificação e isolamento de compostos bioativos em fontes naturais (como frutas, sementes e óleos) é necessária a realização da extração com solventes de polaridades diferentes[26], portanto os solventes orgânicos são freqüentemente utilizados. O rendimento da extração e a determinação da atividade antioxidante dos extratos dependem do tipo de solvente utilizado, dos compostos antioxidantes presentes nos bioativos e da polaridade dos compostos.[26,27]

A extração de substâncias antioxidantes com solventes orgânicos pode ser eficiente para alguns casos, porém torna-se agressiva ao ambiente devido aos resíduos gerados durante o processo. Também exige controle rigoroso de fatores como, a polaridade do solvente

utilizado, o tempo e a temperatura de extração, que se não controlados podem gerar perda ou destruição dos compostos antioxidantes.

A eficácia para o método de extração dos compostos fenólicos a partir do óleo e leite de uva foi testada através da adição de uma quantidade conhecida do padrão catequina, a estes compostos. As misturas formadas foram avaliadas pela metodologia proposta e obteve-se recuperação total da catequina. Na Tabela 1, pode-se verificar a recuperação de 0,033g de catequina no bioativo óleo de semente de uva e 0,023g no leite de uva.

Tabela 1. Compostos fenólicos no óleo de semente de uva e leite de uva com e sem adição do padrão catequina.

Bioativos	**PT ± DP*** **(g/L de catequina)**	**FT ± DP** **(g/L de catequina)**	**HCT ± DP** **(g/L de ácido caféico)**
OSU	4,86 ± 0,015	0,856 ± 0,004	0,309 ± 0,001
OSU + C	4,89 ± 0,014	0,858 ± 0,002	0,308 ± 0,001
LUV	5,59 ± 0,004	0,606 ± 0,000	0,264 ± 0,004
LUV + C	5,61 ± 0,006	0,603 ± 0,003	0,265 ± 0,003

DP* = Desvio padrão. Onde: PT = Polifenóis Totais; FT = Flavonóides Totais; HCT = Hidroxicinamatos Totais; OSU = Óleo de semente de uva; C = Catequina; LUV = Leite de Uva.

Determinação de compostos fenólicos

Os bioativos da uva também são fontes de várias combinações de compostos fenólicos que despertam muito interesse devido a suas propriedades antioxidantes e seus efeitos benéficos para a saúde humana.[18,28] A Figura 1 expressa a concentração de compostos fenólicos no extrato de semente de uva. Pode-se observar que os compostos fenólicos mais encontrados no extrato são os polifenóis totais apresentando 5,330 ± 0,002 g/L de catequina. O extrato também apresentou flavonóides totais (0,360 ± 0,010 g/L de catequina) e hidroxicinamatos (0,067 ± 0,000 g/L de ácido caféico).

Concentrações similares às encontradas em nosso estudo foram obtidas em um recente trabalho, onde foi realizada a caracterização química de extratos de semente e de casca de uva e obteve-se 4 g/L de compostos fenólicos expressos em ácido gálico[29]. Outro estudo realizado com o extrato de semente de uva obteve cerca de 1 a 4 g/L de ácido gálico.[30] Esta similaridade nos resultados comprovam a presença destes compostos fenólicos no extrato de semente de uva.

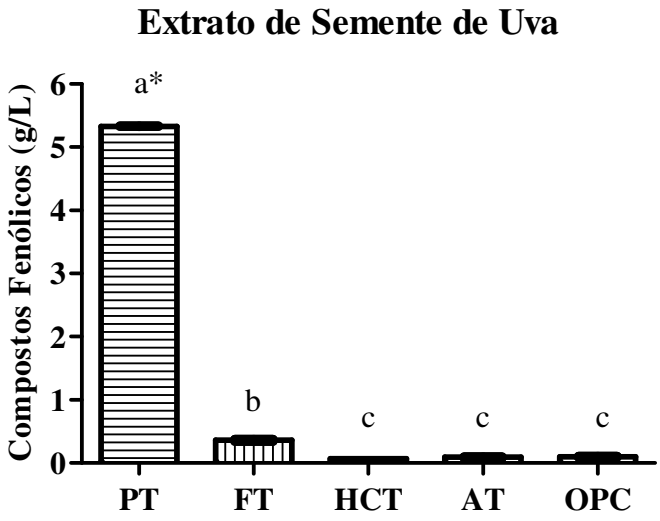

Figura 1. Compostos Fenólicos presentes no Extrato de Semente de Uva. Onde: PT = Polifenóis Totais (g/L de catequina); FT = Flavonóides Totaís (g/L de catequina); HCT = Hidroxicinamatos Totais (g/L de ácido caféico); AT = Antocianinas Totais (g/L); OPC = Oligômeros de Procianidinas (g/L).*Letras distintas diferem significativamente pelo pós teste de *Tukey* (para $p \leq 0{,}001$).

Na literatura, existem inúmeros estudos relacionados ao extrato de semente de uva que confirmam a presença de alto teor de compostos fenólicos nesta matéria-prima.[31,32] Os principais compostos fenólicos encontrados em extrato de semente de uva são catequina, epicatequina, epicatequina-3-O-galato e ácido caféico[33].

Em um estudo realizado com extrato de semente de uva *Vitis vinifera* providas do sul da Grécia, foi obtida uma concentração similar as amostras testadas neste trabalho em relação à hidroxicinamatos, 0,083 g/L de ácido caféico.[32] Com isso, podemos confirmar a presença de hidroxicinamatos no extrato de semente de uva.

Além dos compostos fenólicos citados anteriormente, o extrato apresentou 0,091 ± 0,002 g/L de antocianinas totais e 0,098 ± 0,003 g/L de oligômeros de procianidinas. Um estudo realizou a obtenção do extrato de semente de uva por ultrasom[34]. Com a ajuda deste equipamento e do líquido extrator (etanol), obteve-se cerca de 2g/L de antocianinas. Essa diferença pode ser devido aos diferentes métodos utilizados para obter o extrato de semente de uva, onde o utilizado neste trabalho foi obtido através de extração a frio com sementes de uva moídas e utilizou-se como solventes água e propilenoglicol.

Em outro estudo, também foi realizada a obtenção do extrato através de sementes de uva e etanol como líquido extrator e, em seguida, realizou-se a quantificação de oligômeros de procianidinas através de cromatografia líquida de alta eficiência (CLAE), onde obtiveram 2,83 g/L de oligômeros de procianidinas, um resultado elevado quando comparado com o

obtido neste trabalho, devido a diferentes metodologias de obtenção do extrato de semente de uva.[35]

Nos artigos citados a cima, além da obtenção do extrato de semente de uva ter sido realizado por metodologias diferenciadas, não se pode descartar a possibilidade de que os extratos obtidos possam apresentar essa diferença devida provir de uvas da mesma espécie (*Vitis vinifera*), porém cultivadas em solos, climas e variedades diferentes e que isso pode influenciar tanto a composição fenólica quanto mineral.

A Figura 2 apresenta as concentrações dos compostos fenólicos no óleo de semente de uva, sendo os mais abundantes os polifenóis totais com 4,860 ± 0,015 g/L de catequina, seguido pelos flavonóides totais, com 0,856 ± 0,004 g/L de catequina e hidroxicinamatos com 0,309 ± 0,001 g/L de ácido caféico. Além destes compostos, apresentaram 0,088 ± 0,000 g/L de antocianinas totais e 0,144 ± 0,000 g/L de oligômeros de procianidinas.

Existem inúmeros artigos sobre óleo de semente de uva, porém poucos relacionados à sua composição fenólica. Em um estudo, realizou-se a identificação de compostos fenólicos presentes neste bioativo, onde foi obtido 0,74 g/L de catequina e 0,0169g/L de ácido caféico[6]. Esses resultados demonstram que o processo de extração é importante no que diz respeito ao conteúdo extraído. Com isso, pode-se dizer que o método de extração utilizado neste trabalho foi eficaz por ter possibilitado a extração de um teor mais elevado de compostos fenólicos do que outro estudo realizado em 2009 onde foram utilizados diferentes solventes para a extração, metanol: ácido clorídrico 0,1% (v/v) e etanol: água (3:1, v/v) [6].

A Figura 3 demonstra a quantidade de compostos fenólicos presentes no Leite de Uva. O composto fenólico que apresentou o maior teor foi os polifenóis totais com cerca de 5,590 ± 0,004 g/L de catequina, seguido pelos flavonóides totais com 0,606 ± 0,000 g/L de catequina e hidroxicinamatos com 0,264 ± 0,004 g/L de ácido caféico. Além disso, apresentou 0,097 ± 0,001 g/L de antocianinas e 0,187 ± 0,001 g/L de oligômeros de procianidinas.

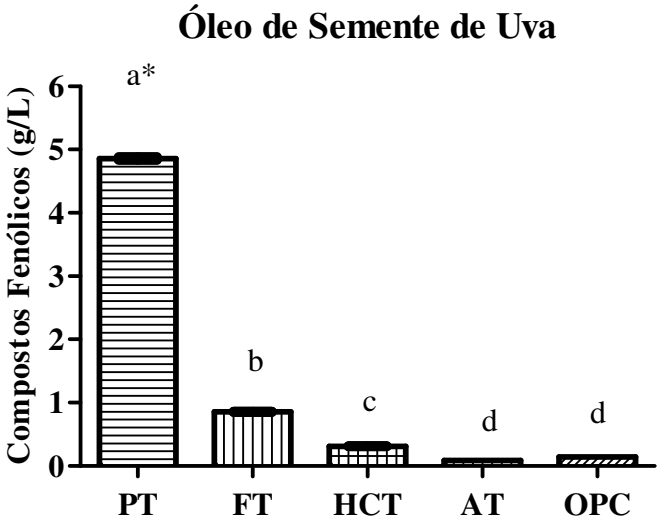

Figura 2. Compostos Fenólicos presentes no Óleo de Semente de Uva. Onde: PT = Polifenóis Totais (g/L de catequina); FT = Flavonóides Totaís (g/L de catequina); HCT = Hidroxicinamatos Totais (g/L de ácido caféico); AT = Antocianinas Totais (g/L); OPC = Oligômeros de Procianidinas (g/L).*Letras distintas diferem significativamente pelo pós teste de *Tukey* (para $p \leq 0{,}001$).

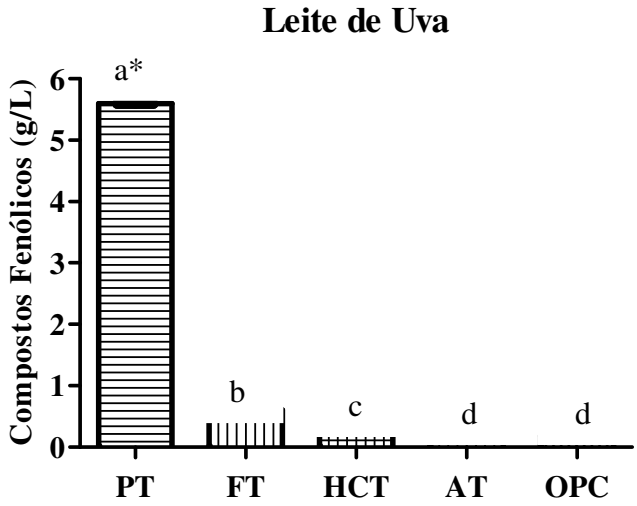

Figura 3. Compostos Fenólicos presentes no Leite de Uva. Onde: PT = Polifenóis Totais (g/L de catequina); FT = Flavonóides Totaís (g/L de catequina); HCT = Hidroxicinamatos Totais (g/L de ácido caféico); AT = Antocianinas Totais (g/L); OPC = Oligômeros de Procianidinas (g/L).*Letras distintas diferem significativamente pelo pós teste de *Tukey* (para $p \leq 0{,}001$).

Até o momento a literatura é bastante escassa sobre o leite de uva sendo este o primeiro trabalho realizado, nas condições testadas, para esta matéria-prima. O leite de uva apresenta em sua composição tanto o óleo quanto o extrato de semente de uva, e pode-se dizer que os resultados obtidos foram satisfatórios comparando com os resultados obtidos no óleo e no extrato.

O leite de uva pode ser amplamente utilizado na área cosmética por ter agregado na mesma base características do extrato e do óleo. Além disso, constitui-se em um sistema estável (emulsão) o que pode ser observado pela dificuldade de desenvolvimento de um método de extração capaz de romper/ desorganizar este sistema. Este sistema emulsionado também poderá garantir/proteger o óleo de processo de oxidação, principalmente se o mesmo se tratar de uma emulsão do tipo O/A, onde o mesmo estará englobado por uma fase aquosa, portanto, comparado ao óleo puro pode ser mais estável.

Para a indústria cosmética, o uso deste tipo de matéria-prima é viável, uma vez que o leite de uva oferece fácil a incorporação nas diferentes bases cosméticas. A emulsão se apresenta mais estável, podendo ainda facilitar uma maior hidratação e penetração dos ativos na pele.[11]

A Figura 4 expressa a quantidade de compostos fenólicos presentes no extrato de vinho, polifenóis totais com 4,660 ± 0,005 g/L de catequina. Além disso, apresentou flavonóides totais de 0,962 ± 0,002 g/L de catequina e de 0,206 ± 0,003 g/L de hidroxicinamatos expressos em ácido caféico. Além destes compostos, ainda obteve-se 0,006 ± 0,000 g/L de antocianinas e 0,040 ± 0,002 g/L de oligômeros de procianidinas.

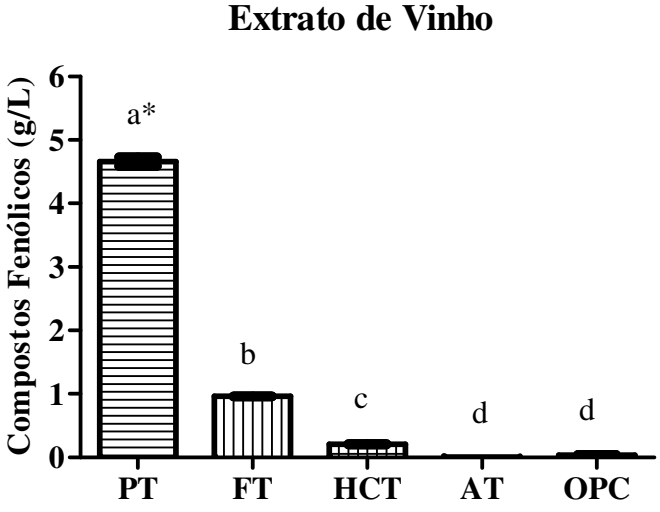

Figura 4. Compostos Fenólicos presentes no Extrato de Vinho. Onde: PT = Polifenóis Totais (g/L de catequina); FT = Flavonóides Totaís (g/L de catequina); HCT = Hidroxicinamatos Totais (g/L de ácido caféico); AT = Antocianinas Totais (g/L); OPC = Oligômeros de Procianidinas (g/L).*Letras distintas diferem significativamente pelo pós teste de *Tukey* (para $p \leq 0,001$).

O extrato de vinho contém grande quantidade de compostos fenólicos, especialmente polifenóis, flavonóides e ácidos hidroxibenzóicos e hidroxicinâmicos.[36] Em um estudo realizado com extrato de vinho foi possível determinar por CLAE 0,79 g/ L de compostos

fenólicos, 0,0054 g/L de ácido caféico, 0,066 g/L de catequina e 0,084 g/L de antocianinas totais, demonstrando valores menores do que nosso estudo, podendo ser justificado pelas seis concentrações que são realizadas no extrato de vinho utilizado neste trabalho.[37]

De acordo com o laudo do fornecedor, a quantidade de hidroxicinamatos tem que ser maior do que 0,12 g/L de ácido caféico, o que confere com o que foi encontrado em nosso trabalho sendo 0,206g/L.[17]

Tabela 2. Compostos Fenólicos presentes nos Bioativos de Uva (*Vitis vinifera*).

Bioativos	**PT ± DP (g/L de catequina)**	**FT ± DP (g/L de catequina)**	**HCT ± DP (g/L de ácido caféico)**	**AT ± DP (g/L)**	**OPC ± DP (g/L)**
ESU	$5{,}330 \pm 0{,}002^{b}$	$0{,}360 \pm 0{,}010^{d}$	$0{,}067 \pm 0{,}000^{d}$	$0{,}091 \pm 0{,}002^{b}$	$0{,}098 \pm 0{,}003^{c}$
OSU	$4{,}860 \pm 0{,}015^{c}$	$0{,}856 \pm 0{,}004^{b}$	$0{,}309 \pm 0{,}001^{a}$	$0{,}088 \pm 0{,}000^{c}$	$0{,}144 \pm 0{,}000^{b}$
LUV	$5{,}590 \pm 0{,}004^{a*}$	$0{,}606 \pm 0{,}000^{c}$	$0{,}264 \pm 0{,}004^{b}$	$0{,}097 \pm 0{,}001^{a}$	$0{,}187 \pm 0{,}000^{a}$
EV	$4{,}660 \pm 0{,}005^{d}$	$0{,}962 \pm 0{,}002^{a}$	$0{,}206 \pm 0{,}003^{c}$	$0{,}006 \pm 0{,}000^{d}$	$0{,}040 \pm 0{,}002^{d}$

DP* = Desvio Padrão. Onde: PT = Polifenóis Totais; FT = Flavonóides Totaís; OPC = Oligômeros de Procianidinas; ESU = Extrato de Semente de Uva; OSU = Óleo de Semente de Uva; LUV = Leite de Uva; EV = Extrato de Vinho. *Letras distintas diferem significativamente pelo pós teste de *Tukey* (para $p \leq 0{,}001$).

Enfim observando a Tabela 2 pode-se concluir que em relação aos polifenóis totais o leite de uva apresentou maior teor de catequina (5,590 ± 0,004 g/L). O extrato de vinho apresentou a maior concentração de flavonóides totais (0,962 ± 0,002 g/L de catequina), para os hidroxicinamatos, o óleo de semente de uva apresentou a maior quantidade (0,309 ± 0,001 g/L de ácido caféico). Além disso, a matéria prima que apresentou o maior teor de antocianinas e oligômeros de procianidinas foi leite de uva com 0,097 ± 0,001 g/L e 0,187 ± 0,001 g/L, respectivamente.

Avaliação da Atividade Antioxidante *in vitro*

O radical DPPH$^{\bullet}$ (1,1-difenil-2-picrilhidrazil) é estável quando em contato com uma substância antioxidante doadora de hidrogênio, pode ser reduzido em meio alcoólico/ metanólico, formando difenil picrilhidrazina.[11] Essa redução é acompanhada pela diminuição da absorbância em comprimento de onda de 517nm, uma vez que ocorre uma mudança de coloração, de violeta (característica do radical) para marrom/amarelado enquanto a reação se processa. A intensidade da coloração é proporcional à concentração da substância com potencial antioxidante.[11,38]

Os resultados apresentados na Figura 5 demonstraram que os bioativos de uva são capazes de reduzir o radical DPPH$^{\bullet}$.

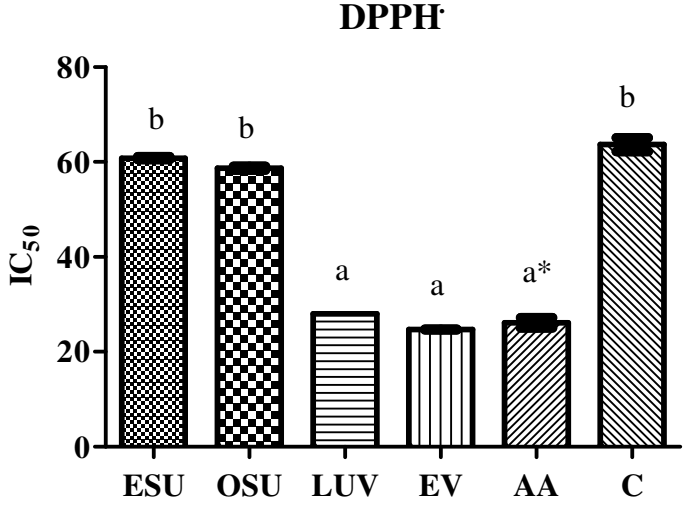

Figura 5. Atividade Antioxidante *in vitro* dos bioativos da uva (*Vitis vinifera*). IC_{50}= quantidade de amostra (%) capaz de reduzir 50% do radical livre DPPH$^{\bullet}$. Onde: ESU = Extrato de Semente de Uva; OSU = Óleo de Semente de Uva; LUV = Leite de Uva; EV =Extrato de Vinho; AA = Ácido Ascórbico; C = Catequina. *Letras distintas diferem significativamente pelo pós teste de *Tukey* (para $p \leq 0,01$).

Os bioativos de uva que apresentaram a maior atividade antioxidante foram o extrato de vinho (IC_{50} = 24,69 ± 0,056) e o leite de uva (IC50 = 28,040 ± 0,017) apresentando atividade similar ao ácido ascórbico (IC_{50} = 26,090 ± 1,050). Essa atividade pode ser explicada devido ao alto teor de compostos fenólicos que estas matérias-primas apresentaram, sendo que o extrato de vinho é concentrado seis vezes para justamente aumentar o seu conteúdo fenólico e sua atividade antioxidante. O leite de uva, por sua vez, apresenta em sua composição dois dos bioativos de uva, o óleo e o extrato, o que poderá contribuir para a atividade antioxidante frente às outras matérias-primas.

O extrato de semente de uva (IC_{50} = 60,670 ± 0,128) e o óleo de semente de uva (IC_{50} = 58,690 ± 0,377) apresentaram uma atividade antioxidante inferior ao leite de uva e ao extrato de vinho. Porém, apresentaram uma atividade antioxidante similar ao padrão catequina (IC_{50} = 63,680 ± 1,420), o que não limita a aplicação destas matérias-primas em formulação cosméticas para obtenção de um efeito antioxidante.

Um outro estudo realizado avaliou a atividade antioxidante do óleo de semente de uva utilizando como método o DPPH$^{\bullet}$, o resultado obtido foi próximo ao encontrado neste trabalho um IC_{50} = 52, comprovando que o óleo apresenta atividade antioxidante[11].

Outra pesquisa enfocando este mesmo tópico avaliou a atividade antioxidante dos compostos fenólicos presentes em subprodutos da uva (extrato de semente e bagaço de uva). O resultado de IC_{50} foi 62,500 ± 0,760, sendo similar ao encontrado neste trabalho.[39]

Com isso, é possível concluir que os bioativos apresentam uma atividade antioxidante relevante e que apresentam potencial para serem incorporados em formulações cosméticas, com objetivo de minimizar os danos causados pelos radicais livres, proteger a pele e mantê-la saudável por mais tempo. No entanto, testes adicionais de verificação da atividade antioxidante da formulação como um todo, após a adição destas matérias-primas, são fundamentais, uma vez que parâmetros como incompatibilidades e instabilidade do sistema podem influenciar a atividade do produto final.

Conclusão

Todos os bioativos apresentaram compostos fenólicos em sua composição. O bioativo da uva que apresentou o maior teor de polifenóis totais expressos em g/L de catequina, foi o leite de uva, podendo ser devido à presença tanto do óleo quanto do extrato de semente de uva em sua composição. O extrato de vinho foi o que apresentou maior quantidade de flavonóides totais (expressos em g/L de catequina). O óleo de semente de uva apresentou o maior teor de hidroxicinamatos (expressos em g/L de ácido caféico). Em relação às antocianinas totais (g/L) e os oligômeros de procianidinas (g/L) o leite de uva foi o que apresentou a maior concentração.

Todos os bioativos apresentaram atividade antioxidante, o que apresentou o maior efeito foi o extrato de vinho, seguido pelo leite de uva apresentando uma atividade antioxidante similar ao ácido ascórbico.

Neste contexto, pode-se inferir que as matérias primas podem ser utilizadas como componentes de formulações cosméticas com objetivo de acentuar e prolongar a saúde e a beleza natural da pele e do cabelo protegendo-os contra as agressões diárias.

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